SPECIATION AND SPECIES DELIMITATION IN INSULAR AND CONTINENTAL SYSTEMS: THE CASES OF THE COMOROS ISLANDS AND AUSTRALIA.

DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES DER FAKULTÄT FÜR BIOLOGIE
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VORGELEGT VON

PRESENTED BY

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AM (*ON*): 03/04/2013

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"Überall habe ich auf den ewigen Einfluss hingewiesen,
welchen die physische Natur auf die moralische
Stimmung der Menschheit und auf ihre Schicksale
ausübt. […] 'Wer sich herausgerettet aus der stürmischen
Lebenswelle', folgt mir gern in das Dickicht der Wälder,
durch die unabsehbare Steppe und auf den hohen
Rücken der Andeskette."

Alexander von Humboldt

SPECIATION AND SPECIES DELIMITATION IN INSULAR AND CONTINENTAL SYSTEMS: THE CASES OF THE COMOROS ISLANDS AND AUSTRALIA.

> "In all probability more paper has been consumed on the questions of the nature and definition of the species than any other subject in evolutionary and systematic biology."
>
> Edward Orlando Wiley III

> "[…] it is not too much to say that when we have mastered the difficulties presented by the peculiarities of island life we shall find it comparatively easy to deal with the more complex and less clearly defined problems of continental distribution […]"
>
> Alfred Russell Wallace

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den

(Oliver Hawlitschek)

Erklärung

Hiermit erkläre ich, dass die Dissertation **nicht** ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich anderweitig einer Doktorprüfung ohne Erfolg **nicht** unterzogen habe.

München, den

(Oliver Hawlitschek)

Liste der Publikationen und Erklärung über den Eigenanteil bei der Autorenschaft an wissenschaftlichen Artikeln

Hiermit erkläre ich, dass ich den im Folgenden angegebenen Eigenanteil an der Arbeit zu den in meiner kumulativen Dissertation enthaltenen wissenschaftlichen Artikeln erbracht habe:

Ich habe für alle Artikel selbstständig unter Anleitung meiner Betreuer Dr. Frank Glaw und Dr. Michael Balke die konzeptionelle Erstellung und Planung, die Sammlung der Daten und Proben, die Verarbeitung der Proben, die Aufbereitung und Auswertung der Daten, sowie die Erstellung der Manuskripte, ihre Einreichung zur Publikation, ihre Überarbeitung nach Begutachtung, sowie alle weiteren Arbeiten durchgeführt.

Dabei kennzeichne ich folgende **Ausnahmen** (ausführende Autoren sind mit Initialen angegeben):

HAWLITSCHEK O., NAGY Z.T., BERGER J., GLAW F. (submitted): DNA Barcoding allows reliable identification of island populations of Comoran squamate reptiles. PLoS ONE.

JB und FG trugen wesentlich zur Probensammlung bei. Ca. 50% der verwendeten DNA-Sequenzen wurden von JB erstellt. Ca. 10% der verwendeten DNA-Sequenzen wurden von ZTN erstellt. Vorläufige Analysen (nicht im Artikel dargestellt) wurden von JB erstellt.

HAWLITSCHEK O., GLAW F. (2012): The complex colonization history of nocturnal geckos (*Paroedura*) on the Comoros Archipelago. Zoologica Scripta 42, 135-150.

Ca. 10% der verwendeten DNA-Sequenzen wurden von ZTN erstellt.

HAWLITSCHEK O., HENDRICH L., ESPELAND M., TOUSSAINT E.F.A., GENNER M.J., BALKE M. (2012): Pleistocene climate change promoted rapid diversification of aquatic invertebrates in Southeast Australia. BMC Evolutionary Biology 12, 142.

Alle Proben und ökologischen Daten wurden von LH gesammelt und bereitgestellt. ME trug wesentlich zu den Diversifikationsanalysen bei. EFAT führte die phylogeographischen Strukturanalysen aus. MJG führte die Tests zu Hybridisierung vs. incomplete lineage sorting aus.

HAWLITSCHEK O., HENDRICH L., BALKE M. (2012): Molecular phylogeny of the squeak beetles, a family with disjunct Palearctic – Australian range. Molecular Phylogenetics and Evolution 62, 550-554.

Alle Proben und ökologischen Daten wurden von LH gesammelt und bereitgestellt. Die Erhebung und Auswertung morphologischer Daten wurde von MB ausgeführt.

HAWLITSCHEK O., NAGY Z.T., GLAW F. (2012): Island evolution and systematic revision of Comoran snakes: why and when subspecies still make sense. PloS ONE 7(8): e42970.

FG trug wesentlich zur Probensammlung bei. Ca. 10% der verwendeten DNA-Sequenzen wurden von ZTN erstellt.

HAWLITSCHEK O., BRÜCKMANN B., BERGER J., GREEN K., GLAW F. (2011): Integrating field surveys and remote sensing data to study distribution, habitat use and conservation status of the herpetofauna of the Comoro Islands. Zookeys 144: 21-79.

BB, JB, KG und FG trugen wesentlich zur Datensammlung bei. BB führte die Satellitenbildauswertung für die Insel Anjouan aus. KG überarbeitete den Artikel sprachlich und stilistisch.

HAWLITSCHEK O., PORCH N., HENDRICH L., BALKE M. (2011): Ecological niche modelling and nDNA sequencing support a new, morphologically cryptic beetle species unveiled by DNA barcoding. PloS ONE 6: e16662.

Alle Proben und ökologischen Daten wurden von LH gesammelt und bereitgestellt. Die Erhebung und Auswertung morphologischer Daten wurde von LH ausgeführt. Die Artbeschreibung wurde von LH erstellt.

Darüber hinaus wurden alle publizierten Artikel entsprechend der Gutachten im Peer-Review-Prozess modifiziert. Alle Autoren trugen zur Überarbeitung der jeweiligen Manuskripte bei. Alle publizierten Artikel bis auf HAWLITSCHEK, BRÜCKMANN, BERGER, GREEN und GLAW (2011) und HAWLITSCHEK und GLAW (2012) wurden von einem professionellen Dienstleister (language editing service) der sprachlichen und stilistischen Bearbeitung unterzogen.

Alle in dieser Dissertation enthaltenen Grafiken und Fotografien, soweit nicht anders angegeben, wurden von mir erstellt.

München, den

(Oliver Hawlitschek)

bestätigt:

(Prof. Dr. Gerhard Haszprunar)

Table of contents:

1 Summaries

1.1 Abstract (English)

Background

The question of the nature and the origin of biological species is one of the most fundamental issues in biology. This so-called 'species problem' has been intensely debated since the formulation of the theory of evolution by Darwin. To date, about 30 concepts have been published that attempt to define, often conflictingly, what a species is and how it can be recognized by scientists, and a general agreement is not in sight. At the same time, taxonomy faces the challenge of a huge amount of global biodiversity that remains to be scientifically described. Therefore, taxonomic methods are required that make the description of new species faster and at the same time make them more reliable and reproducible. DNA barcoding, i.e., the use of a short standardized fragment of DNA for species identification, means to accelerate biodiversity inventories and the recognition of new species. Cybertaxonomy makes the access to taxonomic information easier and faster and helps increasing the efficiency of the taxonomic workflow by making data available online and free. Integrative taxonomy combines different lines of evidence, such as morphological, molecular, and ecological data, to make species delimitation and species descriptions more reliable and reproducible.

In this dissertation I explore two different zoological study systems in order to test current models of speciation and methods of species delimitation. These study systems are the reptiles of the Comoros Archipelago, a group of oceanic islands in the Western Indian Ocean, and aquatic beetles of Australia. The biogeographical backgrounds of these two groups are very different: The Comoros are relatively young volcanic islands whose native terrestrial and non-flying fauna originates exclusively from overseas dispersal. In contrast, Australia is an old isolated landmass whose biota were shaped by past climate change. Oceanic islands have been recognized as prime study systems even by early biogeographers, and my study of these two different systems highlights the common grounds as well as the differences between insular and continental biogeography.

Methods and principal findings

I selected two groups out of the Comoran reptiles (*Paroedura* geckos and *Lycodryas* snakes) and three groups out of the Australian aquatic beetles (family Hygrobiidae and genera *Antiporus* and *Sternopriscus*, Dytiscidae) as study groups for this dissertation. In both cases, the data fundament for subsequent studies was laid by DNA barcoding, as included for reptiles in this dissertation. I then conducted analyses of several mitochondrial and nuclear genetic markers to reconstruct the phylogenies of the study groups and, in Hygrobiidae, estimate the divergence times within the phylogeny in a molecular clock approach. In Comoran reptiles, I attempted to correlate phylogenetic hypotheses with the geological history of island emergence and dispersal to and within the archipelago. In Australian *Antiporus* and *Sternopriscus* beetles, I attempted to correlate phylogenies with past climate change, the genesis of the Australian arid zone, and the Pleistocene climate oscillations. I used Ecological Niche Modeling to corroborate these hypotheses with evidence for ecological diversification in Australian beetles.

Based on the results of DNA barcoding and molecular phylogenies, I used an integrative taxonomic approach to revise the taxonomy of the study groups accordingly. The lines of evidence I used were morphological data, mitochondrial molecular markers, nuclear molecular markers, and categorical and quantitative ecological data. This approach led to the description of one new species of beetle (*Antiporus occidentalis* HAWLITSCHEK, HENDRICH, PORCH, & BALKE, 2011) and two new species (*Paroedura stellata* HAWLITSCHEK & GLAW, 2012 and *Lycodryas cococola* HAWLITSCHEK, NAGY & GLAW, 2012), and one subspecies (*Lycodryas cococola innocens* HAWLITSCHEK, NAGY & GLAW, 2012), of reptiles, as well as to the confirmation or resurrection of the previously described taxa *Lycodryas maculatus* (GÜNTHER, 1858) and *Lycodryas maculatus comorensis* (PETERS, 1874). All taxonomic acts followed a cybertaxonomic concept by using LSID numbers, online databases, and, as far as possible, open access publication.

Additionally, I used data collected in the course of this dissertation for estimating the conservation status of Comoran reptiles and for the development of SmartHerper Comoros, a field guide to the herpetofauna of the Comoros as a mobile application for smartphone.

Conclusions

The results of my studies show complex biogeographic patterns in both the insular and the continental study system. According to these results, the ancestors of native reptiles have colonized the Comoros Archipelago in a very complex pattern, including several events of extinction and re-colonization, e.g., in the case of the gecko genus *Paroedura*, with little correlation to the geographic positions or geological ages of the islands. Many endemic species show possible morphological adaptations to the island environment. Molecular data of reptiles suggest that Grand Comoro, the presumably geologically youngest island, may be considerably older than previously estimated. In Australian aquatic beetles, speciation events were shown to be of very different ages from Mesozoic (Hygrobiidae) to Pleistocene (*Antiporus*) and very recent (*Sternopriscus*). Molecular divergences indicate that speciation in the *Sternopriscus tarsalis* radiation was one of the fastest speciation events so far described among insects.

I applied an integrative taxonomical approach in the delimitation of all newly described taxa and in the confirmation of previously described taxa. This approach provided sufficient evidence for species delimitation even in the absence of morphological differentiation (*Antiporus*), or when genetic data did not provide any clear evidence (*Sternopriscus tarsalis* radiation). In these cases, ecological data, particularly such data from Ecological Niche Modeling, was shown to be highly useful in integrative species delimitation. In the same approach applied to *Lycodryas* snakes, I argued for the usefulness of the subspecies rank for infraspecific entities with some degree of differentiation.

I conclude that my research in the study systems I investigated in this dissertation are but small pieces that nevertheless advance our understanding of speciation and species delimitation by contributing to the ongoing debate on the species problem. My dissertation presents these results and represents my position in the debate. I see this debate as a very fruitful process that is highly important for the current development of evolutionary biology and biodiversity research.

1.2 Zusammenfassung (Deutsch)

Hintergrund

Zu den grundlegendsten Fragestellungen in der Biologie gehört die Frage nach der Natur und Entstehung biologischer Arten. Dieses Problem der Artdefinition (Engl. "Species Problem") war der Ursprung weitläufiger und kontroverser Diskussionen seit der Formulierung der Darwin'schen Evolutionstheorie. Bis heute wurden etwa 30 verschiedene und zum Teil gegensätzliche Konzepte zur Definition und wissenschaftlichen Abgrenzung der Art veröffentlicht. Eine Einigung ist nicht in Sicht. Gleichzeitig ist die Taxonomie mit der Herausforderung konfrontiert, dass ein immenser Teil der weltweiten Artenvielfalt wissenschaftlich noch nicht erfasst und beschrieben ist. Dies erfordert Methoden, die die Beschreibung neuer Arten beschleunigen und gleichzeitig deren Zuverlässigkeit und Nachvollziehbarkeit wahren. DNA-Barcoding, d.h. Artbestimmung an Hand eines kurzen standardisierten Fragments der DNA, soll die Erfassung der Artenvielfalt und das Erkennen unbekannter Arten beschleunigen. Die so genannte "Cybertaxonomie" erlaubt leichteren und schnelleren Zugriff auf vorhandene taxonomische Informationen, indem Daten online und kostenfrei zur Verfügung gestellt werden. Dies trägt zur Steigerung der Effizienz taxonomischer Prozesse bei. Integrative Taxonomie kombiniert verschiedene Beweislinien, wie zum Beispiel morphologische, molekulare und ökologische Daten, um die Zuverlässigkeit und Nachvollziehbarkeit bei der Abgrenzung und Beschreibung von Arten zu erhöhen.

In dieser Dissertation untersuche ich zwei verschiedene Studiensysteme, um derzeit als gültig angesehene Modelle der Artbildung und Methoden der Artabgrenzung zu testen. Bei diesen Systemen handelt es sich um die Reptilien der Komoren, einer Gruppe ozeanischer Inseln im westlichen Indischen Ozean, und australische Wasserkäfer. Die Biogeographie dieser beiden Gruppen ist durch höchst unterschiedliche Faktoren geprägt: Die Komoren sind vergleichsweise junge vulkanische Inseln, deren einheimische, landbewohnende und flugunfähige Faunenelemente ausschließlich auf Besiedelung durch Drift über das offene Meer zurückgehen. Dagegen stellt Australien eine alte und isolierte Landmasse dar, deren Lebensgemeinschaften durch Klimaveränderungen in der Erdgeschichte geprägt sind. Ozeanische Inseln wurden schon von frühen Forschern als wichtige Systeme zum Studium der Biogeographie erkannt, und meine Untersuchung dieser beiden so unterschiedlichen Systeme stellt sowohl die Gemeinsamkeiten als auch die Unterschiede der Biogeographie von Inseln und Kontinenten heraus.

Methoden und wesentliche Ergebnisse

Als Fallbeispiele zur Untersuchung im Rahmen dieser Dissertation wählte ich zwei Teilgruppen der komorischen Reptilien (Geckos der Gattung *Paroedura* und Schlangen der Gattung *Lycodryas*) sowie drei Teilgruppen der australischen Wasserkäfer (die Familie Hygrobiidae und die Gattungen *Antiporus* und *Sternopriscus* aus der Familie Dytiscidae) aus. In beiden Fällen wurde der Grundstein für weitere Untersuchungen durch DNA-Barcoding gelegt, wie für die Reptilien als Teil dieser Dissertation beschrieben. Als nächsten Schritt führte ich Untersuchungen an mehreren mitochondrialen und nukleären Genmarkern durch, um die Phylogenien der jeweiligen Gruppen zu rekonstruieren und, im Fall der Hygrobiidae, das Alter der Phylogenie durch eine molekulare Uhr abzuschätzen. Ich versuchte, die Phylogenien komorischer Reptilien mit geologischen Daten über die erdgeschichtliche Entstehung der Inseln sowie die Ausbreitungsmöglichkeiten zu und zwischen den Inseln in Verbindung zu bringen.

Bei Phylogenien australischer Käfer der Gattungen *Antiporus* und *Sternopriscus* suchte ich nach Korrelationen zu Klimaveränderungen in der Erdgeschichte, der Entstehung der australischen Trockengebiete und den Eiszeiten im Pleistozän. Diese Hypothesen konnte ich durch Belege für die ökologische Diversifikation australischer Käfer aus meinen Ökologischen Nischenmodellierungen untermauern.

Auf der Grundlage der Ergebnisse von DNA-Barcoding und molekularen Phylogenien unternahm ich taxonomische Revisionen der betreffenden Gruppen nach Methoden der integrativen Taxonomie. Als Beweislinien verwendete ich Daten aus morphologischen Untersuchungen, mitochondrialen und nukleären Genen, sowie kategorische und quantitative ökologische Daten. Dieser Ansatz führte zur Beschreibung einer neuen Art von Käfern (*Antiporus occidentalis* HAWLITSCHEK, HENDRICH, PORCH, & BALKE, 2011), zweier neuer Arten (*Paroedura stellata* HAWLITSCHEK & GLAW, 2012 and *Lycodryas cococola* HAWLITSCHEK, NAGY & GLAW, 2012) und einer Unterart von Reptilien (*Lycodryas cococola innocens* HAWLITSCHEK, NAGY & GLAW, 2012), sowie zur Bestätigung oder Wiederherstellung der Gültigkeit der zuvor beschriebene Taxa *Lycodryas maculatus* (GÜNTHER, 1858) und *Lycodryas maculatus comorensis* (PETERS, 1874). Alle taxonomischen Handlungen wurden gemäß dem Konzept der Cybertaxonomie ausgeführt: es wurden LSID-Nummern vergeben, Einträge in Online-Datenbanken vorgenommen, und nach Möglichkeit Publikationsmodi mit freiem Zugang für Leser gewählt.

Zudem verwendete ich die im Rahmen meiner Dissertation gesammelten Daten zur Abschätzung des artenschutzfachlichen Status der Reptilien der Komoren. Außerdem dienten sie als Basis für die Entwicklung von SmartHerper Comoros, einem Naturführer zur Herpetofauna der Komoren als Applikation für Smartphone.

Schlussfolgerungen

Die Ergebnisse meiner Untersuchungen weisen auf komplexe biogeographische Muster sowohl im insulären als auch im kontinentalen Untersuchungsgebiet hin. Demzufolge haben die Stammformen der dort heimischen Reptilien die Komoren in einem sehr komplizierten Muster besiedelt, das z.B. im Fall der Gecko-Gattung *Paroedura* mehrere Aussterbe- und Wiederbesiedlungsereignisse beinhaltet und kaum mit der geographischen Lage und dem geologischen Alter der Inseln korreliert. Viele endemische Arten zeigen mögliche morphologische Anpassungen an den Insellebensraum. Molekulare Daten komorischer Reptilien legen nahe, dass Grand Comoro, zuvor als geologisch jüngste Insel angesehen, möglicherweise weit älter ist als bislang angenommen. Über australische Wasserkäfer erhobene Daten zeigten, dass Artbildungsereignisse innerhalb dieser Gruppe von höchst unterschiedlichem erdgeschichtlichem Alter sind und vom Mesozoikum (Hygrobiidae) über das Pleistozän (*Antiporus*) bis in die jüngste erdgeschichtliche Vergangenheit (*Sternopriscus*) reichen. Molekulare Unterschiede weisen darauf hin, dass die "*Sternopriscus tarsalis* radiation" einen der am schnellsten verlaufenen bislang beschriebenen Artbildungsprozesse innerhalb der Insekten darstellt.

Der integrativ-taxonomische Ansatz erwies sich in meinen Augen bei der Abgrenzung aller neu beschriebenen Taxa wie auch bei der Bestätigung bestehender Taxa als höchst erfolgreich. Durch diesen Ansatz standen Belege für die Artabgrenzung auch bei unzureichender morphologischer oder genetischer Differenzierung in ausreichendem Maße zur Verfügung. Ökologische Daten, insbesondere solche, die bei Ökologischer Nischenmodellierung gewonnen wurden, haben sich in diesen Fällen als höchst aussagekräftig bei der Artabgrenzung erwiesen. Bei der Anwendung des integrativ-taxonomischen Ansatzes auf Schlangen der Gattung *Lycodryas* argumentierte ich, den Rang der Unterart auf infraspezifische Einheiten mit einem gewissen Grad der Differenzierung anzuwenden.

Schlussendlich liefern die Ergebnisse der Untersuchungen in meiner Dissertation nur einen kleinen, aber meiner Meinung nach dennoch nützlichen Beitrag zu unserem Verständnis darüber, wie biologische Arten entstehen und wie sie wissenschaftlich erfasst werden können. Meine Dissertation präsentiert diese Ergebnisse im Kontext der Debatte über die Artdefinition und stellt auch meine Meinung und Position darin dar. Meiner Ansicht nach ist diese äußerst fruchtbare Debatte von hoher Bedeutung für die zeitgenössische Entwicklung der Evolutionsbiologie und Biodiversitätsforschung.

Auf Nachfrage beim Autor ist eine deutsche Übersetzung erhältlich, die Kapitel 2.1, 8.4 und 9, die Abstracts aller enthaltenen wissenschaftlichen Artikel, die Zitate und die Abbildungsbeschreibungen des allgemeinen Teiles dieser Dissertation enthält.

2 Background of this work

2.1 Aims and overview

I prepared this dissertation 'Speciation and species delimitation in insular and continental systems: the cases of the Comoros Islands and Australia' at the Zoologische Staatssammlung (Bavarian State Collection of Zoology), in collaboration with the Ludwig-Maximilians-Universität, Munich, between 2009 and 2013. The work was supervised by Prof. Dr. GERHARD HASZPRUNAR, Dr. FRANK GLAW, and Dr. MICHAEL BALKE.

This work has two major components, the 'Diversification of Australian aquatic beetles' and 'Reptiles on Indian Ocean islands: phylogeny, biogeography, and the impact of environmental changes'. Both originally separate projects yielded satisfactory output, and I realized that they had a common ground. The discussion on speciation, species delimitation, and species concepts was important in all major papers produced in both projects. I am happy that I was given the chance to merge these two rather different study subjects in one dissertation. This allowed me to focus my discussion on the underlying major questions concerning not only a specified zoological study system, but all systematic and evolutionary biology.

First, in Chapter 3, I present an introduction to speciation, species delimitation, and the importance of biogeography for the understanding of these issues. I also introduce the two study systems of this dissertation, Comoran reptiles and Australian aquatic beetles. Second, in Chapters 4 to 6, I present selected case studies from both study systems. Third, in Chapter 8, I discuss the importance of the insights gained in these case studies for our knowledge of biogeography, speciation, and the definition of species.

I am aware that much of what we believe to know on many of the topics that are discussed in this dissertation – the origin of species, the species problem, the definitions of taxonomic ranks – is not based on 'pure' evidence from observations. Rather, the answers to questions concerning these topics are inferred, if not speculated, from the little (but increasing) amount of evidence we have. What evidence we have allowed for the forming of conflicting opinions among scientists and sparked ongoing intense debates on the origin, definition, and nature of species. This dissertation reviews the debates led so far and also reflects my own opinions on these fundamental questions of biology.

> Of course not. After all, I may be wrong.
>
> Bertrand Arthur William Russell

2.2 Acknowledgements / Danksagung

I have a vast number of people to thank for supporting me not only during the more than four years of preparing this thesis, but also during earlier phases of studying and learning. Without them, this work would have either been completely impossible for me to accomplish, or it would at least have taken a very different form.

My first thanks go to the people who had contributed most to shaping this work and, at the same time, shaping me in the ways of a scientist. All of them work at the Zoologische Staatssammlung, Munich, and are the main reason why I feel that this institute is my professional home. Dr. Frank Glaw, curator of herpetology, already advised me in my diploma thesis and continued to do so in my dissertation. If I ever leave any significant scientific traces in the field of herpetology, I shall remember that it was first and most of all him who set me on this path. It was and will always be a pleasure to work with you! Dr. Michael Balke, Curator of Coleoptera, turned from my 'mere' employer into the second adviser of the work for this dissertation. Maybe more importantly on the long run, he taught me some priceless lessons on how to handle a molecular genetic laboratory without despairing, on how I had to set up my mind to tackle the daily challenges that a scientist faces, and that beetles are more than just frog food. Prof. Dr. Gerhard Haszprunar steers not only the ship of the ZSM, but also those of the SNSB and of the Chair of Systematic Biology at the LMU, but he still found time to observe my progress and listen to my sorrows. Dear Herr Haszprunar, thank you for allowing me to conduct this dissertation at the ZSM, and for your kind and generous advice concerning all matters. Vielen herzlichen Dank!

Many more people have contributed significantly to my work. They are acknowledged in the correspondent sections of the chapters of this dissertation. However, I wish to repeat some of my thanks in this place because it is the least I can do to express my gratitude. I am grateful to people who accompanied me on my field trips in the course of this work, particularly to Bastian Brenzinger, Johannes Berger und Boris Brückmann. Es war super mit Euch, jederzeit wieder! Likewise, many thanks to all the people from the Union of the Comoros and Mayotte. Merci beaucoup pour vôtre assistance au terrain! Special thanks also to Katie Green and Hugh Doulton, I wish you all the best for your future projects – maybe we meet again in one of them. Furthermore, I thank the referees Prof. Dr. Susanne Renner, Prof. Dr. Gert Wörheide, Prof. Dr. Günther Heubl and Prof. Dr. Dirk Metzler who kindly agreed to review my dissertation.

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I visited many museums in the course of my work and found great hospitality everywhere. Therefore, and for granting me access to their collections and loaning specimens, I am very indebted to the curators and technicians of these institutes. They are namely acknowledged in the specific chapters of this work. Further thanks go to Phil Bowles (IUCN) who reviewed the IUCN Red List assessments of Comoran reptiles, to Dr. Sara Rocha (Porto) who is among the few people worldwide who share my interest in Comoran reptiles, and to DANIEL GUGGENBICHLER und RENÉ RÖSNER (both FH Rosenheim) who did all the technical work for SmartHerper Comoros. I also send special thanks to AXEL BEUTLER (Munich) for showing me some sides of herpetology, and biology (and life) in general, that without him I would not have experienced in this time. Thanks to Prof. Dr. ERNST-GERHARD BURMEISTER (Munich) for taking me to the desert, it was an experience that formed me as a biologist. Herzlichen Dank an alle!

Fig. 1 displays logos of the bodies that provided financial and other support for my work. I am very grateful and indebted to all of them. Most important were the Deutsche Forschungsgemeinschaft (DFG) and the Universität Bayern, who paid my wages, and the Mohamed bin Zayed (MBZ) Species Conservation Fund who provided the bulk of financing for my fieldwork. Additional funding was provided by the Münchner Universitätsgesellschaft, the Freunde der ZSM, EES travel grants, and Synthesys. I thank ESRI Germany and Creaso for providing student licenses of their softwares ArcGIS and ENVI. Additionally, I am grateful to authorities of the Union of the Comoros, France (for Mayotte), and the Federal Republic of Germany, who granted me permissions for research and field work, as well as capturing, export, and import of animals and samples, in their countries.

Zu guter Letzt, aber vielleicht an wichtigster Stelle, möchte ich meiner Familie, TÜLIN YÜCEARDA, meiner Mutter INGE HAWLITSCHEK, BERND HELLBACH, und meinen Großeltern FRANZ HAWLITSCHEK und IDA HAWLITSCHEK, danken. Danke, dass Ihr immer für mich da wart, mich unterstützt habt und – von meinen ersten Anwandlungen im zarten Alter bis jetzt – immer an mich geglaubt habt!

Figure 1: Funding bodies and other organizations that are acknowledged for their contributions to this dissertation.

2.3 Curriculum vitae

Name: Oliver Hawlitschek, Dipl.-Biol. (eq. M.S.)
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Born: 25th July 1981 / Amberg, Germany
Nationality: Germany

Education:

Dissertation: *Speciation and species delimitation in insular and continental systems: the cases of the Comoros Islands and Australia.* Ludwig-Maximilians-Universtät München (LMU, University of Munich); supervisors: Gerhard Haszprunar, Frank Glaw, Michael Balke.

Since 2009/02, submitted 2013/04/03

Undergraduate thesis: *Reptiles and Amphibians of the Comoro Islands: An integrative approach of zoological systematics and remote sensing to assess biodiversity, distribution and conservation of a threatened island herpetofauna.* Supervisors: Gerhard Haszprunar, Frank Glaw

2002/10–2008/09	Study of biology (Diploma, eq. B.S./M.S.), LMU Munich

Professional experience:

Since 2011/05 (ends 2013/04)	Graduate fellowship of the state of Bavaria (BayEfG, https://www.elitenetzwerk.bayern.de/) for a dissertation at the University of Munich (Ludwig-Maximilians-Universität München, conducted at the Zoologische Staatssammlung München – Bavarian State Collection of Zoology, department Herpetology, http://www.zsm.mwn.de/)
Since 2008/10	Scientific assistant (part time) at the Planungsbüro Beutler (private institute for impact studies, ecology, conservation planning and management) (http://www.pb-beutler.de/)
2009/01–2011/03	Scientific assistant (part time, DFG) at the Bavarian State Collection of Zoology, department Coleoptera, project "Diversification of Australian Diving Beetles" (DFG-PI: M. Balke: BA2152/7-1).
2004/05–2008/09	Student assistant at the Planungsbüro Beutler

Internships:

2006/10–2006/12	Internship at the Instituto Nacional de Pesquisas da Amazônia (National Institute for Amazon Research) in Manaus, Brasil; topic: assemblage and trophic structuring of fish communities in the lake Lago Tupé, Manaus, Amazonia.
2003/02	Internship in the zoological garden Hellabrunn in Munich.

Grants and awards:

Total sum: 63,888 EUR = 83,466 US$

Mohamed bin Zayed Species Conservation Fund (http://www.mbzspeciesconservation.org/): project 11253064 "Biodiversity of the Sorata region, Madagascar" (2012-01-04, co-applicant; sum: 10,000 US$).

Graduate fellowship of the state of Bavaria (BayEFG, Bayerisches Eliteförderungsgesetz, https://www.elitenetzwerk.bayern.de/): project "Reptiles on Indian Ocean islands: phylogeny, biogeography and conservation" (2011-05-01 – 2013-04-30; sum: 29,500 EUR).

DAAD-grant (German academic exchange service, http://www.daad.de/de/index.html): thesis grant: dissertation on the Comoros (2010-01-11; sum: 3,300 EUR).

Mohamed bin Zayed Species Conservation Fund (http://www.mbzspeciesconservation.org/): project 0925157 "Reptiles on the Comoro Islands" (2009-11-25, co-applicant; sum: 20,000 US$).

EES Travel Grant (Ecology, Evolution and Systematics Board of the University of Munich; http://www.eeslmu.de/eeswiki/Main_Page): Travel grant for a flight from the Comoros to Madagascar (2009-11-16; sum: 500 EUR).

EES IRT3-Trial (Ecology, Evolution and Systematics Board of the University of Munich; http://www.eeslmu.de/eeswiki/Main_Page): for satellite imagery for the diploma thesis "Systematics and biogeography of the herpetofauna of the Comoro Islands" (2008-02-04; sum: 500 EUR).

Münchener Universitätsgesellschaft (Society of the University of Munich; http://www.unigesellschaft.de/home.php): project "Systematics and biogeography of the herpetofauna of the Comoro Islands" (2007-11-27; sum: 2,400 EUR).

DAAD-grant (German academic exchange service, http://www.daad.de/de/index.html): Internship grant: project "Biotupé" at the Instituto Nacional de Pesquisas da Amazônia (National Institute of Amazon Research; 2006-09-21; sum: 1,825 EUR).

Synthesys-Grant (http://www.synthesys.info/): FR-TAF-2988: Systematic review and biogeography of the herpetofauna of the Comoro Islands (2006-09-14; sum: 2,900 EUR).

Research interests and skills:

Reptiles: Phylogeny, biogeography, taxonomy, and conservation of the herpetofauna of the Comoros archipelago (with Frank Glaw, Zoologische Staatssammlung München)

Diving beetles: DNA-Barcoding, cybertaxonomy, ecological niche modeling of Australian diving beetles (with Michael Balke and Lars Hendrich, Zoologische Staatssammlung München)

Barcoding Fauna Bavarica (orthopterans, amphibians, reptiles: sampling, preparation, data processing)

SmartHerper Comoros, a field guide to the herpetofauna of the Comoros as a mobile application (app) for smartphones (in collaboration with the University of Applied Sciences Rosenheim)

Geographic information systems (GIS), remote sensing, satellite imagery (ENVI), ecological niche modeling (Maxent)

Reviewer for scientific journals:

PLoS One, Biodiversity and Conservation, African Journal of Ecology, Herpetological Conservation and Biology, Journal of Natural History, Asian Herpetological Research

Field work:

As manager (funding, planning, logistics, obtaining of permits etc. mainly organized by myself):

Mayotte (Comoros Archipelago): reptiles and amphibians, collection of field data for the delimitation of monitoring zones (natural regions of ecological, faunistic, and floristic importance, ZNIEFF, 4 weeks, 2013)

Comoros: reptiles and amphibians, collection of field data, samples and vouchers; additional collection of diving beetles, insect varia, crustaceans, molluscs; administration, organization, supervision of students (PhD thesis, 12 weeks, 2010)

Comoros: reptiles and amphibians, collection of data, samples and vouchers; additional collection of insects, molluscs (Diploma thesis, 6 weeks, 2008)

Numerous short excursions for regional conservation monitoring in various regions of Germany (amphibians, reptiles, orthopterans, odonates, lepidopterans, total of 2 – 4 weeks / year)

As participator (funding, planning, logistics, obtaining of permits etc. mainly organized by others):

Madagascar – Sorata region: reptiles and amphibians, collection of data, samples and vouchers; additional collection of phasmids, diving beetles and aquatic hemipterans (5 weeks, 2012)

Costa Rica: reptiles and amphibians (3 weeks, 2011)

Pemba: reptiles and amphibians, collection of data, samples and vouchers (3 weeks, 2009)

Brazil: fishes, collection of data on biodiversity and trophic structures (internship, 8 weeks, 2006)

Various student excursions to Libya, France, Tenerife, and various regions of Germany 2004 – 2008

University teaching and tutoring:

Tutor for the class "Artenvielfalt Zoologie" (zoological biodiversity) at the LMU Munich (2004 – 2007)

Co-supervision of student theses at the department of herpetology of the Zoologische Staatssammlung München:

Investigation of *Pelophylax*-species (green frogs) in various populations in Bavaria by means of an integrative approach. Bachelor thesis by Martin Mayer, Albert-Ludwigs-University Freiburg, department of Evolutionary Ecology, supervisor: Martin Schäfer, 2012

Reassessing the taxonomic status of the endemic Malagasy blind snake *Xenotyphlops mocquardi* (Serpentes: Xenotyphlopidae) with synonymization of *X. mocquardi* with *X. grandidieri*. Student project in the Masters School Ecology, Evolution and Systematics, supervisor: Frank Glaw, 2011

Pelophylax populations in the Munich area: distribution and evidence for species invasion. Student project of the MEME-Program by Glib Mazepa and Biofagri Rachmayuningtyas, supervisor: Frank Glaw, 2011

Species Distribution Modeling of the Lungless Frog, *Barbourula kalimantanensis*. Student project of the MEME-Program by Biofagri Rachmayuningtyas, supervisor: Frank Glaw, 2011

Phylogeny and barcoding of the Comoroan reptiles, with a focus on the genera *Amphiglossus*, *Furcifer*, *Hemidactylus* and *Ramphotyphlops*. Diploma thesis by Johannes Berger, supervisors: Gerhard Haszprunar, Frank Glaw, 2010

Ecological niche modelling of the genus *Phelsuma* with view on invasive species and climatic change. Diploma thesis by Boris Brückmann, supervisors: Christian LaForsch, Frank Glaw, 2010

Membership in professional societies and NGOs:

LBV – Landesbund für Vogelschutz Bayern (http://www.lbv.de/; since 1992)

LARS – Landesverband für Amphibien- und Reptilienschutz Bayern (http://www.lars-ev.de/; since 2005)

Freunde der Zoologischen Staatssammlung München (http://www.zsm.mwn.de/freunde/; since 2005)

ZGAP – Zoologische Gesellschaft für Arten- und Populationsschutz (http://www.zgap.de/; since 2007)

DGfO – Deutsche Gesellschaft für Orthopterologie (http://www.dgfo-articulata.de/; since 2010)

IUCN Snake and Lizard Red List authority (member since 2011; http://cmsdata.iucn.org/downloads/snake_and_lizard_rla_proofed.pdf)

GfBS – Gesellschaft für Biologische Systematik (http://www.gfbs-home.de/; since 2012)

Society of the NaMu (Natural History Museum) of Bavaria (http://www.namu-bayern.de/; since 2012, founding member)

Languages:

German (native), English, Spanish, French, Portuguese

Publications:

Scientific Publications, peer-reviewed:

Hawlitschek O., Yamamoto K.C., Carvalho-Neto, F.G.M.R. (in press): Diet composition of fish assemblages of Lago Tupé, Amazonas, Brasil. Revista Colombiana de Ciencia Animal.

Hawlitschek O., Nagy Z.T., Berger J., Glaw F. (submitted): DNA Barcoding allows reliable identification of island populations of Comoran squamate reptiles. PLoS ONE.

Mayer, M., Hawlitschek O., Glaw F., Zahn A. (submitted): Composition of various green frog populations (*Pelophylax*) in Bavaria, Germany. Salamandra.

Wegener J.E., Swoboda S., Hawlitschek O., Franzen M., Wallach V., Vences M., Nagy Z.T., Hedges S.B., Köhler J., Glaw F. (under review): Morphological variation and taxonomic reassessment of the endemic Malagasy blind snake family Xenotyphlopidae (Serpentes, Scolecophidia). Spixiana.

Hawlitschek O., Glaw F. (2012): The complex colonization history of nocturnal geckos (*Paroedura*) in the Comoros Archipelago. Zoologica Scripta 42, 135-150.

Hawlitschek O., Hendrich L., Espeland M., Toussaint E.F.A., Genner M.J., Balke M. (2012): Pleistocene climate change promoted rapid diversification of aquatic invertebrates in Southeast Australia. BMC Evolutionary Biology 12, 142.

Hawlitschek O., Hendrich L., Balke M. (2012): Molecular phylogeny of the squeak beetles, a family with disjunct Palearctic – Australian range. Molecular Phylogenetics and Evolution 62, 550-554.

Hawlitschek O., Nagy Z.T., Glaw F. (2012): Island evolution and systematic revision of Comoran snakes: why and when subspecies still make sense. PloS ONE 7(8): e42970.

Hawlitschek O., Brückmann B., Berger J., Green K., Glaw F. (2011): Integrating field surveys and remote sensing data to study distribution, habitat use and conservation status of the herpetofauna of the Comoro Islands. Zookeys 144: 21-79.

Hendrich L., Faille A., Hawlitschek O., Tänzler R. (2011): Wiederfund des Schwimmkäfers *Graphoderus bilineatus* (DeGeer, 1774) nach über 25 Jahren in Bayern (Coleoptera, Dytiscidae). [*Re-discovery of the diving beetle* Graphoderus bilineatus *(DeGeer, 1774) in Bavaria after more than 25 years (Coleoptera, Dytiscidae).*] Nachrichtenblatt der bayerischen Entomologen 60: 59-65.

Hawlitschek O., Porch N., Hendrich L., Balke M. (2011): Ecological niche modelling and nDNA sequencing support a new, morphologically cryptic beetle species unveiled by DNA barcoding. PloS ONE 6: e16662.

Hájek J., Hendrich L., Hawlitschek O., Balke M. (2010): *Copelatus sibelaemontis* sp. nov. (Coleoptera: Dytiscidae) from the Moluccas with generic assignment based on morphology and DNA sequence data. Acta Entomologica Musei Naturae Pragae 50: 437-443.

Rödder D., Hawlitschek O., Glaw F. (2010): Environmental niche plasticity of the endemic gecko *Phelsuma parkeri* Loveridge, 1941 from Pemba Island, Tanzania: a case study of extinction risk on flat islands by climate change. Tropical Zoology 23: 35-49.

Hendrich L., Hawlitschek O., Balke M. (2009): The epigean Australasian species of *Neobidessodes* gen.n. diving beetles – a revision integrating morphology, cybertaxonomy, DNA taxonomy and phylogeny (Coleoptera: Dytiscidae, Bidessini). Zootaxa 2288: 1-41.

Expert reports for conservation projects, international:

Since 2012: Expertise et mission d'inventaire 'Reptiles et Amphibiens' dans le cadre de la délimitation des Zones Naturelles d'Intérêt Ecologique Faunistique et Floristique (ZNIEFF) de Mayotte [*Expert report with field surveys 'Reptiles and amphibians' for a delimitation of natural regions of ecological, faunistic, and floristic importance on Mayotte*]. Zoologische Staatssammlung München on behalf of the Prefectorate Mayotte (France), department of environment, landscape, and housing development (DEAL).

2011: Assessments of 11 Comoran endemic species of amphibians and reptiles for the IUCN Red List (http://www.iucnredlist.org/).

Expert reports for conservation projects, national (Germany; selection):

Beutler A., with assistance of Hildenbrand A., Distler H., Distler C., Waeber G., Hawlitschek O., Schilling D., Koslowski S., Aßmann O., Gnoth-Austen F., Völkl W., Möhrlein E., Hintsche S., Gässler S., Zahn A., Diemer S., Wirth P., Peters B., Schindler K., Stegherr J., Brückmann B., Hildenbrand R. (2011): FFH-Stichprobenmonitoring für sieben Amphibienarten in der kontinentalen biogeographischen Region (KBR) in Bayern. [*Monitoring of random samples of areas of the Habitat Directive for seven species of amphibians in the continental biogeographic region in Bavaria.*] Planungsbüro Beutler on behalf of the Bavarian Environmental Office. Final report, 49 pp.

Heckes U., Hess M., Hawlitschek O. (2011): Neubau eines Druckzentrums im Industriegebiet Nonnenwald der Stadt Penzberg: Spezielle artenschutzrechtliche Prüfung [saP]. [*Construction of a printing centre in the industrial area Nonnenwald of the municipality of Penzberg: special report with regard to wildlife conservation law.*] Büro H2 on behalf of the Municipality of Penzberg. 36 pp.

Stegherr J., Hintsche S., Beutler A., Klingshirn C., Gässler S., with assistance of Schilling D., Hawlitschek O., Hildenbrand A. (2011): Flughafen München: Ökologische Ausgleichs- und Ersatzmaßnahmen. Faunistische Erfolgskontrolle 2011. [*Munich Airport: ecological compensatory and mitigating measures. Faunistic monitoring of success 2011.*] Planungsbüro Beutler on behalf of the Munich Airport GmbH. 73 pp.

Hawlitschek O. (2009): Bestandserhebung von *Bryodemella tuberculata* an der Isar unterhalb des Sylvensteinspeichers. [*Population survey of* Bryodemella tuberculata *(Orthoptera: Caelifera: Acrididae) at the river Isar below Lake Sylvenstein.*] Planungsbüro Beutler on behalf of the Water Management Office Weilheim, Bavaria. 25 pp.

Diemer S., Wirth P., Peters B., Hawlitschek O., Beutler A., Gnoth-Austen F., Waldert R., Weiss I., Hagenguth A., Katzer H. (2009): Pflege- und Entwicklungsplan NSG "Teufelsmauer" (Landkreis Harz) einschließlich Erarbeitung von Managementmaßnahmen für die FFH-Gebiete "Teufelsmauer nördlich Thale" und "Bode und Selke im Harzvorland" (Bereich NSG). [*Conservation and management outline of the nature reserve "Teufelsmauer" with a proposal of management measures for the Habitat Directive areas "Teufelsmauer nördlich Thale" and "Bode und Selke im Harzvorland".*] Yggdrasil – Institute for geology, environmental and conservation planning, and Planungsbüro Beutler, on behalf of the State of Sachsen-Anhalt. 169 pp.

Hawlitschek O., Beutler A. (2009): Faunistische Kartierungen zum Projekt Hochwasserschutz zwischen Straubing und Vilshofen – Deichrückverlegung Natternberg: Fachberichtsteil Springschrecken. [*Faunistic surveys for the flood control project between the towns of Straubing and Vilshofen – modification of the Natternberg levee: chapter Orthoptera.*] Planungsbüro Beutler on behalf of the Federal Republic of Germany and the State of Bavaria. 62 pp.

Beutler A., Hawlitschek O., Schilling D. (2007): Nordumgehung Pasing – Faunistische Bestandsaufnahme und naturschutzfachliche Angaben zur speziellen artenschutzrechtlichen Prüfung gemäß FFH-Richtlinie für Reptilien, Fledermäuse und Brutvögel. [*North bypass road Pasing – faunistic surveys and conservation proposals for the special report with regard to the Habitat Directive for reptiles, bats and birds.*] Planungsbüro Beutler on behalf of the City of Munich. 71 pp.

Popular publications:

Hawlitschek O., Glaw F., Rödder D. (2012): Pemba: herpetologische Fundgrube im Indischen Ozean. [*Pemba: herpetological treasure trove in the Indian Ocean.*] Reptilia 97: 97-109.

Randrianantoandro C., Randrianasolo H., Jenkins R., Rasolofoarimanana T., Hawlitschek O., Brady L., Andriantsimanarilafy R., Glaw F. (2011): Les Caméléons de Madagascar et de l'Archipel de Comores du Genre *Furcifer*: Guide d'ldentification de Poche. [*The chameleons of Madagascar and the Comoros Archipelago, genus* Furcifer*: a pocket identification guide.*] Voakajy Madagasikara.

Hawlitschek O., Rödder D., Glaw F. (2009): Das Lappenchamäleon *Chamaeleo dilepis* auf Pemba: eine eigene Art? [*The Flap-Necked Chameleon* Chamaeleo dilepis *on Pemba: a separate species?*] Chamaeleo 2009, 8-9. (eletced ARTICLE OF THE YEAR 2009)

Hawlitschek O. (2008): *Paroedura sanctijohannis*, un gecko nocturne récemment redécouvert. [Paroedura sanctijohannis, *re-discovery of a nocturnal gecko.*] Univers Maoré 10, 4.

Scientific oral and poster presentations:

Hawlitschek O., Rösner R., Guggenbichler D., Glaw, F. (2012): SmartHerper Comoros, a free field guide to the herpetofauna of the Comoro archipelago as a mobile application. Talk, symposium "Online Resources in Herpetology, Ichthyology and (vertebrate) taxonomy", 7th World Congress of Herpetology, Vancouver, Canada (http://wch2012vancouver.com/).

Hawlitschek O., Nagy Z.T., Glaw, F. (2012): Comoran Tree Snakes: Why and when subspecies still make sense. Talk, 13th annual meeting of the GfBS, Bonn, Germany (German Society for Systematic Biology, http://www.gfbs-home.de/).

Hawlitschek, O. (2011): Herpetofauna of the Comoros – integrative taxonomy, phylogeography and conservation. Talk, SEH European Congress of Herpetology, Luxembourg (http://www.symposium.lu/herpetology/).

Hawlitschek O. (2010): Reptiles des Îles des Comores – Phylogenie, Biogeographie et des effets du change de l'environnement. [*Reptiles of the Comoros Islands – phylogeny, biogeography, and the effects of environmental changes.*] Invited talk at the Université des Comores, Moroni, Comoros.

Hawlitschek O., Glaw F. (2009): Phylogeny of the *Ebenavia inunguis* species complex (Reptilia: Squamata: Gekkonidae): Preliminary results. Poster, symposiums "Evolution and extinction of the terrestrial biota of the western Indian Ocean archipelagos", Linnean Society, London (http://www.linnean.org/).

Popular presentations, press releases, and media coverage:

Treuil F., Louis G., Hermile J., with contributions of Hawlitschek O. and Charpentier M. (2013): Deux chercheurs allemands inventorient reptiles et amphibiens [*Two German researchers survey reptiles and amphibians*]. TV coverage in Journal de Mayotte, Mayotte 1ère, 2013/03/14.

Piolat J., with contributions of Hawlitschek O. and Glaw F. (2013): Deux chercheurs allemands inventorient reptiles et amphibiens [*Two German researchers survey reptiles and amphibians*]. Mayotte Hebdo 605, 16.

Hawlitschek O., Glaw F. (2012): Gesucht: die Ameisengrille. [*Wanted: the Ant Cricket*.] Press release.

Schreglmann I., with contributions of Glaw F. and Hawlitschek O. (2012): Glatt, glitschig und genial vielseitig. [*Smooth, slippery, and brilliantly versatile.*] Broadcasting program of Radio Bayern 2 on amphibians and reptiles (http://www.br.de/radio/bayern2/sendungen/radiowissen/amphibien-reptilien100.html).

Hawlitschek O. (2011): Die Komoren und ihre Phelsumen. [Phelsuma *species (Squamata: Gekkonidae) of the Comoros.*] Talk, annual meeting of the German *Phelsuma* Breeders Group (IG-Phelsuma), Göttingen, Germany (http://www.ig-phelsuma.de/).

Hawlitschek O. (2010): Expedition auf die Komoren – Biologische Vielfalt, Land und Leute. [*Expedition to the Comoros – Biodiversity, Culture, People.*] Talk, Society of the Bavarian State Collection of Zoology, Munich, Germany (http://www.zsm.mwn.de/freunde/).

Hawlitschek O. (2009): Pemba: herpetologische Fundgrube im Indischen Ozean. [*Pemba: herpetological treasure trove in the Indian Ocean.*] Talk, DGHT (German Society for Herpetology and Herp Breeding, http://www.qmvet.de/reptilien/ stadtgruppe_MUC.htm).

Entries at the Blog of the Zoologische Staatssammlung München (http://www.zsmblog.de/)

2.4 Abbreviations and Glossary

Abbreviations:

COI	Cytochrome C Oxidase subunit I, the standard marker for DNA barcoding
IUCN	International Union for Conservation of Nature
LGM	Last Glacial Maximum, the latest cold period of the Ice Ages
LSID	Life Science Identifier, a unique identification number for taxa
mtDNA	mitochondrial DNA
my	million years
mya	million years ago
nDNA	nuclear DNA
ZSM	Zoologische Staatssammlung München (Bavarian State Collection of Zoology, Munich)

Glossary:

Allopatry	Species or populations occuring in geographically separate areas
Biogeography	The discipline of biology that studies the past and present geographical distribution of organisms.
Conspecific	Belonging to the same species.
Cryptic species	Species that are discovered as new although they were previously not recognized as separate species, usually because they are genetically distinct but morphologically very similar.
Cybertaxonomy	A term subsuming some recent developments that are supposed to make → taxonomy more efficient and to increase the availability of the results of taxonomic work, i.e., internet databases, online publication, etc.
Dispersal	Movement of an organism away from its population or parent. Dispersal allows for the founding of new populations in previously uncolonized areas and maintains gene flow within a → metapopulation.
DNA barcoding	Method of identifying organisms by sequencing a short standardized fragment of their DNA, the 'DNA barcode'.
Ecological Niche Modeling	Method of estimating the potential distribution of an organism by using mathematical models to extrapolate from known occurrence localities and ecological background data, e.g., climate.
Endemic	Organism that lives only in a clearly circumscribed region, e.g., an island
Entomology	The discipline of biology that studies insects.
Epigean	Living on the surface of the earth, not in subterranean habitats.
Habitat island	A patch of any kind of habitat surrounded and isolated by a different kind of habitat, e.g., a mountain surrounded by plains, a patch of forest surrounded by grassland, a lake surrounded by land.
Herpetology	The discipline of biology that studies reptiles and amphibians.
Integrative taxonomy	→ Taxonomy that is based on evidence of more than one kind, e.g., genetic, ecological, bioacoustics.
Island	In → biogeography: a patch of land surrounded by water, but also a → habitat island.
Metapopulation	A group of populations which are isolated from one another to a certain degree, but not completely. → Dispersal of individuals allows for the exchange of genes between the populations and for the re-colonization of areas that were previously occupied by extinct populations.

Molecular clock	Technique that uses rates of mutation (molecular change) to estimate at what point in geological history lineages of organisms diverged
Monophyletic	→ Taxonomic group that comprises a common ancestor and all of its descendants.
Morphology	In biology: The study of characters of the form and structure of organisms
Nomenclature	In biology: The discipline of biology that studies how → taxa should be named. Closely related to → taxonomy. Biological nomenclature is determined by the rules of internationally accepted codes, e.g., the International Code of Zoological Nomenclature.
Oceanic island	An → island that has never had any connection to any other landmass. Mostly of volcanic origin.
Pangea	Ancient 'supercontinent' that existed between ca. 300 and 200 mya and comprised all later separate continents in a single landmass.
Parapatry	Species or populations occurring in geographically adjacent areas.
Paraphyletic	→ Taxonomic group that comprises a common ancestor and some, but not all, of its descendants.
Peripatry	A species or population occurring in a small area geographically or ecologically isolated from the original area.
Phylogeny	A hypothesis about the evolutionary history of a → taxonomic group, including information on ancestral relations between → taxa.
Pleistocene climate oscillations	The Ice Ages. Repeating shifts between warmer and colder climate in the Pleistocene period (2.6 to 0.01 mya).
Polyphyletic	Any → taxonomic group that is neither → mono- nor → paraphyletic, usually comprising some forms but not their common ancestor.
Speciation	The process of evolution that leads to new species.
Species concept	Scientific theory that attempts to define what a species is. About 30 species concepts are recognized at present.
Sympatry	Species or populations occurring in the same geographical area.
Taxon	A group of organisms that are considered as a group based on common ancestry or similarity. Taxa usually have ranks, such as species, subspecies, genus, family, etc. According to many authors, taxa must be → monophyletic.
Taxonomy	The discipline of biology that studies which organisms belong to which → taxon and describes new taxa (species and others) scientifically.
Tectonic	Here: referring to the theory of Plate Tectonics which describes movements of the Earth's crust in geological history.
The Species Problem	The problem of how to define what a biological species is, and how to agree on which organisms belong to which species.
Unified Species Concept	→ Species concept that sees all species – rather abstract – as 'separately evolving metapopulation lineages' and accepts all previous species concepts as 'operational criteria' that help delimiting species.
Vicariance	The phenomenon of related species that are geographically isolated but descend from an earlier common population that was split by geological events (e.g., plate tectonics or an increase of sea levels).

3 Introduction

3.1 Speciation

"That mystery of mysteries"

Charles Darwin on the origin of species, referring to John Herschel

"The law of the higgledy-piggledy"

John Herschel on Darwin's theory on the origin of species

One of the most fundamental functions of human perception and communication is to classify objects by assigning them categories for memorization and names for communication. Therefore, human beings have always classified and recognized organisms they shared their environment with. They found that individual organisms can be assigned to a variety of kinds that are called species in biology (WILLIAMS & BAINES 1993).

Antique philosophers already posed the question whether organisms were fixed to 'eternal' forms or whether there was some kind of gradual development between these forms. Pre-Socratic philosophers in Greece of the 6th century BC, such as ANAXIMANDER OF MILETUS and EMPEDOCLES, proposed that one type of animal could descend from another, and that humans originated from another kind of animal (KIRK et al. 1983). In the Chinese Taoist philosophy, constant transformation is one of the fundamental principles, and records of the ideas of changing species are dated back as far as to the 4th century BC (NEEDHAM & RONAN 1995). Other philosophers, such as PLATO and ARISTOTLE, opposed the concept of changing species, believing that species had a fixed form unchanging throughout time. This so-called essentialist view often attributed to an 'ideal form' or 'final cause' of existence to species (SINGER 1931).

In the Medieval, Renaissance, and Enlightenment periods, Christian European scholars generally regarded the Genesis creation narrative of the Bible rather as an allegory than as an account of historical facts, and nature was seen as a subject to change and instability (FORSTER & MARSTON 1999, BOWLER 2003). This thinking was influenced by antique philosophers and Islamic scholars (LOVEJOY 1936). Essentialism gained importance only after the Protestant Reformation that promoted a more literal interpretation of the Bible (HARRISON 2007). Early taxonomy, consequently, was based largely on the assumption that species were fixed and unchanging categories of organisms (JOHNSTON 1999, but see WILKINS 2006).

With the publications of DARWIN (1859, 1871) and WALLACE (1870, 1889), evolution through natural selection was formed as a scientific theory. This theory initially faced many opponents. It was rejected because it disagreed with the contemporary religious and essentialist views that species were unchanging and divine creation (DEWEY 1910, LARSON 2004), but also because it was considered too speculative to meet the requirements of contemporary strict empiric science (SARASIN 2009): JOHN HERSCHEL, who greatly influenced Darwin's work, initially called the theory on the origin of species the "law of the higgledy-piggledy" because of its lack of empirical proof. Nevertheless, Darwin's examples – and the support of those who initially agreed with his theory, such as THOMAS H. HUXLEY and ALFRED R. WALLACE (BOWLER 2003) – convinced the majority of biologists in the following decades that species originated in the process of evolution. However, the idea that this evolution was caused by natural selection was not widely accepted. In the 20th century, the development of genetics as a discipline of biology, following the rediscovery of MENDEL'S work (BOWLER 2003), gave rise to the discipline of population genetics. Population genetics finally determined natural selection as the driving factor of evolution and tied it to a genetic fundament (HALDANE 1924, FISHER 1930, WRIGHT 1932). In the 1930s and 1940, the previous mainly theoretical works on population genetics were backed by examples from the real world and connected with insights from other biological disciplines in the modern evolutionary synthesis. The most prominent works were published by DOBZHANSKY (1937), who provided evidence that natural selection was based on a far higher amount of genetic diversity in natural populations than previously believed, and MAYR (1942), who emphasized the importance of geographical isolation for the evolution of species. The modern synthesis gave rise to the discipline of evolutionary biology. The present-day view of evolution in biology is principally based on the modern evolutionary synthesis, with contributions from molecular genetics, epigenetics, and genomics.

The modern evolutionary synthesis addressed, among many others, the question on the geographic and environmental settings under which speciation, i.e., the evolutionary process by which new species originate, can happen. The four general modes of speciation recognized are (see also COYNE & ORR 2004): (1) Allopatric speciation: The population of a species is split into two geographically isolated populations. In separation, these populations diverge due to different selection regimes, genetic drift, and different mutations. If the populations come into contact at a later point, they may diverge so strongly that they are no longer capable of cross-breeding and exchanging genes. (2) Peripatric speciation: A species colonizes a geographically isolated (smaller) area or an ecologically isolated niche. If there is little or no gene flow between the original and the new peripheral population, speciation occurs as in the allopatric model. (3) Parapatric speciation: This model is similar to allopatric speciation, but populations inhabit adjacent instead of completely isolated areas or niches. Nevertheless, if gene flow is sufficiently reduced, the populations will eventually diverge and become species. (4) Sympatric speciation: In this mode of speciation, species form without any kind of geographical barrier. Reproductive isolation occurs through sexual selection, assortative mating, niche diversification, genomic differences such as polyploidization, or hybrid formation (COYNE & ORR 2004). MAYR and other evolutionary biologists rejected the idea that sympatric speciation might play more than a minor role in the origin of species. In the last decades, many researchers found evidence that, while allopatric speciation is the more common pattern, sympatric speciation happens more often than previously believed (FUTUYMA & MAYER 1980, SCHLIEWEN et al. 1994, VIA 2001).

Speciation under any of the models introduced above does not take place at constant pace in geological history (BARRACLOUGH & NEE 2001). Many species were found to be the product of radiations, i.e., events in which many new species develop from one ancestral species, usually in relatively short evolutionary time (BOAG & GRANT 1981). The radiation of the famous Galápagos finches is often cited for its major impact on DARWIN'S theory of natural selection. Most authors speak of 'adaptive radiation' to denote that speciation in this case is promoted by divergent adaptions of the incipient species. However, as argued by GITTENBERGER (1991), radiations need not necessarily be adaptive, and some examples for such non-adaptive radiation events have been described (GITTENBERGER 1991, SCHLUTER 2000, SCHÖN & MARTENS 2004).

3.2 Species concepts and species delimitation

3.2.1 The species problem – a century of debate

One of the necessary prerequisites for the study of speciation is that biologists recognize species as distinct entities. In pre-Darwinian systematic biology, beginning with LINNAEUS (1758), species were defined, delimited, and described based on the expert knowledge and estimation of the taxonomists – thus, in a systematic, but arbitrary fashion. DARWIN (1859) centered his work on species, but did not provide a clear definition of species, writing: "No one definition has satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species". With an increasing number of known and described species, however, researchers found that individual taxonomists might disagree on the definition of species: where some recognized distinct species, others saw mere intraspecific variation (MAYR 1942). At this point, the 'species problem' was recognized, and the debate on the definition of species was initiated. This debate has been led by generations of scientists in the past and now (SIMPSON 1961, MAYR 1963, DE QUEIROZ & DONOGHUE 1988, O'HARA 1993, BAUM & SHAW 1995, RICHARDS 2010). Today, taxonomists are facing the task of recording an estimated 2 to 5 million (or more) species of organisms on earth, of which only around 1.5 million have been described so far (COSTELLO et al. 2013). Many species remain to be discovered even in regions that are thought to be well known (VAN ACHTERBERG et al. 2012). Therefore, the species problem deserves and receives increased attention.

The first modern definition of what a species is, i.e., the first species concept, was the biological species concept of MAYR (1942) and DOBZHANSKY (1950) (see also WRIGHT 1940). MAYR defines: "Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups". This reproductive isolation is primarily based on intrinsic barriers to reproduction, e.g., incompatibility of genitals or decreased fitness of offspring resulting from interbreeding, as opposed to extrinsic, i.e., geographic, barriers to reproduction. The biological species concept has been accepted widely for a long time, but it is connected with a number of drawbacks. Hypotheses on the existence or absence of intrinsic reproductive barriers are hard to falsify and were tested only in a minor number of cases, organisms with asexual reproduction cannot be distinguished using the biological species concept, and the many cases of hybridization in nature present problems for the application of this concept (DE QUEIROZ 2005). PATERSON (1985) and MASTERS et al. (1987) extended the biological species concept by the component of mate recognition, but this did not solve the problems just stated.

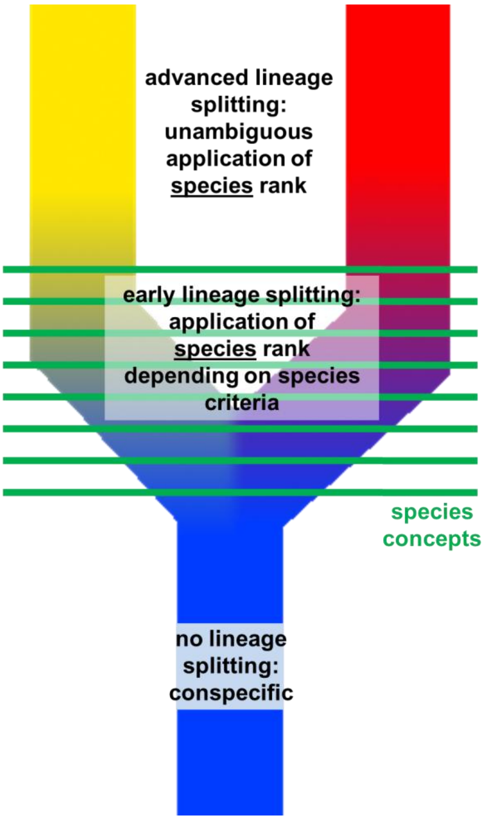
In the 1970s, biologists began to debate other means of defining species, and many new species concepts have been presented until now. These species concepts were based on phenetic, ecological, genetic, or phylogenetic criteria (Table 1). The advantage of these species concepts is that they, in addition to theoretical definitions of what a species is, attempt to use some kinds of quantitative or distinct qualitative data to delimit species. Therefore, I use the term **'empiric species concepts'** for these concepts, with respect to the theory of knowledge in which "knowledge comes only or primarily from sensory experience" (BAIRD & KAUFMANN 2008). None of these concepts found acceptance as wide as the biological species concept because the application of different species concepts, based on different kinds of data, often led to contradictory results (HAUSDORF 2011). Other authors attempted to overcome this disadvantage by publishing concepts that defined species on a much more abstract level, e.g., in the evolutionary species concept (SIMPSON 1951, WILEY 1978, MAYDEN 1997) and the general lineage concept (DE QUEIROZ 1998; Fig. 2). These species concepts focused much more on the theoretical development of the understanding of what a species actually is, and they could be applied to almost any kind of organism. I therefore call them **'rationalist species concepts'**, with respect to the theory of knowledge in which "the criterion of the truth is not sensory but intellectual and deductive" (BOURKE 1962; see also 'lineage-based species concepts: HAUSDORF 2011). Rationalist species concepts considerably improved our understanding of how to define species. However, testing these proposed criteria in practice and applying them to species delimitations and descriptions is difficult because these concepts do not directly include the possibility of supporting the species delimitations with any kind of data.

Table 1: An overview over species concepts, or operational criteria of the Unified Species Concept according to DE QUEIROZ *(2007).*

Concept	**Species definition**	**Advocates**	**Type**
Biological	Reproductive isolation through intrinsic barriers	MAYR (1942), DOBZHANSKY (1950)	empiric
Recognition	Reproductive isolation through mate recognition	PATERSON (1985), MASTERS et al. (1987)	empiric
Phenetic	Quantitative detectable difference in phenotype	MICHENER (1970), SOKAL & CROVELLO (1970), SNEATH & SOKAL (1973)	empiric
Diagnosability	Qualitative detectable difference in phenotype	NELSON & PLATNICK (1981); CRACRAFT (1983); NIXON & WHEELER (1990)	empiric
Ecological	Ecological niche or adaptive zone	VAN VALEN (1976), ANDERSSON (1990)	empiric
Genotypic cluster	No genetic intermediates, e.g., heterozygotes, between species	MALLET (1995)	empiric
Cohesion	Genetic and / or demographic exchangeability	TEMPLETON (1989, 1998)	empiric
Monophyly	Monophyly	DONOGHUE (1985), MISHLER (1985)	empiric
Coalescence	Exclusive coalescence of alleles	BAUM & SHAW (1995)	empiric
Genic	No sharing of genes that control adaptive characters	WU (2001), WU & TING (2004)	empiric
Differential fitness	Characterized by features that would have negative fitness effects in other species	HAUSDORF (2011)	empiric
Evolutionary	Unique evolutionary role, tendencies, and historical fate	SIMPSON (1951), WILEY (1978), MAYDEN (1997)	rationalist
General Lineage	Separately evolving metapopulation lineage	DE QUEIROZ (1998)	rationalist

In his review of species concepts, DE QUEIROZ (2007) proposed that "the issues of species conceptualization and species delimitation are clearly separated" (see also HART 2010). He argued that already DARWIN'S (1859) concept of species had a conceptual and a taxonomic component. DE QUEIROZ further proposed that all species could be seen as separately evolving metapopulation lineages, as defined in his general lineage concept (DE QUEIROZ 1998) based on earlier evolutionary species concepts (SIMPSON 1951, WILEY 1978, MAYDEN 1997). However, this concept alone (termed 'rationalist' by me) does not provide evidence whether lineages are separated or not. Instead, all 'secondary' species concepts – here termed 'empiric' species concepts – can provide evidence for lineage separation and are therefore maintained as 'operational criteria' for species delimitation. DE QUEIROZ (2007) termed this concept the 'Unified Species Concept'. Criticism of the Unified Species Concept is mostly not directed against the idea of unifying operational criteria, but against the use of the general lineage concept as an overarching concept. HAUSDORF (2011) writes: "It is circular to define a species as a sequence of (meta-)populations, because it is part of the definition of 'population' that the organisms that form a population belong to the same species." Despite this criticism, the Unified Species Concept has found wide acceptance among researchers in systematic and evolutionary biology (PADIAL et al. 2009, 2010).

Figure 2: Simplified sketch showing a speciation event. Modified after DE QUEIROZ (1998, 2007). A single lineage (blue) splits into two divergent lineages (red and yellow). Below the zone of green lines, a single species is recognized unambiguously, while above the zone of green lines, two species are recognized unambiguously. In the zone between the lines, disagreement over the number of species (1 vs. 2) is possible, depending on which species concept is applied.



3.2.2 Species delimitation in an integrative approach

The application of operational criteria based on different kinds of evidence, as suggested in the Unified Species Concept, is similar to the ideas at the base of integrative taxonomy, and many authors who advocated any integrative taxonomic approaches referred to the Unified Species Concept (PADIAL et al. 2009, 2010).

The idea of using evidence from different kinds of data, e.g., on morphology, genetics, ecology, etc., is not particularly new (PORTER et al. 1991, ARNTZEN & WALLIS 1999). However, the demand that "an integrative taxonomy grounded on evolutionary theory is necessary to accomplish a reliable inventory of Earth's biodiversity" (PADIAL et al. 2009) was clearly formulated and widely accepted only in the 21st century. Its roots, as made clear by the authors, are to be found in (1) the lineage-based (i.e., rationalist) species concepts which imply that no single trait (e.g., reproductive isolation, morphological differentiation) can be considered necessary to define a species, and (2) the 'taxonomic renaissance' accompanied by the shift in taxonomic science from an often purely descriptive to a hypothesis-based discipline (MALLET & WILLMOTT 2003, MILLER 2007, HASZPRUNAR 2011) and facilitated by technological advances and easier access to taxonomic information (PADIAL et al. 2010).

PADIAL et al. (2010) distinguish between two basic approaches in integrative taxonomy, called integration by cumulation and integration by congruence. In the former, all lineages that can be distinguished in at least one of the lines of evidence studied will be recognized as species. In the latter, a selected minimum number of lines of evidence (e.g., 2 or 3) must provide congruent evidence for the differentiation of a lineage from other lineages to allow its recognition as species. Consequently, integration by cumulation is prone to lead to alpha-errors in species recognition: lineages may be erroneously recognized as species due to detected differences in a line of evidence, even if these differences represent only intraspecific variation. Integration by congruence, on the other hand, is prone to lead to beta-errors: cryptic or young species may not be detected because evidence for differentiation cannot be found in a sufficient number of lines of evidence.

Table 2: Definitions of categories of candidate species after VIEITES et al. (2009).

Unconfirmed Candidate Species (UCS)	**Confirmed Candidate Species (CCS)**	**Deep Conspecific Lineage (DCL)**
Default category for detectable genealogic lineages of unknown status, usually detected via genetic methods (e.g., DNA Barcoding or 16S rRNA screening) and via a threshold of divergence known to be typical for the molecular marker and the respective group. Data deficient for other lines of evidence, e.g., morphology, ecology, other molecular characters.	Lineages characterized by a detectable genetic divergence (not necessarily above the threshold) from all described species and at least one of the following criteria: a) Distinct differentiation in a character that mediates a reproductive barrier (e.g., advertisement calls, genital morphology, mode of reproduction) b) Diagnostic morphological difference in a character that is known to be of high value to discriminate species in the respective group c) Sympatric occurrence with another lineage without admixture and with at least some phenotypic difference	Genealogic lineages found to diverge from other lineages above a threshold of divergence known to be typical for the molecular marker and the respective group. One of the following must apply: a) No distinct differentiations in any characters that mediate a reproductive barrier b) No differences in any other line of evidence, or only subtle differences in characters that are known to be of low value to discriminate species in the respective group c) Indications for genetic admixture with other lineages

PADIAL et al. (2010) also proposed a 'Consensus Protocol' of integrative taxonomy that incorporates aspects of the Candidate Species Approach by VIEITES et al. (2009). This protocol if different from the basic approaches of cumulation and congruence in that it does not require any a-priori selection of the numbers of lines of evidence that have to be met for the recognition of a lineage as species. Instead, the quality of lines of evidence is taken into account (Table 2). Any character that mediates sexual isolation, or that distinguishes syntopic lineages, or that can clearly be attributed to the separation of evolutionary lineages, is sufficient for the recognition of a lineage as species. The problems connected to this approach are that closely related species often do not live in syntopy, and that the value of most characters for sexual isolation or lineage separation is not clear. Therefore, the application of the Consensus Protocol to taxonomic studies on many groups of organisms will most likely be inhibited by a lack of knowledge on the quality of the characters studied.

In such cases in which the quality of taxonomic characters for lineage separation is not clearly known, a simpler approach based on the basic methods of cumulation and congruence is required. MIRALLES et al. (2011) used the simplest agreement between these two methods, an approach of integration by partial congruence. They studied three lines of evidence (mtDNA, nDNA, morphology) and recognized all lineages that were congruently differentiated in at least two of these lines of evidence as species. Additionally, they recognized all lineages with differences in only one line of evidence as subspecies (Table 3). The a-priori selection of a certain number of lines of evidence for species recognition is necessarily arbitrary. However, in combination with taxonomic expertise it still reduces the probability of errors in taxonomic descriptions. The approach of MIRALLES et al. (2011) was successfully applied in a subsequent case study (VASCONCELOS et al. 2012).

Table 3: The integrative Lines of Evidence approach for the delimitation of species and subspecies after MIRALLES *et al. (2011).*

Match in 0 lines of evidence	**Match in 1 line of evidence**	**Match in 2+ lines of evidence**
conspecific	subspecies	species

3.2.3 Taxonomy in the 21st century – DNA barcoding and cybertaxonomy

In most studies with an integrative taxonomic approach, some kind of genetic data is used for at least one line of evidence. Often, candidate species will even be detected by preliminary genetic screening (proposed in VIEITES et al. 2009; see also MILLER 2007, ASTRIN et al. 2012). In the last decade, this method of genetic survey was institutionalized as DNA barcoding. HEBERT et al. (2003) established the mitochondrial Cytochrome C Oxidase subunit I (COI) gene as standard DNA barcoding region for animals. In the following years a multitude of studies on various applications of DNA barcoding was published (ARMSTRONG & BALL 2005, SAVOLAINEN et al. 2005, BALL & ARMSTRONG 2006, SCHEFFER et al. 2006, HAJIABAEI et al. 2007, RATNASINGHAM & HEBERT 2007, GONZALEZ et al. 2009, JANZEN et al. 2009, HASZPRUNAR 2009, LOWENSTEIN et al. 2010, HAUSMANN et al. 2011). The method has become popular as a quick and technically easy approach that does not require expert knowledge once reliable databases have been established. However, it has been shown to be of limited value to infer phylogenetic relationships (MEYER & PAULAY 2005, VENCES et al. 2005). Nevertheless, the first step in many phylogenetic studies is DNA barcoding of the samples to provide a 'preview' of the topology and set the course for a multigene analysis (MONAGHAN et al. 2006, HENDRICH et al. 2010). Many studies in biodiversity research have demonstrated that DNA barcoding is also extremely helpful to analyze large datasets that are assumed to comprise many unknown species; known species can be identified with high reliability, and sequences of unidentified specimens can be clustered to form operational taxonomic units (or 'barcoding species'; HEBERT et al. 2003, 2004).

Many case studies on DNA barcoding focused on insects, likely because this group of animals poses the strongest challenges to modern taxonomy (ARMSTRONG & BALL 2005, BALL & ARMSTRONG 2006, SCHEFFER et al. 2006, NOVOTNY et al. 2007). A DNA barcoding study on Australian diving beetles, one of the two focus groups of this dissertation, was conducted by HENDRICH et al. (2010). This study was important for this dissertation because it highlighted potential cryptic species, as well as morphologically clearly defined species that could not be identified by DNA barcoding. In herpetology, the DNA barcoding approach as proposed by

HEBERT et al. (2003) remained less popular until the last few years, mainly because of the lack of a standardized and reliable protocol for COI (VENCES et al. 2005). NAGY et al. (2012) published one of the first larger-scale DNA barcoding studies on reptiles, focusing on the fauna of Madagascar. This study was important for this dissertation because it (already before its own publication) provided sequence data that was important to study the phylogenetic and biogeographic relationships of Comoran reptiles, the second focus group of this dissertation.

Scientists welcomed DNA barcoding because they had understood far earlier that the traditional methods of taxonomy alone, i.e., identifying species using keys based on morphological characters, would not be sufficient to cope with the task of describing and identifying an unknown, but definitely overwhelming, number of species on Earth (PIMM et al. 1995, WHEELER 2004). Integrative taxonomy appears to be an extremely helpful approach in reliably delimiting species, but does not accelerate the process of species identification and description. Therefore, in the last decade, taxonomists began to promote the use of online resources for species identification and description, subsumed under the term 'Cybertaxonomy' (HENDRICH et al. 2009, WINTERTON 2009, VENIN et al. 2010, HENDRICH & BALKE 2011, PENEV et al. 2011). Cybertaxonomy aimed at making taxonomic work easier and more efficient by increasing the availability of taxonomic resources and facilitating the propagation of new taxonomic data.

Online databases were among the first steps that were taken in this approach. The deposition of newly produced DNA sequence data in GenBank (2013) has been accepted as a universal standard in molecular biology for many years. Many biological collections likewise put effort into making their data available online, as demonstrated in HerpNet (2013). Independent databases such as AmphibiaWeb (2013) and the Reptile Database (UETZ et al. 2013) provide taxonomic, nomenclatural, and distributional information on the organism groups they cover. Species-ID (2013) attempts to provide reliable information on the biology and taxonomy of species that is contributed by experts and backed by references. Identification keys to species are available online, e.g., at online-keys.net (2013). Finally, many peer-reviewed scientific journals publish online and under the open-access policy. All these developments contribute towards making access to the global vast amount of taxonomic information, formerly one of the major impediments to taxonomic and nomenclatural research (see, e.g., GLAW et al. 2007, CADLE & INEICH 2008), easier and more efficient.

The online distribution of previously available taxonomic information is limited only by the motivation of experts to put effort into this issue, and by the copyrights on taxonomic publications retained by publishers. In contrast, the descriptions of new species were until very recently tied to publication in printed form, as demanded by the codes of the ICZN (International Commission on Zoological Nomenclature) and ICBN (International Commission on Botanical Nomenclature). With the amendment to the International Code of Zoological Nomenclature (ICZN 2012: 1–7) of 4th September, 2012, the descriptions of new species of animals in electronic media only are now valid if the new species are given an LSID at ZooBank (2013) (see also PYLE & MICHEL 2008 and ZHANG 2012). ZooBank is a database that aims at collecting information on described species of animals and tagging these species with a unique identification number and a permalink that will allow their permanent and unambiguous identification. With this new option, taxonomists will not only have easier access to previously generated taxonomic information, but will also have it easier to distribute newly generated taxonomic information by themselves. It therefore marks another important step in the 'taxonomic renaissance' (MILLER 2007).

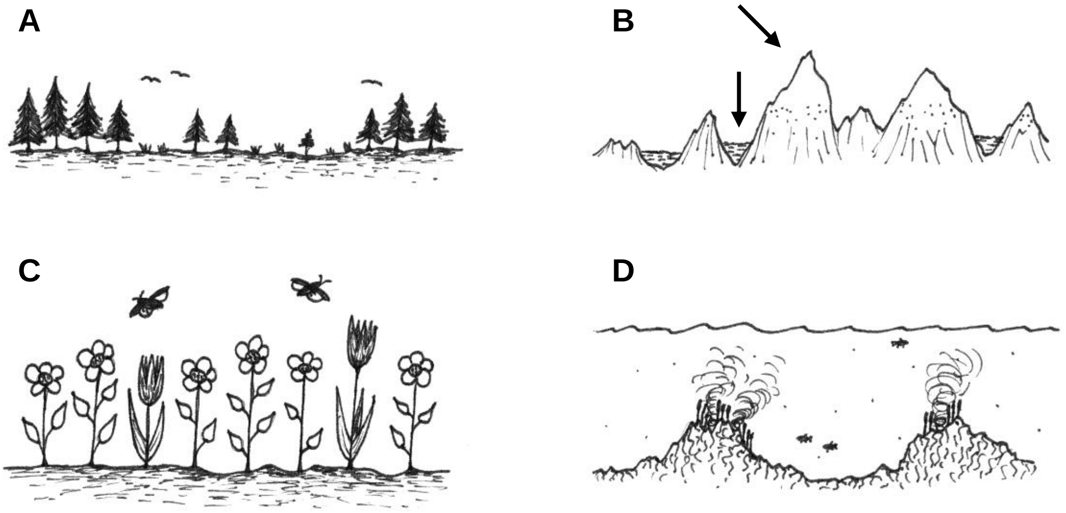
3.3 Biogeography of insular and continental regions

The discipline of biogeography addresses questions on how organisms are distributed on the planet, and how these distributions have developed in geological history. The study of biogeography goes back to the turn of the 18th and 19th centuries, when von Humboldt (1805) worked on the geographical distributions of plants. Soon the potential of islands as study systems of biogeographic questions was recognized (Darwin 1845, Wallace 1876). The 'Theory of Island Biogeography' (MacArthur & Wilson 1963, 1967) established island biogeography as a field of study in its own right. Whittaker & Fernández-Palacios (2007, p.3) summarize the importance of islands in biogeography as follows:

> "It is that islands, being discrete, internally quantifiable, numerous, and varied entities, provide us with a suite of natural laboratories, from which the discerning natural scientist can make a selection that simplifies the complexity of the natural world, enabling theories of general importance to be developed and tested."

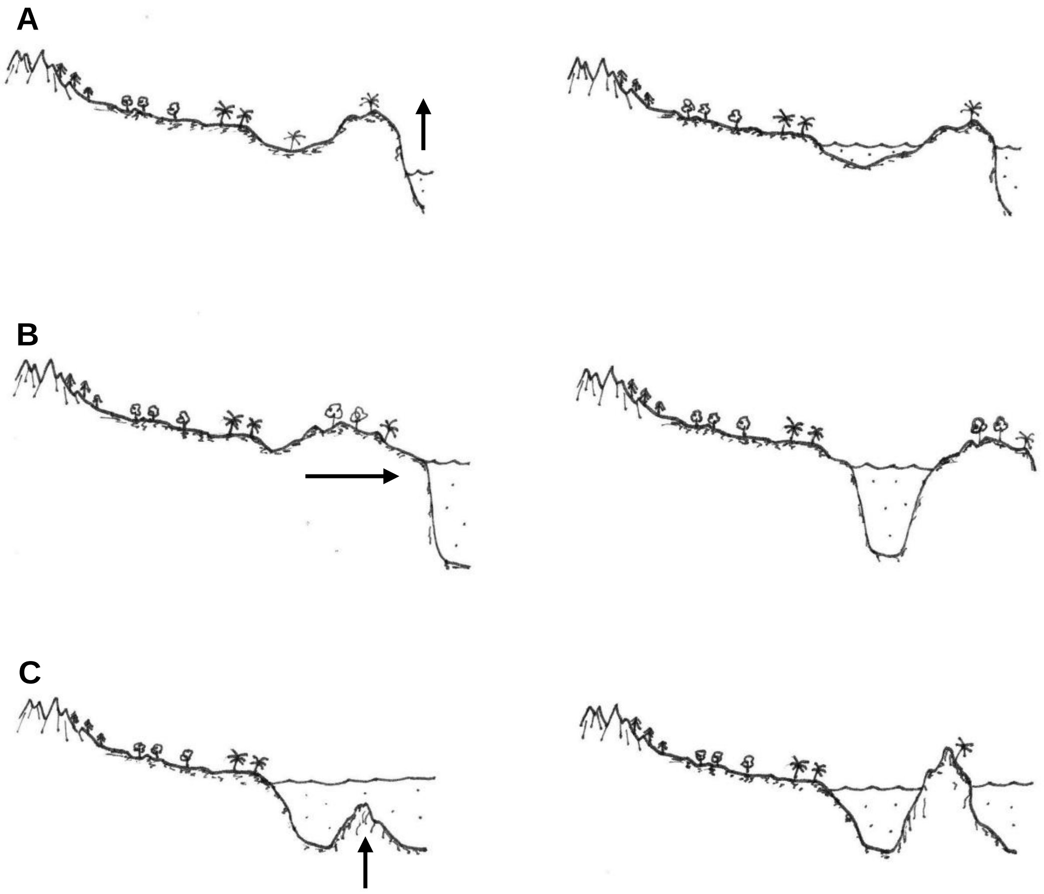
Among the most important insights in biology gained from the study of island systems is that true islands, i.e., small areas of land surrounded by water, are very similar to patches of any kind of habitat that are isolated from one another by a different type of habitat (Fig. 3). These may be patches of forest isolated by natural or anthropogenic forest-free area, coral reefs isolated by open sea, lakes or ponds isolated by dry land, or mountains isolated by valleys (Wilson & Bossert 1971). As described in MacArthur & Wilson (1967), such habitat islands basically follow the same rules as 'true' islands in the sea: the compositions of their biotas are mainly defined by the size of the islands, the degree of isolation in space and time, and the dispersal abilities of potentially colonizing species.

Figure 3: Types of 'islands' in biogeography. A: Stands of trees isolated by grassland represent islands for tree-dwelling mammals. Stands of only few trees may be too small to support a population. B: Two types of islands are shown in this mountain range. For terrestrial plants adapted to high-altitude climate, the mountaintops represent islands. For freshwater species, the lakes in the valleys represent islands. C: For insects that depend on a certain plant for larval nutrition, these plants (if isolated in a meadow) represent islands. D: Hydrothermal vents are islands under the ocean.



Wallace (1902) distinguished three major types of islands (Fig. 4). (1) Continental islands, e.g., Britain, Zanzibar, and Borneo, are islands that are situated on the continental shelves. They are isolated from the continent and from other islands at higher sea levels, but are connected during periods of lower sea levels. (2) Continental fragments, e.g., Sicily, Cuba, and Madagascar, are landmasses that once were connected with a continent. Through tectonic drift they were cut off from the continent and are now isolated by open sea. (3) Oceanic islands, e.g., the Canaries, the Galápagos, and the Comoros, have originated mostly from volcanic activity and have never had any connection to any other landmass. Because of these properties, such islands can be colonized in natural ways only by organisms that are capable of dispersal across the open ocean. This makes oceanic islands (also called 'true oceanic islands') particularly interesting for studies on the genesis and function of organism communities (MacArthur & Wilson 1967, Emerson 2002, Heaney 2007, Whittaker et al. 2008).

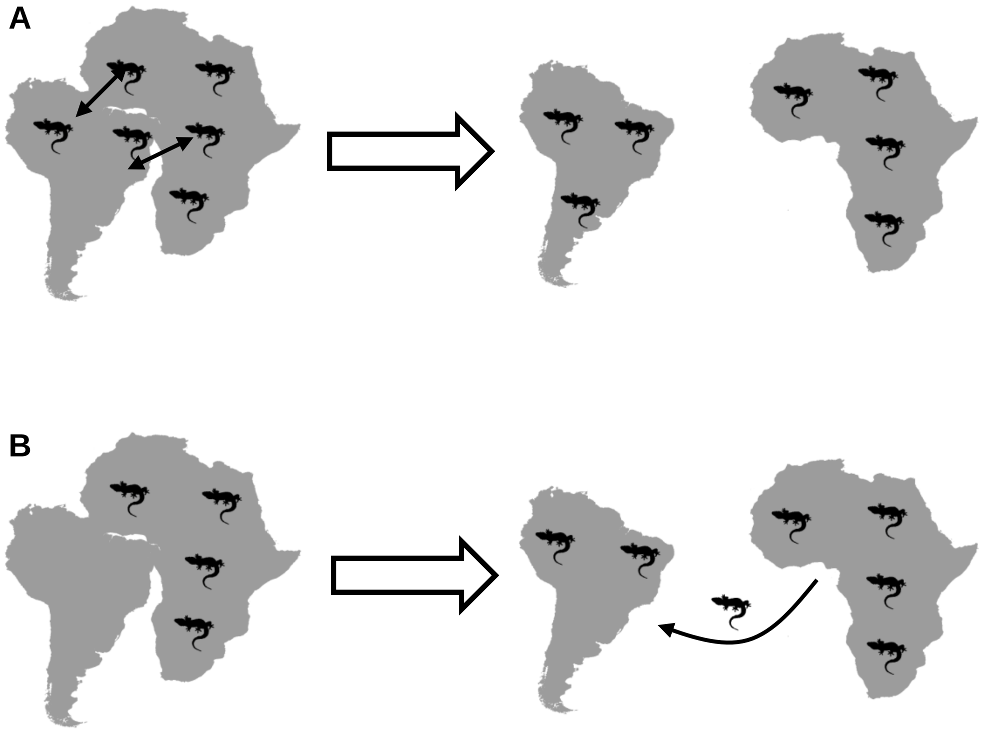
Figure 4: Types of islands in the sea after Wallace (1902). A: Continental islands are situated on the continental shelves and are only isolated from the continent during periods of higher sea level. B: Continental fragments are parts of continents that disconnect from the continent through tectonic drift, thus forming islands. C: 'True oceanic islands' are created by the activity of marine volcanoes. They emerge from the sea and later sink (after erosion) without ever having been connected to any other landmass.



Continents present much more complex biogeographic scenarios than islands in the sea because continents are larger, older, and have a much more complex habitat structure than islands (EMERSON 2002). As described above, island-like scenarios may also be represented by habitats in continental settings. Large-scale studies on continental biogeography, however, show that the factors mainly influencing island biogeography are of lower importance in continental settings, if compared with climate and climate change (PRENTICE et al. 2000, RIBERA & VOGLER 2004) and plate tectonics (COX 2000, UPCHURCH et al. 2002, SANMARTÍN & RONQUIST 2004).

In continental and island scenarios alike, disjunct distributions can be explained by dispersal or vicariance scenarios (Fig. 5). During the late 20th century vicariance was seen as the far more common explanation for disjunct distributions (ROSEN 1975, PLATNICK 1976, WILEY 1988). The importance of dispersal was deemed to be low for all systems except oceanic islands (CROIZAT 1958, CRAW 1984, HEADS 2004), in part because dispersal scenarios cannot easily be falsified: almost any kind of distribution pattern can theoretically be explained by dispersal (MORRONE & CRISCI 1995). Most likely this was a drastic underestimation, as shown in many studies of the last decades (WAGNER & FUNK 1995, WINKWORTH et al. 2002, VENCES et al. 2003, ESPELAND & MURIENNE 2011, RENNER et al. 2010). Not only has dispersal been demonstrated to be of higher importance in non-oceanic island systems, but evidence has also been provided that long-distance dispersal across oceans – although still considered unlikely by some authors (HEADS 2009) – can explain the biogeography of many continental organisms (CARRANZA et al. 2000, RENNER et al. 2010).

Figure 5: Vicariance and dispersal. A: The ancestors of vicariant species formerly inhabited a large range comprising the now disjunct ranges of the extant species. B: Alternatively, the ancestral range comprised only one part of the extant disjunct range, and the other parts were colonized via dispersal from the inhabited range after disjunction.



Thus, biogeographic studies on continents, continental fragments, and continental islands are often complicated by many unknown parameters of paleoclimate, tectonics, and the modes of colonization of their inhabitants. In oceanic islands, these parameters are of lower relevance. Due to the volcanic nature of most oceanic islands, estimates of the geological age are available for many of them, and biologists often relied on these data in attempts to time biogeographic events connected with the islands (HO & PHILLIPS 2009, VANDERPOORTEN et al. 2010). In particular, many molecular clock studies have used endemic populations from oceanic islands as calibration points, under the assumptions that (1) the split between such a population and its ancestral population cannot be older than the island it lives on and (2) the ancestors of extant endemics likely colonized 'their' islands shortly after emergence because later colonizers would be unable to establish a population due to competition with already present organisms (see review in HEADS 2011). Assumption (2) stands in contrast to MACARTHUR & WILSON (1967) who stated that island biotas were highly influenced by extinction and species turnover. However, some studies apparently provided evidence that islands of oceanic archipelagoes were indeed colonized, at least by some organism groups, in the order of their geological age. This was termed the 'Progression Rule' (FUNK & WAGNER 1995). HEADS (2011) argued that, although the biogeography of some organisms on oceanic islands appears to follow the Progression Rule, the pre-assumptions connected to this rule often lead to erroneous results because clades may be older or younger than the island they are endemic to, and because the geological age estimates for the islands may be incorrect. FUNK & WAGNER (1995) and WHITTAKER & FERNÁNDEZ-PALACIOS (2007) also propose a number of scenarios alternative to the progression rule (Table 4).

Table 4: Biogeographical-evolutionary patterns of organisms on oceanic islands after FUNK & WAGNER (1995) and WHITTAKER & FERNÁNDEZ-PALACIOS (2007).

Pattern	**Explanation**
Progression Rule	Phylogeography corresponding to the geological age of the islands of the archipelago
Intraisland radiation	(Sympatric) speciation within a single island
Stochastic	Apparent stochasticity in distribution in relation to the geological history of the archipelago
Back-dispersal	Dispersal from geologically younger to older island
Progressive clades and grades	Generally following progression rule, assemblages of species endemic to the same island corresponding to clades or to grades within the group
Terminal resolution	Phylogeography of basal taxa in the study group unresolved, possibly because of evolution in now submerged islands
Recent colonization	Colonization of the archipelago much more recent than the geological origins of the islands, therefore no relation between phylogeography and geological history
Extinction	Phylogeography influenced by extinction events
Unresolved	Available data allowing no clear estimation of the biogeographical-evolutionary scenario
Repeated colonization	Study groups does not form any monophyletic group, but is the result of more than one colonization events of the archipelago
Fusion of paleoislands	Sister taxa originated on formerly separate islands that fused to a single extant island; now occurring there in sympatry

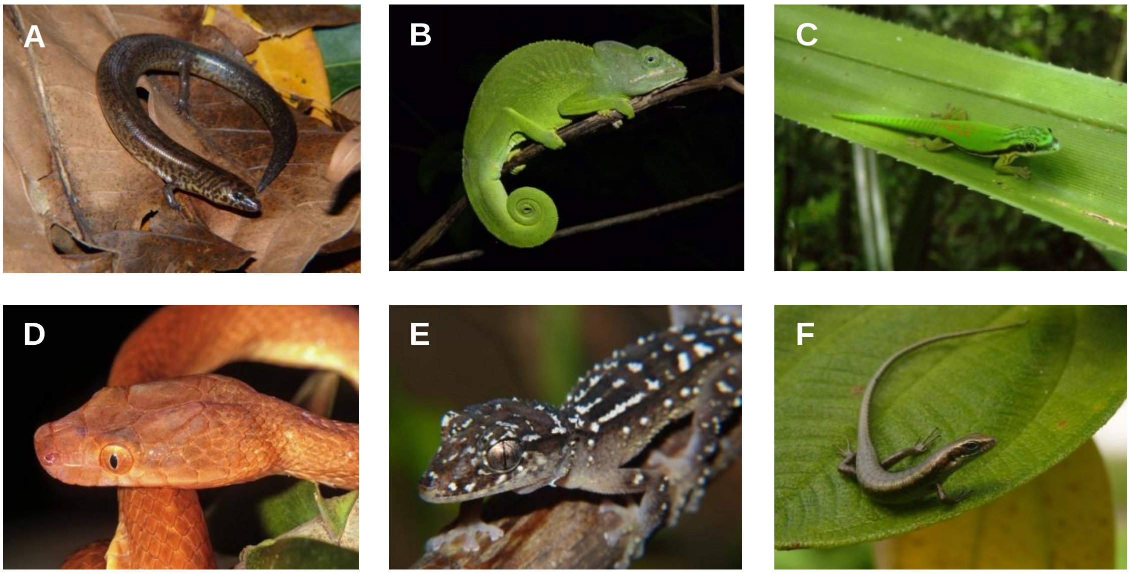
I will attempt to find evidence in this dissertation whether the Progression Rule or any other biogeographical-evolutionary pattern can be applied to Comoran reptiles. Additionally, I will explore the various factors that influence island and continental biogeography.

3.4 Study system I: reptiles of the Comoros Islands

3.4.1 *Reptiles in systematic biology*

In traditional taxonomy, the vernacular term 'reptiles' was applied to the taxon 'Reptilia', a taxon of the class rank within the vertebrates (Laurenti 1768). Originally, they were even grouped with the amphibians by Linnaeus (1758). In a modern taxonomy, which is based on the insights gained from cladistics and phylogenetic systematics (Hennig 1966), 'Reptilia' cannot any longer be maintained as a taxon that meets the criterion of monophyly. 'Reptilia', including turtles, tortoises, crocodylians, and all groups of lizards and snakes, is paraphyletic with respect to dinosaurs and birds. 'Reptiles' circumscribes just the same assemblage of tetrapods as the old 'Reptilia' but, being a merely vernacular term, does not imply any taxonomical significance. Nevertheless, mainly to avoid misunderstandings, many authors argued that 'reptiles' was too similar to the invalid 'Reptilia' and should therefore not be used in the scientific communication (Tudge 2000).

Figure 6: Native reptiles of the Comoros Archipelago. A: Amphiglossus johannae *(Scincidae). B:* Furcifer polleni *(Chamaeleonidae). C:* Phelsuma nigristriata *(Gekkonidae). D:* Lycodryas cococola *(Lamprophiidae). E:* Paroedura stellata *(Gekkonidae). F:* Trachylepis comorensis *(Scincidae). Photograph D by Frank Glaw, F by Bastian Brenzinger.*

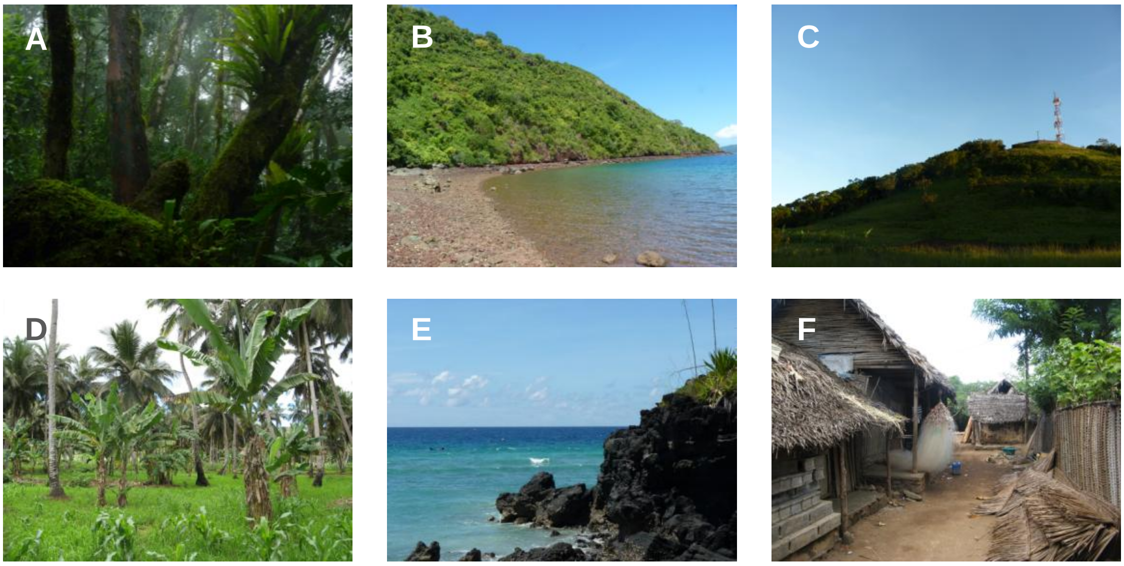


The term 'non-avian reptiles' was proposed to circumscribe the assemblage of turtles, tortoises, archosaurians, and squamates (Tudge 2000). It conveniently subsumes all extant groups that have traditionally been regarded as reptiles and includes dinosaurs, although the border between dinosaurs and birds is not clarified. However, 'non-avian reptiles' is a term not commonly understood in the public, and its length and negative phrasing make it unhandy to use. Additionally, the term suggests that 'reptiles' is commonly applied to a group comprising traditional 'Reptilia' plus dinosaurs and birds, which is not the case.

All work on any kind of reptiles that is presented in this dissertation refers to squamate reptiles (taxon Squamata). These comprise all kinds of lizards, amphisbaenians, and snakes, but exclude Sphenodontida (the Tuatara), archosaurians (including crocodylians, dinosaurs, pterosaurs, and birds), testudines (turtles and tortoises), as well as mammals and all kinds of non-amniotes ('amphibians' and 'fishes') (Vidal & Hedges 2009, Wiens et al. 2012). While I use the term 'squamates' or 'squamate reptiles' in some manuscripts and publications to meet requirements of the peer-review process, I will also maintain 'reptiles' as vernacular term to circumscribe the traditional 'Reptilia'. This use of 'reptiles' is in congruence with the taxonomic sub-discipline of herpetology (comprising the study of reptiles and amphibians), the organization of most taxonomic collections, a large part of popular and scientific literature (e.g. Glaw & Vences 2007, Vitt & Caldwell 2009), and important online databases such as HERPnet (2013) and The Reptile Database (Uetz et al. 2013).

Currently 9,547 species of reptiles are known to science (Uetz et al. 2013), which is more than in amphibians (7,085, AmphibiaWeb 2013) and mammals (5,488, IUCN 2012), but less than in birds (10,596, Gill & Donsker 2012). Due to the relatively high rate of species descriptions, reptiles may soon be the most species-rich group of tetrapods: in the period from 2000 to 2010, only 3 to 8 new species of birds were described per year (Gill & Donsker 2012), but ca. 60 to 160 species of reptiles (Uetz 2010). Reptiles inhabit every continent except Antarctica and are found on many islands. The tropics are the regions of highest reptile diversity, while only few species live in regions beyond the polar circles (Vitt & Caldwell 2009; Figs. 6,7).

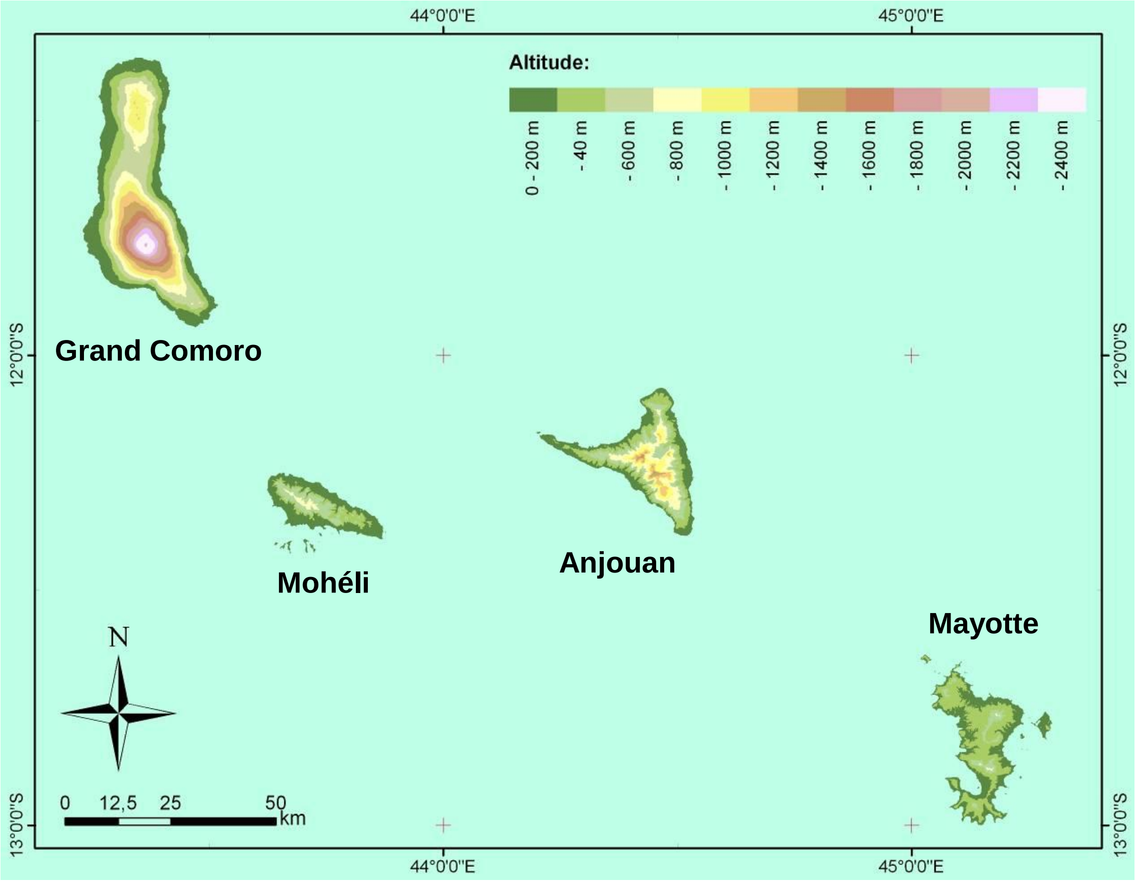
Figure 7: Habitats of Comoran reptiles A: Tropical humid forest at the central mountain ridge of Mohéli. B: Tropical dry forest and bush at Chissioua Mbouzi, Mayotte. C: Degraded forest at La Grille, Grand Comoro. D: Plantations of coconut and banana on Mohéli. E: Volcanic coastal rocks of Grand Comoro. F: Village, Anjouan.



3.4.2 Reptiles of the Comoros Islands

Reptiles have been the objects of many studies in evolutionary biology and biogeography. Some projects focused on evolution, speciation, and biogeography of reptiles in continental regions such as Australia (e.g., Moritz et al. 2009), on large islands such as Madagascar (e.g., Vences 2004, Crottini et al. 2012), or on transoceanic dispersal between continents (Gamble et al. 2008). More studies were undertaken concerning reptiles on small, often oceanic islands. Due to their ability to reproduce independently from water and their low rate of metabolism, reptiles are particularly suitable for overseas dispersal (Censky et al. 1998, Schoener et al. 2001). Even small islets surrounded by hundreds of kilometers of open ocean are inhabited by endemic reptile species (CARRANZA & ARNOLD 2003, NAGY et al. 2003). Archipelagoes consisting of several islands are often the homes of entire radiations of endemic species, and many of these radiations have been the subject of intensive studies. Among others, the anoles of the Bahamas and the Caribbean (LOSOS 1992, LOSOS et al. 2006), the iguanas of the Galápagos (RASSMANN 1997), the chuckwallas of the Baja California islands (PETREN & CASE 1997, GRISMER 1999), the lizards and geckos of the Canaries (BROWN & PESTANO 1998, CARRANZA et al. 2000), many groups of reptiles on Mediterranean islands (HENLE & KLAVER 1986, SALVADOR 1986, CAPULA 1994), the geckos of the Seychelles and Mascarenes (RADKEY 1996, ROCHA et al. 2013), and the entire herpetofauna of the Oceanian islands (PREGILL & STEADMAN 2009, HAMILTON et al. 2010) found the interest of biologists.

Figure 8: The Comoros Archipelago. Map from HAWLITSCHEK (2008).



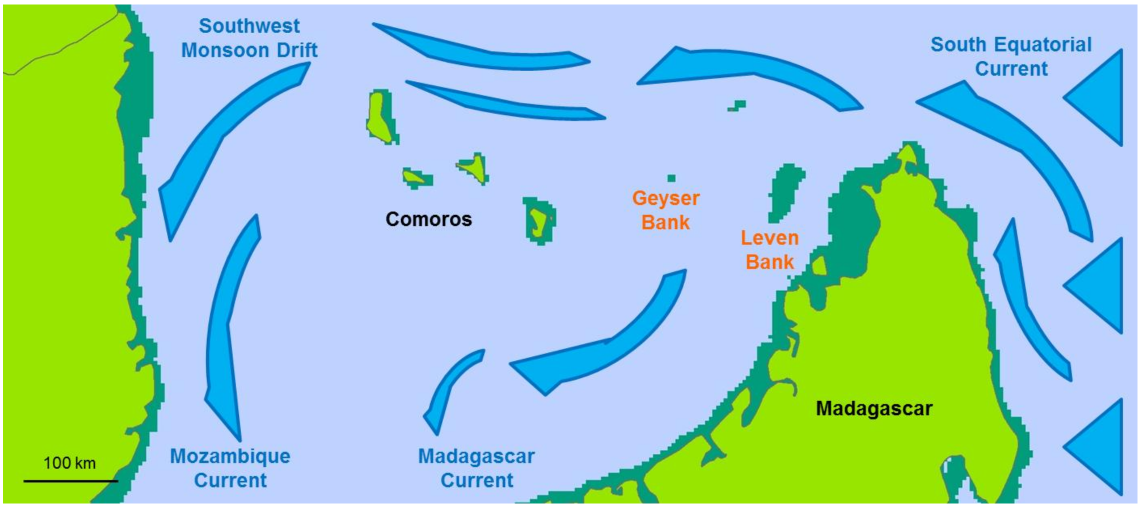
Unlike the island groups listed above, the Comoros archipelago (Fig. 8) in the Western Indian Ocean (WIO) has received relatively little attention from scientists of all disciplines. One possible reason for this is that the degree of endemism in the Comoran biota is considerable, but lower that of comparable biota of fully oceanic islands. 28.3% of breeding birds and 15% of the terrestrial plants are endemic (LOUETTE et al. 2008, PASCAL 2002). In the Galápagos 60.4% of all birds and 45% of terrestrial plants are endemic (GOSLINER 2009), in the Mascarenes 66.7% of birds and 72% of plants (THÉBAUD et al. 2009). 13 of the 28 currently recognized species of Comoran reptiles are endemic (46.4%). If introduced species are not taken into account, the degree of endemism is 76.5% (see chapters 4 to 0). In contrast, 100% native terrestrial reptiles on the Canaries and Galápagos, and 98% of the native species on the Mascarenes are endemic (GILLESPIE & CLAGUE 2009).

Table 5: A summary of geological age estimates of the Comoros Islands. Modified after Hawlitschek (2008).

Grand Comoro	Anjouan	Mohéli	Mayotte
0.5 my (Montaggioni and Nougier 1981) 0.13 ± 0.02 my (Emerick and Duncan 1982) 0.13 to 0.01 my (Bachéllery and Coudray 1993) 0.01 ± 0.10 my (Hajash and Armstrong 1972)	11.5 my (Montaggioni and Nougier 1981) 5.0 my (Battistini 1996) 3.9 my (Nougier et al. 1986) 3.5 my (Emerick and Duncan 1982; inferred estimation) 1.52 ± 0.10 my (Hajash and Armstrong 1972) 1.20 ± 0.03 my (Emerick and Duncan 1982)	5.0 my (Nougier et al. 1986) 3.9 my (Battistini 1996) 2.81 ± 0.08 my (Emerick and Duncan 1982) 1.52 ± 0.10 my (Hajash and Armstrong 1972)	About 15 my (Debeuf et al. 2005) 15 to 10 my (Montaggioni and Nougier 1981) 15 to 8 my (Battistini 1996) 7.7 my (Nougier et al. 1986) 5.40 ± 0.01 my (Emerick and Duncan 1982) 3.65 ± 0.10 my (Hajash and Armstrong 1972)

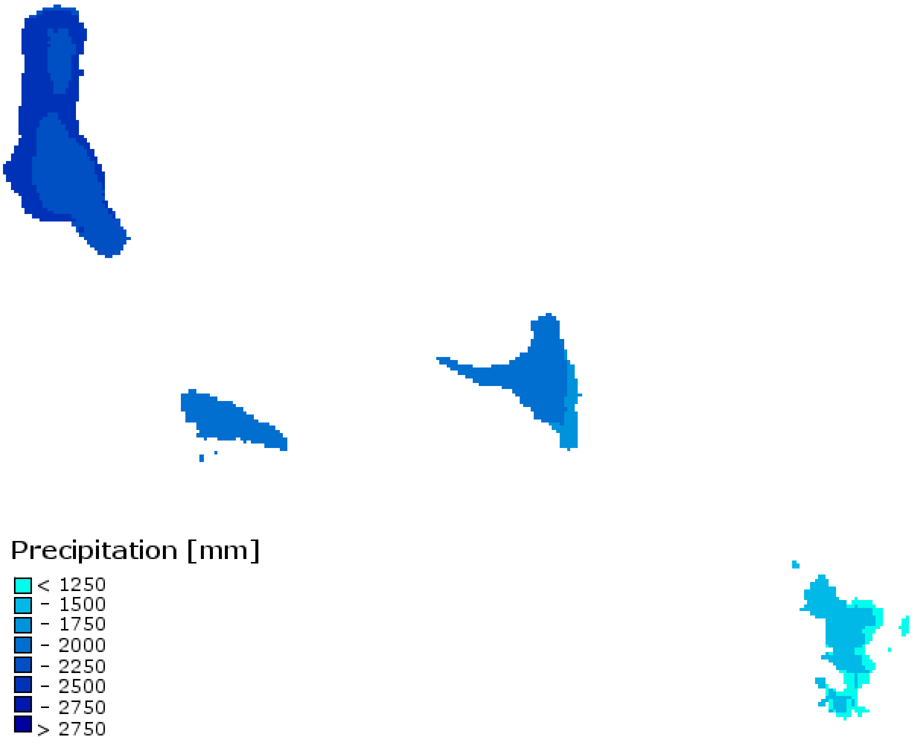
The relatively low degree of endemism is probably owed to the short distance of little more than 300 km between the Comoros and Madagascar, the ancestral origin of most its biota (Louette et al. 2004). This was even shortened by stepping-stone islands; the extant Geyser and Leven Banks are so shallow that they emerged from the ocean during the Pleistocene regressions before re-submerging and cut the distance into stages of around 100 km (Battistini & Cremers 1972, Zinke et al. 2003, Fig. 9). Marine currents and winds also support the transport from Madagascar to the Comoros and between the islands (Louette et al. 2004). For comparison, the Galapágos are situated at a distance of 1,000 km from the mainland, the Mascarenes at more than 700 km.

Figure 9: A sketch of the geographical background of the biography of the Comoros islands. Major marine currents and their directions are symbolized by blue arrows. Present-day terrestrial areas are filled with bright green, terrestrial areas of the last glacial maximum (LGM) are filled with dark green. The sketch shows that none of the Comoros Islands was connected to any other landmass. However, the Geyser and Leven Banks are submerged now but were emerged during the LGM and could thus function as stepping stones for dispersal. Resolution of LGM terrestrial areas 2.5 arc-minutes (ca. 4.65 km). Graphic created in ArcGIS 10.0, LGM data from WorldClim (Hijmans et al. 2005).



The four major islands of the Comoros Archipelago, Grand Comoro (1,100 sqkm), Anjouan (425 sqkm), Mohéli (210 sqkm), and Mayotte (375 sqkm), are situated at distances of about 60 km from one another (Fig. 8). The archipelago is assumed to be based on hot-spot volcanism (EMERICK and DUNCAN 1982); all islands are fully oceanic and were not connected to each other or any other landmasses at any point in their geological history (COLONNA et al. 1996, BATTISTINI & VERIN 1984). The oldest estimated ages are about 15 my, but most estimates are younger (Table 5). The climate in the Comoros is tropical, with a pronounced rainy season caused by the East-African monsoon from November to April, and a temperate and relatively dry climate created by the southeast trade wind from May to October. The monsoon creates a gradient in rainfall from Northeast to Southwest (Fig. 10).

Figure 10: Annual precipitation on the Comoros Archipelago. The gradient from Northwest to Southeast is determined by the Northwest Monsoon. Resolution 1 km. Graphic created DIVA-GIS 7.1.7.2, LGM data from WorldClim (HIJMANS et al. 2005).



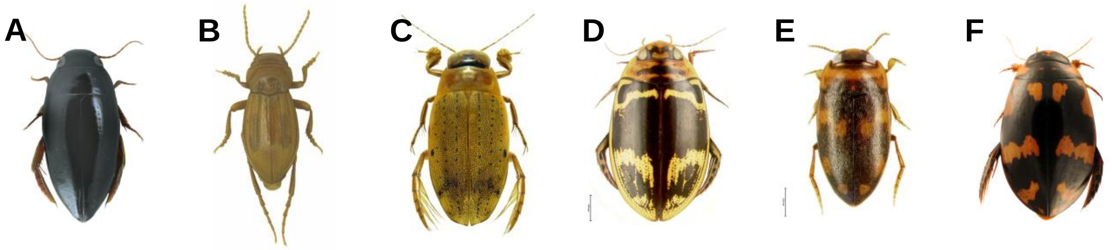
As stated above, the herpetofauna of the Comoros remained poorly studied for very long. A considerable amount of voucher specimens is available in many European museums (HAWLITSCHEK 2008), but much of it has never been revised after the initial species descriptions in historical works of PETERS (1874), BOETTGER (1878, 1913), BOULENGER (1885, 1887, 1893, 1896), and GÜNTHER (1858, 1879). In the 20th century, some herpetological work was conducted by MEIER (1980, 1984, 1986) and MEIRTE (1992, 1993, 1999, 2004). The first comprehensive modern research on the Comoran herpetofauna was conducted in the group of S. ROCHA (ROCHA et al. 2005a, 2005b, 2006, 2007, 2009, 2010a, 2010b, 2010c). Some studies of VENCES et al. (2003, 2004a, 2004b) also include Comoran reptiles. All these works focused on the phylogeny and biogeography of some groups of Comoran reptiles and amphibians, but did not consider taxonomy, ecology, distribution, and conservation. The first comprehensive work that comprises all these aspects was started in HAWLITSCHEK (2008) and is now continued in this dissertation.

3.5 Study system II: aquatic beetles of Australia

3.5.1 Aquatic beetles

Beetles are the most diverse group of organisms, at least regarding the numbers of 350,000 species currently known to science. This represents about one-quarter of all described species of animals (HUNT et al. 2007). The diversification of beetles was likely supported by their sclerotized forewings, a key innovation that provides protection while still allowing active flight (CROWSON 1981), as well as by their co-evolution with other highly diverse groups such as angiosperms and mammals (FARRELL 1998, DAVIS et al. 2002). Evidence has been provided that speciation events within some beetle groups were triggered by past climate change and the accompanying environmental changes (ERWIN 1985, LEYS et al. 2003, RIBERA & VOGLER 2004).

Figure 11: Some species of Australian aquatic beetles. A: Allomatus nannup. *B:* Carabhydrus nullica. *C:* Eretes australis. *D:* Hydaticus bihamatus. *E:* Neobidessodes thoracicus. *F:* Sandracottus bakewelli. *All photographs were provided by LARS HENDRICH.*



About 10,000 beetle species are regarded as aquatic in at least one of their developmental stages. This number seems low, but is comparatively higher than in many other predominantly terrestrial insect orders. Possibly, pre-adaptations such as the protective elytra allowed the colonization of aquatic habitats by adults while maintaining their capability to fly (BALKE et al. 2004). Beetles that spend most of their adult life submerged, or partly submerged, are defined as 'True Water Beetles' by JÄCH (1998). Many of them belong to the suborder Adephaga; the adephagan leg movement mechanism may have been another helpful pre-adaptation to the invasion of aquatic habitats. The largest family of True Water Beetles is the Dytiscidae, the diving beetles. Currently, about 3700 species in about 150 genera are recognized (PEDERZANI 1995, NILSSON 2001). Dytiscids inhabit all continents except Antarctica and are found in almost all kinds of fresh water habitats, including brackish and subterranean waters, although fewer species occur in large water bodies such as larger streams and lakes where larger predators (e.g., fish) are abundant. The diversity is higher in meso- and eutrophic waters (BALKE et al. 2004). A high number of species inhabits seasonal habitats, where they may constitute apex predators.

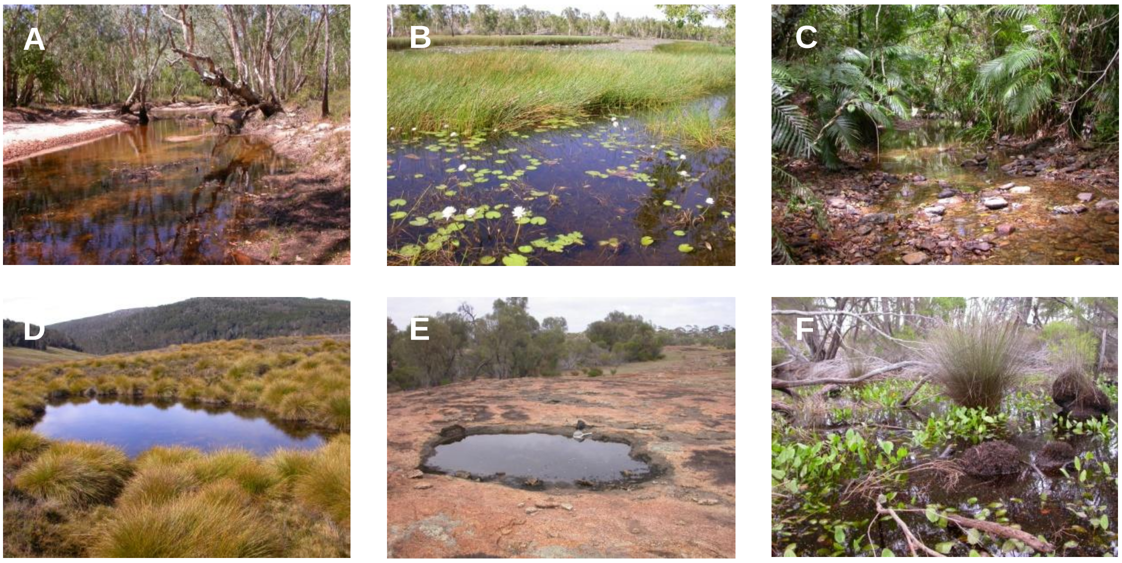
Most species of diving beetles and related families are capable of actively powered flight, and many have good dispersal abilities. Therefore, the availability of suitable habitats is most likely more important in defining the distributions of diving beetle species than drainage systems, as proposed for the freshwater biogeographic regions of ABELL et al. (2004). Notably, widespread and narrowly distributed species may be closely related, as apparent in the genus *Rhantus*, which includes microendemic species on New Guinea mountain chains as well as the near-cosmopolitan *Rhantus suturalis* (BALKE et al. 2001, 2009). These complex and often surprising patterns make dytiscids highly interesting study objects in biogeography.

The monophyly of adephagan aquatic beetles was shown in HUNT et al. (2007) and BALKE et al. (2005). Most families are less species-rich than the Dytiscidae, among them the Hygrobiidae, which are also studied in this dissertation.

3.5.2 The Australian diving beetle fauna

One of the world's most remarkable fauna of diving beetles inhabits Australia (Figs. 11, 12). To date, over 300 species have been described, of which 90% are endemic to the continent. These endemic species belong to 18 or 19 exclusively Australian radiations (COOPER et al. 2002, LEYS et al. 2003, HENDRICH & WATTS 2004, WATTS & HUMPHREYS 2004, BALKE et al. 2007, HENDRICH et al. 2009). As stated above for dytiscids in general, also many Australian species are restricted to small regions, whereas only few are widespread. An interesting aspect of the Australian diving beetle fauna is the high diversity of subterranean species. After these communities were discovered only in the mid-1990s, over 100 species have been described that inhabit groundwater calcrete (terrestrial limestone) aquifers (COOPER et al. 2002, LEYS et al. 2003).

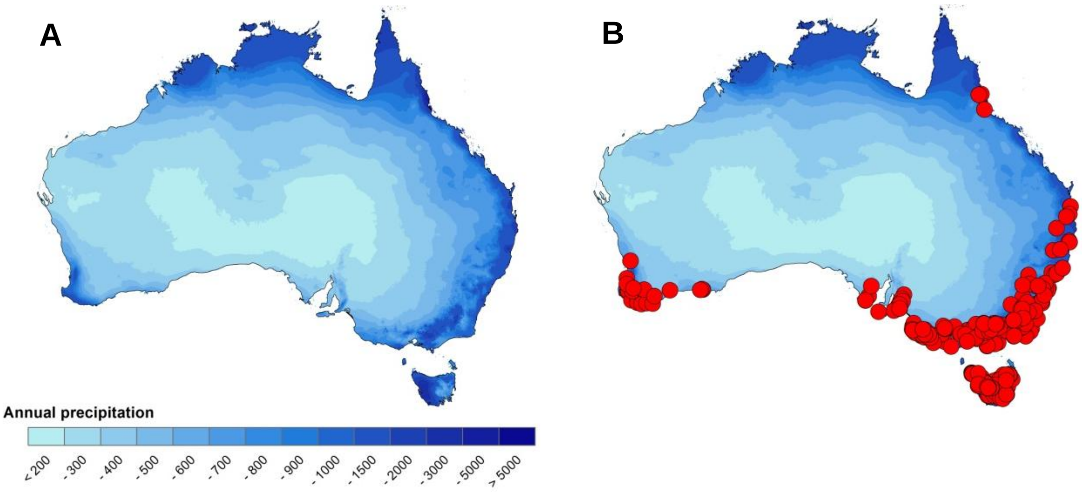
Figure 12: Habitats of Australian aquatic beetles. A: Magela Creek, Northern Territory. B: Coojimba Billabong, Northern Territory. C: Rainforest creek in Queensland. D: Highland pond in Tasmania. E: 'Gnamma' (desert rock puddle), Newman Rocks, Western Australia. F: Swamp in Northcliffe, Western Australia. All photographs were provided by LARS HENDRICH.



Australia is a geologically old continent; much of its surface is composed of Archean, Proterozoic, and Paleozoic rocks (JOHNSON et al. 2009). In contrast, many habitats that cover large areas on the continent are of relatively recent origins (KEMP 1981, FRAKES et al. 1987, BYRNE et al. 2008). It is assumed that the tropical rainforest, now restricted to small refuges in the North of the continent, covered most of Australia until the Miocene (23.03 – 5.33 mya).

Only then, Australia drifted northward, and the global climate began to cool, leading to a shift in the climate of Australia from predominantly humid to predominantly arid (BOWLER 1982). In this period, the mesic biomes along Australia's southern, eastern and northern coasts formed (HILL 1994, SCHODDE 2006). The aridification process was a gradual one, however, and large parts of present-day's deserts formed only during the Pleistocene climate oscillations (i.e., the Ice Ages; FUJIOKA et al. 2005, 2009). All these changes in climate were accompanied by drastic changes in the biota of the continent. Molecular genetic data indicates many speciation events even in geologically very recent times (BYRNE et al. 2008, DUBEY & SHINE 2010a, 2010b, PEPPER et al. 2011a, 2011b, 2011c). Possibly even more massive extinctions are indicated by fossils of vertebrates (MARTIN & KLEIN 1989, PRIDEAUX et al. 2007) and can only be speculated for groups with a poorer fossil record.

Figure 13: The distribution of Australian diving beetles. A: Annual precipitation in Australia. B: Same graphic with sampling localities of diving beetles used in this dissertation. Graphic created in ArcGIS 10.0, climate data from WorldClim (HIJMANS et al. 2005).



In this work I focus on the epigean species of Australian diving beetles, predominantly from the mesic Southeast region (Fig. 13), and on the global Hygrobiidae. I build on a considerable amount of work on the phylogeny, taxonomy, and faunistics of this group that was conducted in the last decades by M. BALKE, L. HENDRICH, and C.H.S. WATTS (WATTS 1978, 1997, BALKE et al. 2004, 2007, 2009, HENDRICH & WATTS 2004, WATTS & HUMPHREYS 2004, HENDRICH et al. 2009, 2010).

4 DNA Barcoding – a first glimpse to set the course of phylogenetic analyses

4.1 MANUSCRIPT: DNA Barcoding allows reliable identification of island populations of Comoran squamate reptiles.

In Chapter 3.2.3, I explained that DNA barcoding was often used for large datasets with unclear identification of at least a part of the included samples. In smaller datasets, barcoding mostly serves to grant a first glimpse to set the course of subsequent phylogenetic or other molecular genetic analyses. However, such datasets may also serve as test cases for the barcoding approach. The dataset of Comoran reptiles had the advantage that almost all samples included were safely identified to species, subspecies, and island population level, and that multigene molecular phylogenies were available at least for some groups included, representing subsets of the barcoding dataset. Therefore, in addition to clarifying the identification of a few dubious samples and hinting at the topology of groups that had not been analyzed with a multigene dataset, this barcoding study allowed me to compare the performance of DNA barcoding vs. multigene pyhlogenies in the reconstruction of topoplogies by quantitative means. It also suggested suitable study systems included in this dataset for future studies.

HAWLITSCHEK O., NAGY Z.T., BERGER J., GLAW F. (submitted): DNA Barcoding allows reliable identification of island populations of Comoran squamate reptiles. PLoS ONE.

DNA Barcoding allows reliable identification of island populations of Comoran squamate reptiles

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Abstract

In the past decade, DNA barcoding became increasingly common as a method for species identification in biodiversity inventories and related studies. However, mainly due to technical obstacles, non-avian reptiles have been the target of few barcoding studies. In this article, we present the results of a DNA barcoding study of squamates of the Comoros archipelago, a poorly studied group of oceanic islands close to Madagascar. The barcoding dataset presented here includes 27 of the 29 currently recognized squamate species of the Comoros, including 17 of the 18 endemic species. Some Comoran species were found to include non-Comoran lineages, which is probably due to poorly resolved taxonomy. All other species for which more than one barcode was obtained formed distinct clusters useful for species identification by barcoding. In most species, even island populations could be distinguished using barcoding. Two cryptic species were identified using the DNA barcoding approach. The barcoding topology, i.e., a Bayesian tree based on COI sequences of 5 genera was compared with available multigene topologies, and in 3 cases, major incongruences between the two topologies became evident. Three of the multigene studies were initiated after initial screening of a preliminary version of the barcoding dataset presented here. We conclude that in the case of the squamates of the Comoros Islands, DNA barcoding has proven a very useful and efficient way of detecting isolated populations and promising starting points for subsequent research.

Introduction

Since the pioneer studies of Hebert et al. [1], DNA barcoding has gained great popularity among biologists as a standardized, quick, and technically easy approach that does not require expert knowledge once reliable databases have been established. DNA barcoding has been applied in a broad range of studies and are helpful at various ends, such as biodiversity inventories of unstudied regions [2,3], species identification through barcode databases [4,5], pest identification and control [6], control of invasive species [7,8], and human health [9]. One of the most common applications in biodiversity research is the use of DNA barcoding for a preliminary biodiversity assessment of a certain organism group in a certain region. This may range from very narrowly circumscribed target groups (e.g., [10]) to a broad range of organisms in large areas [11-13]. Despite its various uses and applications, DNA barcoding data have limited value to elucidate phylogenetic relationships [14] and sometimes 'disguise' species that cannot be identified by barcoding [15].

In animals, the cytochrome *c* oxidase subunit I (COI) was established as the universal barcoding marker [1], mostly using the universal primers LCO and HCO [16]. However, COI is not equally easy to amplify in all taxonomic groups of animals. Until recently, non-avian reptiles were among the animal groups that were hard to barcode, and few studies focused on their DNA barcoding [17]. Nagy et al. [18] published a barcoding study of the squamates and turtles of Madagascar. This was the first large-scale barcoding attempt targeting this group of vertebrates. The study focused on testing the efficiency of new primers for non-avian reptile barcoding, on detecting cryptic diversity, and on providing a barcode database for easier identification of Malagasy species.

Like many other studies on Malagasy organisms, the DNA barcoding study [18] did not include the fauna of the Comoros archipelago. This group of four volcanic and hence fully oceanic islands is situated in the Western Indian Ocean halfway between the East African coast and Northwest Madagascar. Because of prevalent oceanic currents and winds, much of the Comoran biota originates from Madagascar, but is rich in endemic species [19,20]. Nevertheless, only relatively few modern studies focused on Comoran organisms. Recent works on the phylogeny, biogeography and taxonomy of Comoran squamates were published by the group of S. Rocha [21-28] and by our group [20,29,30].

In our work with Comoran squamates, we initially used preliminary genetic screening, including DNA barcoding, to receive a preview on genetic divergences between species and island populations, to distinguish whether species were more likely native or introduced, and to detect possible cryptic species. Then, we used multigene approaches to study groups of squamates that were found to be interesting by our initial screening. In this article, we present the results of our DNA barcoding approach and, wherever possible, compare them with the results of available multigene phylogenies. We also tested the performance of DNA barcoding to correctly identify island populations of native species.

Material and methods

Sampling, permits, and ethics statement

No experiments were conducted using living animals. Furthermore, none of the samples were specifically collected for this project, but for an earlier study on Comoran reptiles [20] by 3 of the 4 authors of this paper (OH, JB, FG). We exclusively used museum samples which were already available and were deposited in a tissue bank at the Zoologische Staatssammlung München (ZSM), Germany. For all species and 176 out of 217 specimens, not only tissue samples but also voucher specimens were available (Tables 1 and S1). All samples and voucher specimens were analysed with permission of the ZSM. Voucher specimens were euthanized using approved methods (e.g. anaesthesia with ketamine, followed by ketamine overdose) that do not require approval by an ethics committee according to national law on the Comoros.

Collection and transport of specimens was conducted with the following permits: (1) Issued by the Direction Générale de l'Environnement, Moroni, Union des Comores: research and export permit (no permit number, 1st March 2000), research permit (02/121/MPE/DGE, 12th April 2002), export permit (02/141/MPE/DGE, 2002), research and export permit (no permit number, 12th March 2008), research permit (CNDRS/08/2010, 22nd January 2010), export permit (CNDRS/030/2010, 5th April 2010). (2) Issued by the Direction de l'Agriculture et de la Forêt, Mayotte, France: research and export permit (no permit number, 23rd February 2000), research and export permit (24/DAF/SEF/2008, 19th March 2008), research and export permit (2010-13/DAF/SEF, 30th March 2010). Import of species protected by CITES into Germany was approved by the German authorities (Bundesamt für Naturschutz).

Laboratory protocols

Total genomic DNA was extracted using the standard protocols of the NucleoSpin ® 96 Tissue kit (Macherey-Nagel) and the DNEasy Tissue Kit (Qiagen, Hilden, Germany). We amplified the 5’ half of COI using the primers RepCOI-F / RepCOI-R [18] or LCO / HCO [16] and the corresponding PCR protocols. Table 1 lists which primer combination was more successful for each species. Sequencing was conducted using the BigDye ® Terminator v1.1 Cycle Sequencing Kit on ABI 3730 and ABI 3130xl capillary sequencers (Life Technologies). Sequence data were deposited in BOLD and GenBank and are available under accession numbers XXX to XXX (Table S1).

Barcoding tree reconstruction

We used Sequencher 4.9 © for editing and quality checking of the chromatograms, Mesquite 2.72 [31] for additional quality checking, including inspection of protein translations, and MAFFT 6 [32,33] for alignment of the COI dataset. In addition to the sequences produced from Comoran samples, we added 34 sequences from related species obtained from GenBank (most originate from [18]) for comparison with the barcoding dataset. We selected sequences that were found to be most similar to the Comoran sequences in BLAST searches. We then conducted a test of substitution saturation [34,35] in DAMBE v5.2.34 [36] and plotted transitions and transversions against Kimura 2-parameter (K2p) divergences to visualize possible saturation at a higher divergence level.

We calculated pairwise K2p-distances in MEGA 5.0 [37]. We partitioned the dataset according to codon position. To identify appropriate substitution models for the maximum likelihood (ML) and Bayesian analyses, we used jModeltest 0.1.1 [38]. We assessed AIC and BIC results, giving BIC preference over AIC. Subsequently, we conducted (1) ML analyses with 1,000 fast bootstrap repeats in raxmlGUI 1.0 [39,40] and (2) Bayesian analyses in MrBayes 3.1.2 [41] on the CIPRES portal 2.2 [42] with two runs and four chains with 30,000,000 generations (samplefreq=1,000, 25% burnin). MrBayes runs were checked for convergence and normal distribution in Tracer v1.5 [43].

One aim of this analysis was to test the performance of DNA barcoding versus multigene phylogenies, wherever available. We used data for the genera *Cryptoblepharus* (766 bp) [32], *Ebenavia* (1894 bp) [44], *Lycodryas* (3498 bp) [29], *Phelsuma* (2872 bp) [25], and *Paroedura* (3174 bp) [30]. Then, we estimated trees using MrBayes with the setting described above, but only run for 10,000,000 generations. The subsets of our barcoding dataset and the corresponding multigene datasets contained only the genus in question and related taxa.

Clustering and species identification by barcoding

To measure the success of the identification of species and island populations of native species in our dataset using DNA barcodes we used an objective clustering approach as implemented in SpeciesIdentifier [45]. This software clusters sequences using p-distances, thus allowing the comparison of clusters with the existing taxonomy [10,46]). Species names and clustering thresholds are preset by the user. We conducted clustering analyses with thresholds of 5% to 15% for delimitation of 'barcoding species', and 0.2% to 2.0% for delimitation of island populations. Additionally, we conducted query identification analyses of the dataset with the 'best match' and 'best close match' criteria [45]. Under the 'best match' criterion, any query sequence is assigned the species name of its best matching barcode (i.e., reference sequence). If this analysis is run in SpeciesIdentifier, the output shows how many sequences were assigned to a matching sequence in agreement with their pre-assigned species name. Obviously, the sequences of species of which only a single sequence is included in the dataset are automatically misidentified because their best matching sequence belongs to a different species. Applying the 'best close match' criterion, the same analysis is refined with a user-defined cutoff distance. Sequences that do not match within the defined cutoff distance are not assigned to the species of their best matching sequence, but to a species of their own. For the clustering analyses for species identification, we used a dataset from which all identical haplotypes were cropped using the software Collapse 1.2 [47]. All sequences from non-Comoran species were removed manually. The cropped dataset consisted of 130 sequences. In the dataset for the identification of island populations, we additionally removed all species that were considered non-native [20], leaving 61 sequences.

Results and Discussion

The DNA barcoding dataset

We produced a total of 168 DNA barcodes for 27 out of the 29 currently recognized species of Comoran squamates (Table 1) including 2 recently described species, *Lycodryas cococola* [29] and *Paroedura stellata* [30]. We also included all recognized subspecies of Comoran species, including *Cryptoblepharus boutonii ater* (Grand Comoro, corresponding to *C. ater* according to Horner 2007 [48]), *C. b. degrijsii* (Anjouan, corresponding to *C. quinquetaeniatus*), *C. b. mayottensis* (Mayotte, corresponding to *C. gloriosus mayottensis*), *C. b. mohelicus* (Mohéli, corresponding to *C. g. mohelicus*), *Lycodryas cococola cococola* (Grand Comoro), *L. c. innocens* (Mohéli), *L. maculatus maculatus* (Anjouan), *L. m. comorensis* (Mayotte), *Phelsuma v-nigra v-nigra* (Mohéli), *P. v. anjouanensis* (Anjouan), and *P. v. comoraegrandensis* (Grand Comoro).

A single barcode sequence was obtained for 5 species, 2 or more DNA barcodes for all other species, with an overall high success rate of 100% in 12 species and >70% in further 7 species (excluding the species for which a single sample was available and successfully sequenced). The highest success rates in PCR amplification and sequencing were achieved using the primers RepCOI-F and RepCOI-R [18]. However, for a number of species LCO and HCO [16] worked better (Table 1). Notably, both primer pairs failed to produce readable sequences in the most common Comoran reptile species, *Trachylepis comorensis*. Only a single sequence could be produced for this species, based on a sample of an egg; all of the numerous samples of muscle tissue failed. Neither could any sequence be produced for the related, non-native *T. striata*. Furthermore, a sample of an undescribed species of *Typhlops* could not be sequenced. *Ramphotyphlops braminus* and *Typhlops comorensis* were the only species in which no sequence was produced with RepCOI-F / RepCOI-R, but LCO / HCO performed well.

K2p-distances are given in Table 2 for families and in Table 3 for species or clades endemic to the Comoros. In many species inhabiting more than one island of the archipelago, genetic divergences range from 4.8% to 9.4% (K2p distance), with an average around 3%. Notably, this comprises endemic clades whose lineages can be clearly attributed to islands (*Phelsuma v-nigra*, *Geckolepis maculata*, *Cryptoblepharus boutonii*), as well as non-endemic groups whose lineages are mixed between the islands

(*Hemidactylus* spp.). Other introduced taxa (*Phelsuma laticauda, P. dubia, Ramphotyphlops braminus*) show much lower divergences from 0.02% to 1.3%. *R. braminus* is the only all-female snake known to reproduce parthenogenetically, which means that a single specimen can found a population with its clonally produced offspring and may explain the exceptionally low haplotype diversity [49].

In the analysis of substitution saturation in DAMBE, the index of substitution saturation Iss was always significantly below its critical value Iss.c. This indicates an overall low saturation in the dataset. The plotting of transitions and transversions against divergence indicated saturation at higher levels of divergence (results not shown).

Clustering, identification of species and island populations

The objective clustering analysis for species identification under thresholds from 5% to 15% yielded a varying number of clusters, ranging from 25 to 37. The number of clusters never corresponded exactly to the number of species included (27). The best results were achieved under thresholds of 8% to 11% with a total of 28 clusters, 24 of which were in correspondence to the currently valid taxonomy. Because of the high divergences between the island populations of *Ebenavia inunguis*, these samples did not form a common cluster at thresholds that yielded appropriate results for other species. At the same level, however, the 2 Comoran species of *Lycodryas* formed a common cluster.

The 'best match' query analysis correctly linked 124 out of 130 barcoding sequences to species. The remaining 6 sequences refer to species that are represented by a single sequence only in the clustering dataset, and are thus automatically misidentified by the 'best match' analysis. The 'best closest match' query analysis correctly linked 123 sequences at thresholds of 8% to 11%. This supported the view that all Comoran squamate species included in this study are monophyletic, if sequences of non-Comoran origin are excluded.

The objective clustering analyses for the identification of island populations of native species yielded 27 to 48 clusters. Thus, the number of clusters corresponded to the 27 island populations of 9 included species at thresholds from 1.6% to 2.0%. However, the highest number of clusters corresponding directly to actual island populations was 24 at a clustering threshold of 1.2%. At higher thresholds, island populations were lumped. The 'best match' query linked 50 out of 61 barcoding sequences to the correct island populations. The 'best closest match' query correctly linked 47 sequences at thresholds of 1.4% or higher. Thus, the combination of all objective clustering criteria not only allows the identification of barcodes to species level, but also to the level of island populations, with good performance as long as native species with monophyletic island populations are concerned.

Topologies constructed in DNA barcoding vs. multigene phylogenies

The barcoding topology based on a Bayesian tree is shown in Fig. 1. All genera, including Comoran species and selected related species, were retrieved monophyletic. As in the clustering analysis, all species were retrieved monophyletic, with the exceptions of *Phelsuma dubia* and *Amphiglossus johannae*. In our trees, a sequence of the Malagasy *P. ravenala* is nested within the branch comprising Comoran samples of *P. dubia*. In *Amphiglossus*, the sequences of the Malagasy *A. ardouini* are nested within the Comoran endemic *A. johannae*.

Fig. 2 shows 5 subsets of the barcoding topology. The subsets were cropped so that only representatives of major clades are displayed. A comparison of the trees with topologies of multigene phylogenies shows major incongruences between the topologies in 3 of these 5 cases. However, these incongruences are often poorly supported, and the support values for the nodes concerned in the barcoding topology are generally poor. In the topologies of *Cryptoblepharus*, differences to the barcoding tree are based only on differences in the resolution: some nodes are better resolved in the multigene topology, some in the barcoding topology. The sister relationship between *C. boutonii ater* (Grand Comoro) and *C. boutonii mohelicus* (Mohéli) is not retrieved with high support in the barcoding tree, however, this relationship is neither highly supported in the multigene analysis [23]. The relatively poor support values in the multigene analysis may be due to the comparably small number of nucleotides studied. In *Ebenavia*, the multigene analysis [44] finds a sister-group relationship of the Anjouan sample with a clade of the Grand Comoro + Mohéli samples, which is not retrieved in the barcoding tree. In the other 3 genera studied, major incongruences between the barcoding tree and the multigene trees are found. In *Lycodryas*, our barcoding analyses place the Malagasy *L. gaimardii* within Comoran *Lycodryas*, whereas it is retrieved as sister group to Comoran *Lycodryas* in the multigene analyses [29]. Comoran *Paroedura* are retrieved paraphyletic, as in the multigene analysis by [30], but the Malagasy *P. lohatsara* is the sister taxon to *P. sanctijohannis* in our barcoding analyses instead of a clade comprising *P. stellata* + *P. lohatsara* (Madagascar) + *P. stumpffi* (Madagascar), albeit with poor support. In our DNA barcoding study, the Malagasy *Phelsuma laticauda* (together with *P. roesleri*) is the sister taxon to the Comoran *P. v-nigra*, and the two endemic species from Mayotte *P. robertmertensi* and *P. pasteuri* form a sister group to this clade, whereas *P. v.nigra*, *P. pasteuri*, and *P. robertmertensi* are comprised in one clade with *P. laticauda* as sister group in the multigene analyses [25]. *P. lineata* + *P. comorensis* are the sister group to this clade in the multigene analysis, but not in our barcoding analyses. In *P. v-nigra*, the population of Anjouan clusters with that of Grand Comoro in the barcoding topology, whereas in the multigene tree [25] it clusters with the Mohéli population.

Patterns of genetic divergence in island populations

As shown in Figs. 1 and 2, and Tables 4 and 5, DNA barcodes of Comoran squamates are in most cases not only useful to identify which species a sample belongs to, but also in which island population it was collected. This was possible for all included samples of the endemics *Lycodryas cococola*, *L. maculatus*, *Phelsuma v-nigra*, *Typhlops comorensis*, and the Comoran clades of *Cryptoblepharus boutonii*, *Ebenavia inunguis*, and *Geckolepis maculata*. In other cases, the most recently diverged island lineages could not be distinguished, whereas the more distant island lineages were distinct. This was found in the endemic *Amphiglossus johannae* and *Paroedura sanctijohannis*, but notably also in the presumably introduced *Hemidactylus frenatus* and *H. mercatorius*. However, because of the low sample sizes for some rarer species we cannot exclude that incomplete lineage sorting between island populations may be higher than shown by our results. This is also the reason why no statement can be made on some species. In some introduced species, haplotypes are mixed over all colonized islands, such as in *Hemidactylus platycephalus*, *Ramphotyphlops braminus*, *Phelsuma dubia* and *P. laticauda*, which supports the view that the Comoran populations of these anthropophilous species originated from recent introduction events.

Notably, all introduced species with comparably high genetic divergences belong to the genus *Hemidactylus*. The nocturnal *Hemidactylus* geckos are commonly attracted by artificial sources of light in settlements and harbours. This may promote the genetic exchange between overseas population mediated by human sea traffic [20,27].

Endemic taxa that are restricted to a single island – and hence probably resulting from isolated colonization events, as shown for the Comoran *Furcifer* species [22], *Paroedura stellata* [30], and *Phelsuma nigristriata* [25] – also show relatively little genetic diversification of 0.2% to 1.3%. A notable exception is *Furcifer cephalolepis* with high intraspecific divergences (up to 2.8%), which has also been shown for an independent set of samples and different molecular markers [22].

The DNA barcoding dataset supports the high genetic divergences in relation to other island populations of the same species that are found in Grand Comoro endemic populations of squamates [22,29,30]. This is remarkable because Grand Comoro is assumed to be the geologically youngest major island of the archipelago [50]. As discussed in Hawlitschek & Glaw [30], reasons for this may be that either Grand Comoro is geologically older than currently estimated, or populations of geologically older islands are younger because these islands were colonized later in geological history, e.g., after the extinction of an earlier island population.

Another remarkable feature of some Comoran endemic squamate populations is that the Comoros were colonized several times by the same genus. This was found for the genera *Phelsuma* [24,25] and *Paroedura* [30] and also suggested for *Furcifer* [22]. A similar pattern is found in the barcoding tree for *Ebenavia inunguis*. Although the Comoran populations are considered conspecific with Malagasy *E. inunguis*, the high genetic divergences between the island populations, and between Comoran and Malagasy samples, suggest that this species colonized at least 3 islands of the Comoros by natural dispersal, and that its taxonomy should be revised (see also [44]).

The barcoding topology at species level and its significance for taxonomy

Of the 22 Comoran squamate species for which at least 2 barcodes were produced, 19 were retrieved as monophyletic units. We explore the cases of the species that were not retrieved monophyletic and examine the reason for this incongruence between DNA barcoding and existing taxonomy.

The Malagasy day gecko *Phelsuma ravenala*, described by Raxworthy et al. [51], is nested within *P. dubia* from Madagascar and the Comoros in our barcoding tree, with K2p distances of less than 1% from all included *P. dubia* sequences. This is congruent with the results of Rocha et al. [25]. In their original description, the authors presented the number of scale rows around midbody as an important character to distinguish between the two species. However, a morphological study of this character in Comoran *P. dubia* [52] showed that these specimens were outside the ranges given for *P. dubia* and *P. ravenala* by Raxworthy et al. [51], suggesting that the validity of the latter species is in need of confirmation.

The Comoran endemic *Phelsuma comorensis* is found nested within the otherwise Malagasy *P. lineata* in our barcoding tree. This is also congruent with the results of Rocha et al. [25]. The minimal K2p distance from *P. lineata* sequences is 3.1%. The polytypic *P. lineata* has been shown to be a species with variable morphology and ecological adaptability [28], and *P. comorensis* could be argued to fall within the ranges of these amplitudes. Future studies should attempt to examine the species status of *P. comorensis*, and search for indications whether this Comoran population represents a case of natural (ancient) or human-mediated (recent) dispersal.

Amphiglossus johannae, a skink considered endemic to the Comoros, is retrieved paraphyletic with respect to the Malagasy *A. ardouini*. While both species are easily distinguished via external morphological characters, the minimal K2p distance between them is 0.5%. The reason for this unexpected position of the 2 species in the barcoding tree is unknown. Future studies should explore the possibility that *A. johannae* represents a case of recent, but natural dispersal from Madagascar to the Comoros with rapid adaptation of the morphological characters to the insular environment (but see [30] for alternative scenarios).

The Comoran populations of the gecko genus *Paroedura* and the snake genus *Lycodryas*, formerly considered as *Paroedura sanctijohannis* and *Lycodryas sanctijohannis*, respectively, are retrieved paraphyletic in our barcoding tree. The paraphyly of Comoran *Paroedura* was confirmed by molecular and morphological data [30], whereas Comoran *Lycodryas* were found to be monophyletic [29].

With the exception of the cases stated so far, all species form monophyletic and clearly distinct clusters. As described, most detected cases of species paraphyly can likely be attributed to poorly resolved taxonomy of the species in question. *Lycodryas gaimardii* is placed within *L. sanctijohannis* due to the small barcoding gap, and the case of *Amphiglossus johannae* remains unclear. This means that – once taxonomy is revised – all the 22 Comoran squamate species for which at least 2 barcodes were produced will be correctly identified by DNA barcoding.

Retrieving higher-ranking taxa

As shown in Table 2, the interspecific divergences found within different families are at very different levels, ranging from an average 14.1% in Lamprophiidae to 28.5% in Gekkonidae. Due to the unbalanced specific diversity of the Comoran herpetofauna in comparison with the Malagasy herpetofauna, the distances found between Comoran species of Chamaeleonidae, Lamprophiidae, and Gekkonidae are lower than the average distances given by Nagy et al. [18] for the same families in Madagascar.

The only case of unexpected placement of a family-level taxon in the barcoding tree is that of *Oplurus* (Iguanidae), which clusters with the genera of the Scincidae (Bayesian PP and ML bootstrap ≥ 90%). This is in contrast to most modern deep phylogenies of non-avian reptiles that place the Iguanidae in the neighbourhood of the Chamaeleonidae and Agamidae [53,54].

Despite the high levels of saturation detected for deeper splits, many higher-ranking taxonomic units are revealed monophyletic in our barcoding tree. 14 of the 16 genera included in the study are retrieved monophyletic, with the exceptions of the genera *Trachylepis* (monophyly not resolved with respect to *Cryptoblepharus*) and *Typhlops* (paraphyletic with respect to *Ramphotyphlops*). However, all Comoran species except for the 2 species that were not included in our study were correctly assigned to genus level in our barcoding topology.

In conclusion, we find that the identification of Comoran squamate species was successful at multiple levels: DNA barcodes always allowed correct identification at the levels of genus and species, and mostly even at the level of island populations.

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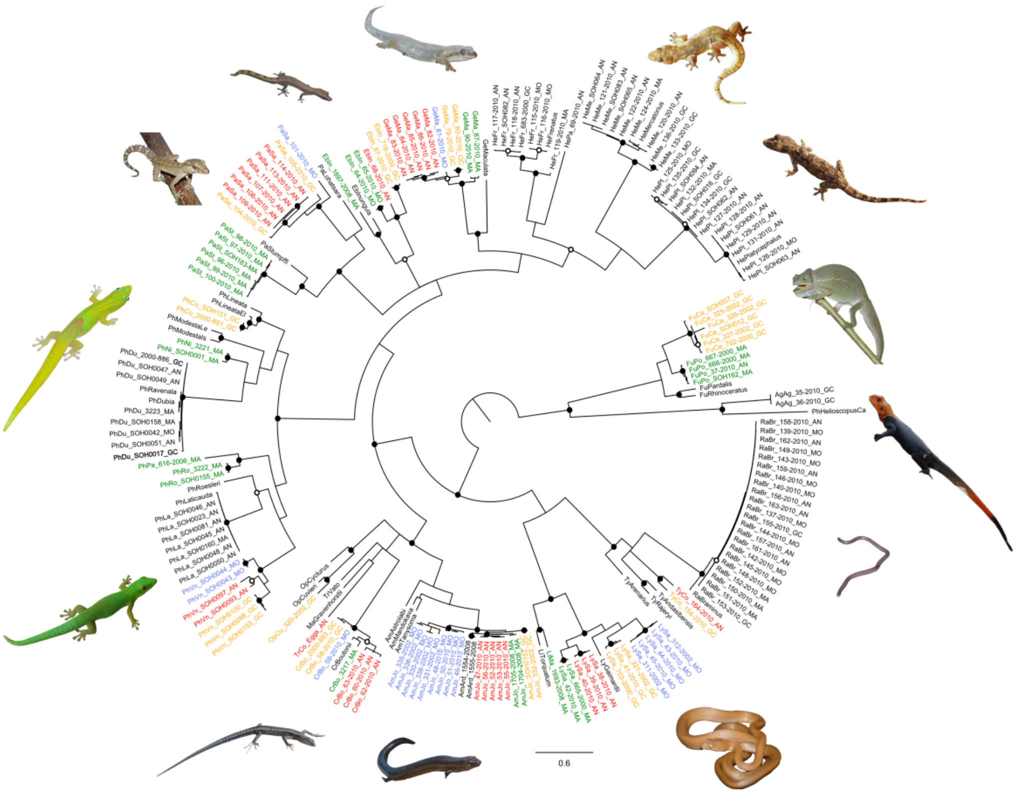
Figure legends



Colors: Anjouan, Grand Comoro, Mayotte, Mohéli, Madagascar

Figure 1: Bayesian tree of the COI dataset. Nodes with Bayesian PP and ML bootstrap support ≥ 90% are marked with filled black circles, nodes with Bayesian PP or ML bootstrap support ≥ 90% are marked with empty black circles. Island lineages of endemic species are marked in colors.

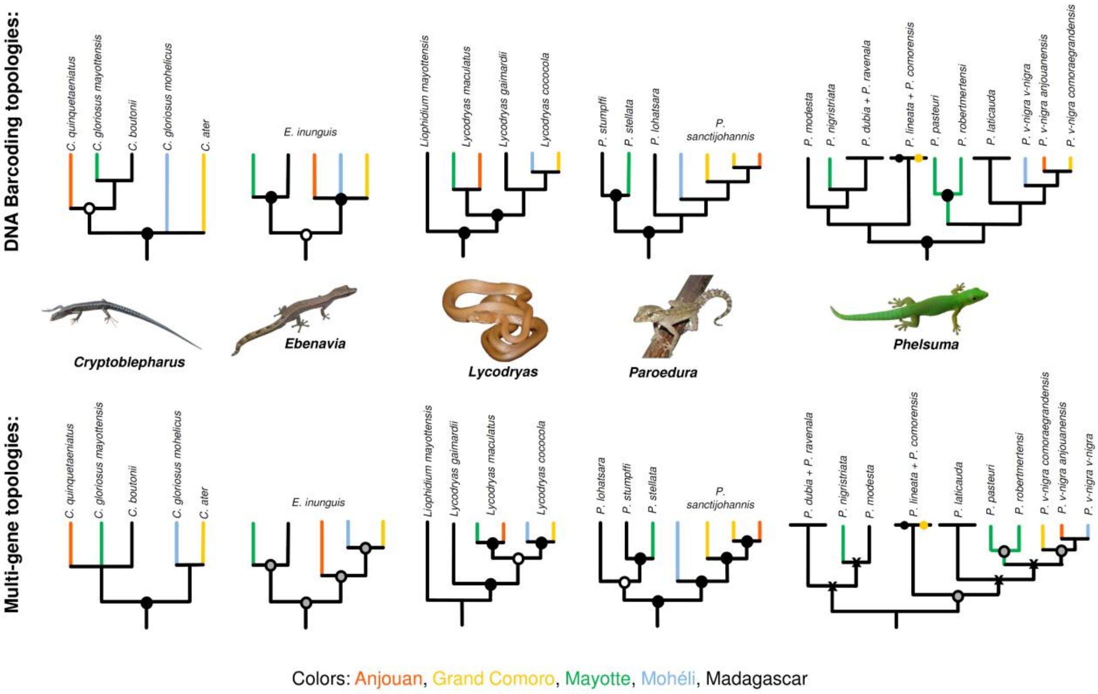


Figure 2: A comparison of topologies from our DNA barcoding analyses with topologies from multigene analyses for 5 genera of squamates with endemic Comoran lineages. Nodes with at least two values out of Bayesian PP, ML bootstrap, or Parsimony bootstrap support ≥ 90% are marked with filled black circles, nodes with at least one value out of Bayesian PP, ML bootstrap, or Parsimony bootstrap support ≥ 90% are marked with empty black circles. If only a single support value is available for the phylogeny, black circles filled with grey mark nodes with support values of ≥ 90%, and 'X' mark nodes with support values of ≥ 80%. The topologies were cropped to highlight lineages that are endemic to a single island, marked by color. Lineages that are present in the Comoros, but not endemic, are not highlighted. The multigene topologies are taken from the following studies: *Cryptoblepharus* [23], *Ebenavia* [44], *Lycodryas* [29], *Paroedura* [30], *Phelsuma* [25].

Tables

Family / Species	Samples	Vouchers	RepCOI	LCO/HCO	Total sequences	Success
Agamidae	2	2	2	0	2	100%
Agama agama	2	2	2	0	2	100%
Iguanidae	1	1	1	0	1	100%
Oplurus cuvieri	1	1	1	0	1	100%
Chamaeleonidae	10	7	10	4(4)	10	100%
Furcifer cephalolepis	6	4	6	2(2)	6	100%
Furcifer polleni	4	3	4	2(2)	4	100%
Typhlopidae	29	29	0	24	24	83%
Ramphotyphlops braminus	26	26	0	22	22	85%
Typhlops comorensis	2	2	0	2	2	100%
Typhlops sp.	1	1	0	0	0	0%
Lamprophiidae	14	14	14	2	14	100%
Liophidium mayottensis	1	1	1	0	1	100%
Lycodryas cococola	8	8	8	1	8	100%
Lycodryas maculatus	5	5	5	1	5	100%
Scincidae	52	52	26	9	26	50%
Amphiglossus johannae	25	25	18	9(9)	18	72%
Crytoblepharus boutonii	8	8	7	0(6)	7	88%
Trachylepis comorensis	18	18	1	0	1	6%
Trachylepis striata	1	1	0	0	0	0%
Gekkonidae	108	81	91	38	91	84%
Ebenavia inunguis	7	7	6	1	6	86%
Geckolepis maculata	12	12	10	9	10	77%
Hemidactylus frenatus	7	6	7	4	7	100%
Hemidactylus mercatorius	9	7	9	0	9	100%
Hemidactylus parvimaculatus	4	4	1	0	1	25%
Hemidactylus	14	14	14	0	14	100%

platycephalus						
Paroedura sanctijohannis	12	12	9	0	9	75%
Paroedura stellata	6	6	6	0	6	100%
Phelsuma comorensis	3	1	2	2(2)	2	67%
Phelsuma dubia	8	2	8	7(7)	8	100%
Phelsuma laticauda	8	1	7	5(7)	7	88%
Phelsuma nigristriata	2	1	2	1(1)	2	100%
Phelsuma pasteuri	2	2	1	0(0)	1	50%
Phelsuma robertmertensi	3	1	2	2(2)	2	67%
Phelsuma v-nigra	12	3	7	7	7	58%

Table 1: Samples used in the DNA barcoding analysis. Sequences of non-Comoran species (mostly from Madgascar; all taken from GenBank, with the exception of *Amphiglossus ardouini*) are not listed. The values given for families are sums of all species comprised. Samples: the total number of samples that were attempted to sequence. Vouchers: the number of samples for which a voucher specimen is available. RepCOI: the number of sequences that were obtained using the primer pair RepCOI-F/RepCOI-R [18]. LCO/HCO: the number of sequences that were obtained using the primer pair LCO/HCO [16]; the number of brackets lists the number of samples attempted to amplify with HCO/LCO, if different from the number given in "Samples". Total sequences: the total number of sequences obtained.

Family	Avg. distance between Comoran species	Avg. distance between Malagasy species (Nagy et al. 2012)
Chamaeleonidae	12.5 (11.6-13.9)	23.7
Typhlopidae	22.5 (22.0-23.0)	18.6
Lamprophiidae	14.1 (10.4-20.3)	20.2
Scincidae	26.8 (23.5-29.4)	22.2
Gekkonidae	28.5 (11.9-35.7)	29.8

Table 2: Genetic divergences within families of Comoran squamates. All genetic divergences are given as K2p-distances. Agamidae and Iguanidae are each represented by a single species only and are not shown.

Species	Max. overall K2p distance	Anjouan	Grand Comoro	Mayotte	Mohéli
Furcifer cephalolepis	2.8	-	2.8 (N=6)	-	-
Furcifer polleni	1.1	0 (N=1)	-	1.1 (N=3)	-
Ramphotyphlops braminus	0.2	0 (N=7)	0.2 (N=2)	0 (N=3)	0 (N=10)
Typhlops comorensis	8.1	0 (N=1)	0 (N=1)	-	-
Lycodryas cococola	6.6	-	0.8 (N=3)	-	0.5 (N=5)
Lycodryas maculatus	6.4	0.3 (N=3)	-	0.2 (N=2)	-
Amphiglossus johannae	6.8	0.5 (N=5)	0.2 (N=2)	0.5 (N=2)	1.4 (N=9)
Cryptoblepharus boutonii	5.3	1.9 (N=3)	0.2 (N=2)	0 (N=1)	0 (N=1)
Ebenavia inunguis	22.0	0 (N=1)	0.4 (N=2)	0 (N=1)	0 (N=2)
Geckolepis maculata	4.8	1.3 (N=5)	0 (N=2)	0.7 (N=2)	0 (N=1)
Hemidactylus frenatus	6.7	0.2 (N=3)	0 (N=1)	0 (N=1)	0 (N=2)
Hemidactylus mercatorius	6.5	2.5 (N=6)	0 (N=2)	0 (N=1)	0 (N=0)
Hemidactylus platycephalus	5.5	5.3 (N=8)	1.1 (N=3)	0 (N=1)	5.5 (N=2)
Paroedura sanctijohannis	8.2	0.4 (N=6)	4.1 (N=2)	-	0 (N=1)
Paroedura stellata	1.3	-	-	1.3 (N=6)	-
Phelsuma comorensis	0	-	0 (N=2)	-	-
Phelsuma dubia	1.3	0.7 (N=3)	0.4 (N=2)	0.9 (N=2)	0 (N=1)
Phelsuma laticauda	0.2	0 (N=6)	-	0 (N=1)	-
Phelsuma nigristriata	0.2	-	-	0.2 (N=2)	-
Phelsuma robertmertensi	0.9	-	-	0.9 (N=2)	-
Phelsuma v-nigra	9.4	4.3 (N=2)	0.4 (N=3)	-	0 (N=2)
Comoran *Phelsuma* radiation*	24.4	4.3 (N=2)	0.4 (N=3)	12.3 (N=3)	0 (N=2)
Comoran *Lycodryas* radiation**	10.4	0.3 (N=3)	0.8 (N=3)	0.2 (N=2)	0.5 (N=5)

Table 3: Maximum genetic divergences between and within island populations of Comoran squamates. All genetic divergences are given as % of K2p-distances. Species for which only a single sequence is available are not included. * This includes *P. v-*

nigra, *P. pasteuri* and *P. robertmertensi*. ** This includes *L. cococola* and *L. maculatus*.

Clustering threshold	No. clusters	No. clusters corresponding to taxonomy	Max. No. species per cluster	No. correct identifications by 'best closest match'
5%	37	20	1	116
6%	36	21	1	116
7%	34	23	1	117
8%	28	24	2	123
9%	28	24	2	123
10%	28	24	2	123
11%	28	24	2	123
12%	25	22	2	123*
13%	25	20	2	123*
14%	25	22	2	123*
15%	25	22	2	123*

Table 4: Results of the objective clustering analyses of species. Clustering was conducted in SpeciesIdentifier with arbitrary thresholds of 5% to 15%. The dataset used here contained 130 sequences belonging to 27 species. 6 species were represented by a single sequence. 124 sequences were correctly identified by the 'best match' criterion. *At these clustering thresholds, the 'best closest match' query criterion yielded 1 misidentification.

Clustering threshold	No. clusters	No. clusters corresponding to island population	Max. No. populations per cluster	No. correct identifications by 'best closest match'
0.2%	48	11	1	23
0.4%	41	18	1	34
0.6%	39	20	1	37
0.8%	34	23	1	42
1.0%	32	23	1	45
1.2%	31	24	1	46
1.4%	29	23	2	47*
1.6%	27	20	2	47**
1.8%	27	20	2	47**
2.0%	27	20	2	47**

Table 5: Results of the objective clustering analyses of island populations. Clustering was conducted in SpeciesIdentifier with arbitrary thresholds of 0.2% to 2.0%. The dataset used here contained 61 sequences belonging to 27 island populations of 9 native species. 9 island populations were represented by a single sequence. 50 sequences were correctly identified by the 'best match' criterion. At higher clustering thresholds, the 'best closest match' query criterion yielded 1 (*) or 2 (*) misidentifications.

Additional files

Table S1: A list of all samples included in the DNA barcoding study of Comoran squamates. The list includes voucher specimens, collecting details, and accession numbers for GenBank and BOLD.

5 Speciation in space and time

5.1 PAPER: The complex colonization history of nocturnal geckos (*Paroedura*) on the Comoros Archipelago.

While island biotas are often less species-rich and complex than continental biotas, they still show a high diversity of phylogenetic and biogeographic patterns. As introduced in Chapter 3.3, many earlier authors (reviewed in FUNK & WAGNER 1995) suggested that the colonization patterns of oceanic islands should often follow the stereotypic 'Progression Rule': Oceanic islands should be colonized by radiations of species whose colonization patterns followed the order of geological age and distance to the mainland of the islands. Most modern studies show that only few radiations follow the Progression Rule (FUNK & WAGNER 1995, WHITTAKER & FERNÁNDEZ-PALACIOS 2007, HEADS 2011); instead, they show that colonization patterns of oceanic islands are often almost unpredictable. This chapter describes the case study of *Paroedura sanctijohannis* GÜNTHER 1879, a nocturnal gecko endemic to the Comoros Archipelago. Previous to my study, authors assumed that this species was present on all four major islands of the archipelago (PARIS 1999, MEIRTE 2004). In HAWLITSCHEK (2008), I suggested that at least some of the island populations should be regarded as separate species that had emerged from a speciation process based on geographic separation. I backed this proposal with morphological data and a preliminary genetic screening that showed considerable divergences between the populations. However, due to the small sample size available then, and because no sequence data of any close outgroups was available, I failed to recognize that *P. sanctijohannis sensu* GÜNTHER (1879) and MEIRTE (2004) is paraphyletic and most likely represents two independent colonization events of the archipelago. The new results based on the data collected in my 2010 expedition to the Comoros are presented in this chapter.

HAWLITSCHEK O., GLAW F. (2012): The complex colonization history of nocturnal geckos (*Paroedura*) on the Comoros Archipelago. Zoologica Scripta 42, 135-150.

Post-publication comments and errata:

p. 1: This paper was published online in the Early View section of the journal website on 2012-11-22. It was printed in the journal issue of March 2013. The species description was published with LSID and Zoobank entry and is therefore valid even if published online only (ICZN 2012). Therefore, the question remains whether *Paroedura stellata* HAWLITSCHEK & GLAW, 2012 or *Paroedura stellata* HAWLITSCHEK & GLAW, 2013 is the correct name of the species. According to LYUBOMIR PENEV (managing director of Pnesoft Publishers; e-mail 2013-03-29), the date of the first (i.e., online) publication should be valid, but the question does not appear to be finally resolved. As long as no further information is available I consider 2012 as the year of publication of the species description and use the name *Paroedura stellata* HAWLITSCHEK & GLAW, 2012.

Zoologica Scripta

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The complex colonization history of nocturnal geckos (*Paroedura*) in the Comoros Archipelago

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Hawlitschek, O. & Glaw, F. (2012). The complex colonization history of nocturnal geckos (*Paroedura*) in the Comoros Archipelago. —*Zoologica Scripta*, *42*, 135–150.

Oceanic islands have attracted special attention from evolutionary biologists because their mostly species-poor, but highly endemic biota are exposed to selection regimes different to those of their mainland relatives. While many groups of oceanic islands worldwide have been used as natural laboratories of evolutionary biology, few such studies have been performed on the Comoros Archipelago in the Western Indian Ocean. We study *Paroedura sanctijohannis* Günther 1879, a nocturnal gecko endemic to this archipelago as only species of an otherwise Malagasy endemic genus. According to our phylogeny, *P. sanctijohannis* is not monophyletic, the population of the geologically oldest island Mayotte is clustering with related Malagasy species. We describe this population as *Paroedura stellata* sp.n. and provide morphological evidence distinguishing it from other *Paroedura* species. A molecular clock analysis shows that genetic divergence within *P. sanctijohannis* of the youngest island Grand Comoro is higher than expected based on its geological age. Additionally, this population is paraphyletic with respect to the population of the older island Anjouan, suggesting that the latter island was colonized long after its initial emergence, possibly after extinction of an original *Paroedura* population. Furthermore, we find that *P. stellata* sp.n. and *P. sanctijohannis* are more similar to each other than to other *Paroedura* species regarding adult coloration and juvenile coloration. Because these two species are not each other's closest relatives, we discuss possible explanations for this pattern and suggest that it represents convergent adaptation to a relaxed insular selection regime.

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Introduction

Oceanic islands have been recognized as the natural laboratories of evolutionary biology by generations of biologists (Darwin 1845; Mertens 1934; Boag & Grant 1981; Price & Clague 2002; Losos & Ricklefs 2009). They were found particularly interesting for biogeographic studies, as the ancestors of all organisms living on these islands must have arrived by crossing the sea, thus creating new assemblages of species often very different from those of the closest continental landmasses (MacArthur & Wilson 1963, 1967; Heaney 2007). In recent decades, oceanic islands have been routinely used as geological calibration points for the molecular dating of phylogenetic events, if geological age estimates based on K–Ar determinations were available (Brown & Pestano 1998; Thorpe *et al.* 2005; Rocha *et al.* 2007).

Studies on the phylogeny and molecular dating of island species and radiations often imply the underlying assumption that oceanic islands are colonized soon after their emergence, the age of island endemics in an archipelago corresponding to the geological ages of the islands and older islands serving as source for the colonization of younger ones (Brown & Pestano 1998; Juan *et al.* 2000; Heaney 2007; Rocha *et al.* 2007; Gillespie *et al.* 2012). In many cases, the same studies have found that such colonization patterns are often more complex than the simple 'island colonized as it emerged' pattern (Juan *et al.* 2000) and that groups of organisms that might have appeared as island radiations sometimes resulted from multiple independent dispersal events (Heaney 2007; Gillespie *et al.* 2012).

In contrast to other archipelagos of oceanic islands, such as the Galápagos, the Canaries or the Hawaiian islands, the Comoros archipelago in the Western Indian Ocean has been the target of relatively few studies in evolutionary biology (Rocha *et al.* 2005a, 2006, 2007; Fuchs *et al.* 2008; Louette *et al.* 2011). This is intriguing, as the Comoros are fully oceanic and have never had any connection with one another or any other landmass, are home to numerous endemic species in many organism groups (Louette *et al.* 2004) and consist of four major age-progressive islands (Mayotte, Anjouan, Mohéli, Grand Comoro) for which K–Ar age determinations are available (Hajash & Armstrong 1972; Montaggioni & Nougier 1981; Emerick & Duncan 1982; Nougier *et al.* 1986; Bachélery & Coudray 1993). Possibly, the scarcity of endemic taxa above species level (Louette *et al.* 2004; Hawlitschek *et al.* 2011), the short distance to continental Africa and Madagascar and the close biogeographic affinity to Madagascar may have worked to reduce the attractiveness of this archipelago to the scientific community.

Among the endemic species of the Comoros is the nocturnal gecko *Paroedura sanctijohannis* Günther, 1879. All other 15 recognized species and several candidate species of this genus are endemic to Madagascar, where it represents the most species-rich genus of nocturnal geckos (Nussbaum & Raxworthy 2000; Glaw & Vences 2007). Fossils of a probably extinct *Paroedura* species are known from the Aldabra atoll (Arnold 1976). *Paroedura* species are medium-sized geckos expressing varying degrees of arboreal to near fully terrestrial lifestyles. Only two species inhabit the otherwise diverse eastern rainforest regions of Madagascar. The majority of both the widespread and the microendemic species are known from the arid west (Glaw & Vences 2007). Several species have been bred in captivity for many generations (Manwaring 1992; Rösler 1998; Glaw *et al.* 2001) and are known to show juvenile coloration strikingly different from that of adults. The diverse juvenile colorations are a character of high taxonomic significance within the genus, although data are not available for all known species.

Günther (1879) described the genus *Paroedura* with the type species *P. sanctijohannis* from the Comoros Islands. The genus was later synonymized with *Phyllodactylus*, but resurrected by Dixon & Kroll (1974). Mocquard (1895) divided *Paroedura* into two groups based on the relative position of the nostril. In the *sanctijohannis* group, the nostril is in contact with the rostral scale, whereas in the *picta* group, the prenasal (or supranasal) scale is positioned between rostral and nostril. This division correlates, as based on the species known by then, with geography: species of the *picta* group inhabit the dry south and south-west, while those of the *sanctijohannis* group inhabit the wetter central west and north. This two-species-group concept was followed by Angel (1942) and Guibé (1956), but Nussbaum & Raxworthy (2000) pointed out that there was no evidence other than the nostril position for the monophyly of the two groups. A molecular study by Jackman *et al.* (2008) provided evidence that these two groups are indeed paraphyletic, although several smaller clades of species with the *sanctijohannis* or *picta* configuration, respectively, exist within the genus. Nussbaum & Raxworthy (2000) recognized the similarity between the Comoran *P. sanctijohannis* and the Malagasy *P. stumpffi* and speculated that the former might be just an isolated geographic race of the latter.

In this article, we test the hypothesis that Comoran *Paroedura* form a monophyletic group with a phylogeny that is congruent with the available geological age estimates of the Comoros Islands, that is, that the population of the oldest island Mayotte is the sister group to all other Comoran *Paroedura*, followed by the other islands in age-progressive order ('island colonized as it emerged'). To test this hypothesis, we provide a phylogeny of Comoran *Paroedura* populations and related species from northern Madagascar, describe a new species and discuss the consequences of our results for the colonization history of *Paroedura* on the Comoros. Additionally, we discuss the conservation status of Comoran *Paroedura* and the significance of the juvenile coloration in the genus.

Materials and methods

Data collection and morphological analyses

Morphological data and tissue samples were obtained from specimens collected in the field and stored at the Zoologische Staatssammlung München, Germany (ZSM). Additionally, we obtained morphological data from specimens of the collection at the Muséum national d'Histoire naturelle, Paris, France (MNHN). A list of all specimens studied is given in Table S1.

To detect the morphological variation between Comoran *Paroedura* populations, we studied 13 morphometric characters (and 15 ratios between these characters), 6 meristic characters and colour (adult and juvenile) of 33 specimens. Unfortunately, the holotype of *P. sanctijohannis* (BMNH 77.8.9.33) has been on overdue loan for many years and was not available for examination (C. McCarthy, pers. comm.), but other specimens from the type locality "Anjouan" were examined. To make comparisons easier, the terminology and abbreviations of characters largely follow Nussbaum & Raxworthy (2000). A list of all characters studied, with abbreviations, is given in Table S1. Snout–vent length and tail length were measured to the nearest millimetre, other morphometric characters to the nearest 0.1 mm, using a digital steel calliper. Meristic characters were examined visually, if necessary using a stereo microscope. Colour was considered a reliable character only if photographs or descriptions of living or freshly dead specimens were available.

To detect significant differences between *Paroedura* species, we used multivariate analysis of variance (MANOVA) in PAST version 1.55 (Hammer *et al.* 2001). A MANOVA allows testing for equality of the means of several multivariate samples. Given overall significance of the MANOVA result, pairwise significance tests with Hotelling's p for every pair of island populations are provided. Canonical variates analysis is employed to visualize the results. We show the results of two runs, one including *Paroedura* from all four Comoros islands and one additionally including *P. stumpffi* and *P. lohatsara* specimens. A third analysis was run to detect the possible significant differences between sexes. We repeated all analyses excluding snout–vent length and tail length to account for allometry. Data on distribution and ecology were taken from Hawlitschek *et al.* (2011), and a map was created using the methodology described there.

DNA extraction and sequencing

DNA was extracted from tissue samples of *Paroedura* using the standard protocols of the Qiagen DNA DNEasy Tissue and Macherey & Nagel NucleoSpin® 96 Tissue, kits. We amplified four mitochondrial gene loci, 12S rRNA (12S), 16S rRNA (16S), cytochrome oxidase subunit 1 (COI), and NADH dehydrogenase 4 (ND4), and two nuclear loci, the recombination activating gene 2 (Rag2) and the prolactin receptor (PRLR), of *Paroedura* specimens from the Comoros ($n = 20$) and from Madagascar ($n = 7$). The standard PCR protocol follows Hawlitschek *et al.* (2012); primer sequences and modifications of the standard PCR protocol are described in Table S2. Sequencing was carried out using the BigDye® TERMINATOR v1.1 Cycle Sequencing Kit on ABI 3730 and ABI 3130xl capillary sequencers. Sequence data were deposited in GenBank and are available under the accession numbers HE967762 to HE967923 (Table S3).

Analyses of molecular data

Our data set consisted of 3174 bp of mitochondrial (2231 bp) and nuclear (943 bp) markers. We included 20 specimens of Comoran *Paroedura* in our data set, representing altogether 8 localities on all 4 major islands of the Comoros archipelago. All localities but one were represented by at least two individuals. We also included samples of *P. lohatsara* and *P. stumpffi*, the sister species to *P. sanctijohannis* according to Jackman *et al.* (2008). Sequence data for outgroups *P. vazimba*, *P. masobe*, *P. gracilis*, *Ebenavia inunguis*, *Uroplatus lineatus* and *Hemidactylus frenatus* were partially retrieved from GenBank.

The sequence data were aligned in MAFFT 6 (Katoh *et al.* 2002; Katoh & Toh 2008) using the default settings of the software. We tested six different partition strategies for the concatenated data set. We either treated (i) all codon positions of protein-coding markers separately, (ii) treated only the 3rd codon position as separate or (iii) lumped all codon positions. For each of these three patterns, we treated either (i) protein-coding mitochondrial, ribosomal mitochondrial and nuclear markers, or (ii) all markers as separate partitions. To identify appropriate substitution models for each partition, we used JMODELTEST 0.1.1 (Posada 2008). We assessed AIC and BIC results, giving BIC preference over AIC. Subsequently, we conducted a Bayes factor test in MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003) on the CIPRES PORTAL 2.2 (Miller *et al.* 2010) and TRACER v1.5 (Rambaut & Drummond 2007). Pairwise distances were calculated in MEGA 5.0 (Tamura *et al.* 2011).

We conducted maximum-likelihood analyses with 1000 bootstrap repeats in RAXMLGUI 1.0 (Stamatakis 2006; Silvestro & Michalak 2011) and Bayesian analyses in MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003) on the CIPRES PORTAL 2.2 (Miller *et al.* 2010), with two runs and four chains with 30 000 000 generations (sample freq = 1000, conservative 25% burnin). MRBAYES runs were checked for convergence and normal distribution in TRACER v1.5 (Rambaut & Drummond 2007). Finally, we conducted parsimony analyses in TNT 1.1, with 1000 jackknife (removal 36%) replications (Goloboff *et al.* 2005; hit best tree 5 times, keep 10 000 trees in memory). Additionally, we ran Bayesian analyses as described above for data sets of single markers. As an alternative to gene trees, we used coalescent-based species tree inference models in *BEAST v. 1.6.1 (Heled & Drummond 2010). *BEAST requires a priori designation of species, which we performed using the results of our gene tree phylogeny and morphological analyses. We conducted 2 runs over 50 000 000 generations (sample freq = 1000 and 10% burnin) and checked for convergence and normal distribution in TRACER v1.5 (Rambaut & Drummond 2007). Finally, we conducted a Shimodaira–Hasegawa Test in PAUP 4.0b10 (Shimodaira & Hasegawa 1999; Swofford 2003) to compare the topology resulting from our analyses with a topology based on our initial hypothesis that all Comoran *Paroedura* form a monophyletic clade.

To infer divergence dates, we used BEAST v.1.6.1. (Drummond & Rambaut 2007). Age estimates for the Comoros Islands are available and could thus be used for calibration of the BEAST run (Hajash & Armstrong 1972; Montaggioni & Nougier 1981; Emerick & Duncan 1982; Nougier *et al.* 1986; Bachélery & Coudray 1993). However, as discussed below, it is debatable whether the geological age of an island can be used as a calibration point for the phylogeny of Comoran *Paroedura*. Therefore, instead of using geological calibration points, we applied a different approach in which the substitution rate and standard error of the 12S marker (0.00755 ± 0.00247) were inferred from fully calibrated lizard phylogenies and used as an informative prior, following Carranza & Arnold (2012).

To provide comparability with other studies using the integrative IPC (Integration by Partial Congruence) approach (Miralles *et al.* 2011; Vasconcelos *et al.* 2012), we constructed haplotype networks using statistical parsimony (Templeton *et al.* 1992) in the software TCS v1.21 (Clement *et al.* 2000) with a connection limit of 95%. This approach produces haplotype networks with independent clusters considered distinctive for recognizing evolutionary significant units (ESUs). Instead of cytochrome b as in Miralles *et al.* (2011) and Vasconcelos *et al.* (2012), which was not available for our study group, we used a concatenated data set of all mitochondrial markers used in our study, following Gómez-Díaz *et al.* (2012). Additionally, we constructed median-joining haplotype networks (Bandelt *et al.* 1999) of the Rag2 and PRLR genes in NETWORK 4.6.1.0 (Fluxus Technology Ltd 2004–2012).

To evaluate the question whether the similar adult coloration and cryptic juvenile coloration of all Comoran *Paroedura* are an ancestral or derived character, we used maximum parsimony and maximum-likelihood ancestral character state reconstruction in MESQUITE v2.72 (Maddison & Maddison 2009).

Integrative taxonomy

In our integrative approach to delimit species, we follow Miralles *et al.* (2011) and Vasconcelos *et al.* (2012). These authors used three equivalent, independent and combinable lines of evidence for taxon recognition and delimitation: mtDNA (independent parsimony networks of a mtDNA marker with 95% connection limit), nDNA (absence of shared haplotypes of a nDNA marker) and morphology (detection of at least one fixed character state: presence/absence, non-overlapping values or combination of characters). We followed these authors in assessing Rag2 as nDNA marker.

Results

Phylogeny of P. sanctijohannis and its relatives

According to the jModelTest results, we selected the following substitution models: HKY for Rag2, HKY + G for 12S, ND4 and PRLR, HKY + I + G for COI and GTR + G for 16S. The results of the Bayes factor test favoured the most complex partition strategy in which all molecular markers, and all three codon positions of protein-coding markers, were treated as a total of 14 partitions.

The phylogram of the studied taxa of *Paroedura*, based on the entire data set, including age estimates from the BEAST analysis, is given in Fig. 1. It displays two clearly supported ingroup clades. The first represents *P. lohatsara*, *P. stumpffi* and *P.* "*sanctijohannis*" from Mayotte and the second *P. sanctijohannis* specimens from Grand Comoro, Anjouan and Mohéli. In the first clade, *P. stumpffi* from Madagascar and *P.* "*sanctijohannis*" from Mayotte each and both together form a monophyletic unit, and subclades of *P. stumpffi* are well supported. In the second clade, specimens from Mohéli form a monophyletic group distinguished from all other specimens. A single Grand Comoro individual (ZSM 105/2010), representing a previously unknown and isolated locality, is the sister group to the remaining specimens, and the remaining Grand Comoro specimens form a sister clade to all Anjouan specimens. The results of the SH test support that this topology is highly significantly better ($P < 0.001^{**}$) than a topology in which all Comoran *Paroedura* form a monophyletic clade.

Genetic distances of all markers analysed are given in Table 1. The TCS analysis reveals seven independent clusters within our data set (Fig. 1). *Paroedura* from Mayotte and Mohéli and *P. lohatsara* each form a cluster. All specimens from Grand Comoro and Anjouan cluster together. Three clusters are detected within *P. stumpffi*. Haplotype networks of nDNA markers, as shown in Fig. 2, broadly support the results of the phylogeny and the mtDNA network analyses.

Morphological analyses

Multivariate analyses of morphometric characters show that *Paroedura* species can be distinguished based on their morphometry, as shown in Fig. 3. The analysis including Comoran *Paroedura*, *P. stumpffi* and *P. lohatsara* yielded highly significant results ($P = 1.084\mathrm{E}{-}18^{**}$). The plot shows that both Malagasy species form clusters very distant from the Comoran forms, while the latter cluster closely. This contrasts with the results of the molecular phylogeny, in which *Paroedura* from Mayotte appear more closely related to *P. stumpffi* than to *Paroedura* from other Comoran islands. The characters with the highest loadings are snout–vent length (axis 1: −0.29, axis 2: +1.46), tail length (axis 1: −0.30, axis 2: +0.40), distance from eye to ear opening (axis 1: −0.24, axis 2: +0.17) and body width (axis 1: −0.08, axis 2: 0.49). In a second analysis excluding the Malagasy species, the specimens from Mayotte and Mohéli each formed separate clusters, while specimens from Anjouan and Grand Comoro clustered with each other. This is congruent with the results of the molecular phylogeny. The results are significant ($P = 0.0013^{*}$). Here, the characters with the highest loadings are snout–vent length (axis 1: −0.67, axis 2: −2.00), body width (axis 1: −0.60, axis 2: −0.15), axilla–groin-length (axis 1: −0.47, axis 2: −0.15) and snout length (axis 1: −0.06, axis 2: −0.71). Both analyses also yielded significant results and a similar cluster structure if snout–vent length and tail length were excluded. The analyses searching for differences in morphometry between sexes, both with and without snout–vent length

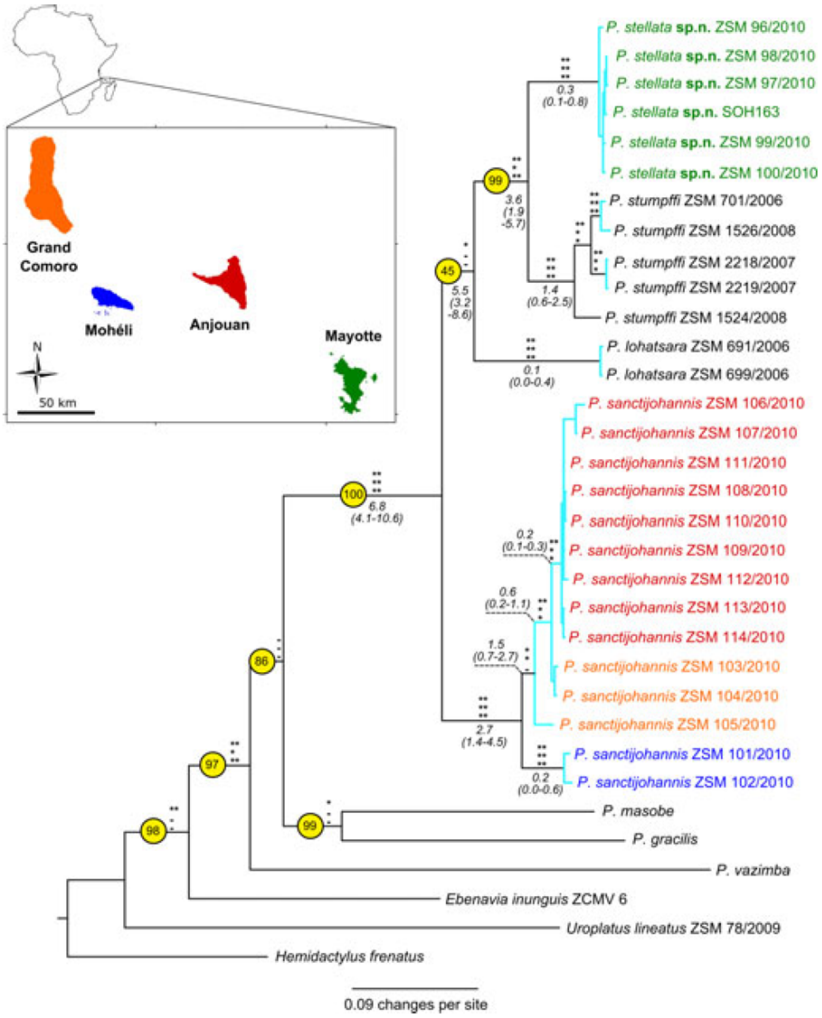

Fig. 1 Phylogram of *Paroedura* species. The gene tree is based on a data set comprising 3174 bp. Numbers in yellow circles represent *BEAST species tree posterior probabilities. Asterisks above nodes denote support values of 100 (**), 90 or more (*) or below 90 (–), with MRBAYES pp (top), RAXML bootstrap (middle) and TNT Jackknife (bottom). Italic numbers below nodes are mean age estimates in million years with 95% confidence intervals in brackets. Geographic affiliations: green, Mayotte; blue, Mohéli; orange, Anjouan; yellow, Grand Comoro; black, Madagascar. Cyan colour of branches marks clades that were retrieved as independent clusters in the TCS analysis.

Table 1 Molecular divergence of *Paroedura* species (p-distances were calculated in MEGA 5)

	12S	16S	COI	ND4	Rag2	PRLR
Within *P. sanctijohannis*	0.0–0.064	0.0–0.031	0.0–0.076	0.0–0.066	0.0–0.003	0.0–0.002
Within *P. stellata* sp.n.	0.0–0.012	0.0–0.004	0.0–0.010	0.0–0.006	0.0	0.0
P. sanctijohannis – *P. stellata* sp.n.	0.113–0.125	0.088–0.100	0.169–0.179	0.106–0.122	0.005–0.008	0.022–0.024
P. stellata sp.n. – *P. stumpffi*	0.061–0.078	0.051–0.063	0.123–0.138	0.0778–0.100	0.005–0.008	0.002
Within *P. sanctijohannis* from Grand Comoro	0.0–0.023	0.0–0.022	0.047	0.034	0.0	0.0
Within *P. sanctijohannis* from Anjouan	0.0–0.003	0.0	0.0–0.004	0.0	0.0	0.0–0.003
Within *P. sanctijohannis* from Mohéli	0.003	0.002	0.0	0.016	0.002	0.0

and tail length, yielded neither significant results nor clearly separated clusters.

Table 2 lists the characters diagnostic to distinguish *P. stumpffi*, *P. lohatsara*, *Paroedura* from Mayotte and *Paroedura* from other Comoran populations. All morphological data are listed in Table S1. Differences in diagnostic characters were detected neither between *Paroedura* from Mohéli, Anjouan and Grand Comoro nor between sexes of any population.

Taxonomy

The results of our integrative approach suggest that the taxonomy of Comoran *Paroedura*, all of which have previously been treated as a single species *P. sanctijohannis*

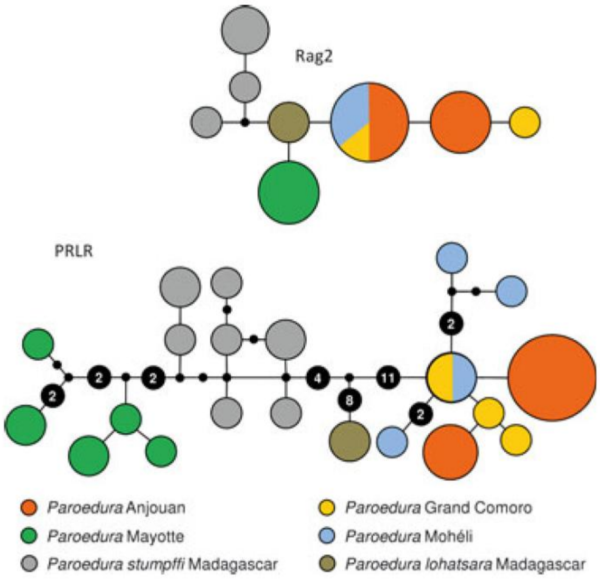

Fig. 2 Haplotype networks of nDNA markers, created in NETWORK. Circles represent haplotypes, their size correlating with the number of individuals sharing this haplotype (given as number in the centre of the circle). Colours code *Paroedura* species and populations. Connecting lines represent each one mutation, black dots or filled circles with numbers represent additional mutational steps.

Günther, 1879, is in need of revision. The TCS analysis shows that there are four independent clusters within Comoran *Paroedura*, one endemic to the island of Mayotte, one to Mohéli and one shared between Grand Comoro and Anjouan. The fourth lineage is represented by a single specimen from Grand Comoro. This line of evidence alone suggests that *P. sanctijohannis* should be divided into four taxa of at least subspecies status. Notably, according to our molecular phylogeny, *P. sanctijohannis* is not monophyletic: the populations of Grand Comoro, Anjouan and Mohéli form a monophyletic sister group to the clade encompassing *P. stumpffi*, *P. lohatsara* and *Paroedura* from Mayotte.

The analysis of nDNA markers supports the taxonomic splitting of Comoran *Paroedura*. No haplotypes are shared between *P. stumpffi*, *P. lohatsara*, *Paroedura* from Mayotte and *Paroedura* from other Comoran populations. Morphological characters also support the splitting of Comoran *Paroedura*. The multivariate analysis of morphometric characters yields three clusters: Mayotte, Mohéli and Grand Comoro + Anjouan. Additionally, specimens from Mayotte are distinguished from other Comoran *Paroedura* by a set of fixed characters (Table 2).

This summary shows that the recognition of *Paroedura* from Mayotte as a species different from *P. sanctijohannis* is clearly supported by all three lines of evidence, as well as by the molecular phylogeny.

Genus Paroedura Günther, 1879

Paroedura stellata sp.n.

Fig. 4

Species-ID page. http://species-id.net/wiki/Paroedura_stellata.

LSID for this journal article. urn:lsid:zoobank.org:pub:28D9F304-F4DD-47F5-A36A-2E15F53AC91E.

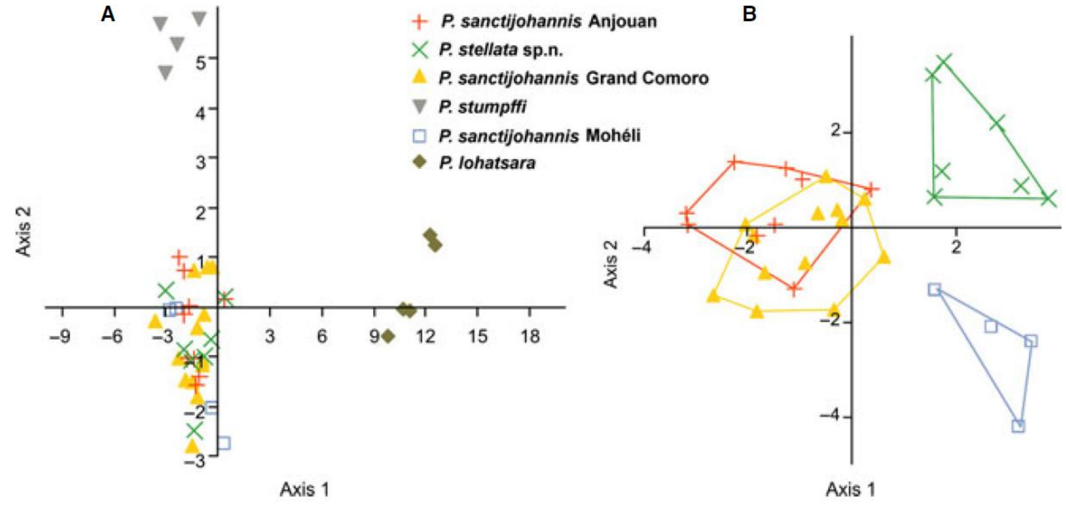

Fig. 3 Canonical variates analysis plot of morphometrical data of *Paroedura* species. 'A' shows the plot of an analysis including Comoran *Paroedura*, *P. stumpffi* and *P. lohatsara*, 'B' shows an analysis of Comoran *Paroedura* only. Measurements included are SVL, snout–vent length; TL, tail length; BW, body width; HL, head length; HH, head height; ED, eye diameter; SL, snout length; EE, distance from eye to ear; DE, distance between eyes; HW, head width; FLM, forelimb; HLM, hindlimb; AG, axilla–groin distance.

Table 2 Morphological variation of Paroedura species. All characters except Juvenile colour and all sample sizes refer to adult specimens

	P. stellata sp.n. (*n* = 7)	*P. sanctijohannis* (*n* = 26)	*P. stumpffi* (*n* = 4)	*P. lohatsara* (*n* = 6)
SVL	50.6 ± 8.1 (34.8–61.6)	56.5 ± 9.7 (34.6–70.0)	65.9 ± 6.5 (59.3–74.9)	73.01 ± 3.0 (69.0–77.5)
LTUB	28.7 ± 1.1 (28–31)	34.5 ± 2.3 (31–40)	26.8 ± 1.9 (24–28)	25.2 ± 0.8 (24–26)
Tubercle form	Tetrahedral to spiny	Oval to tetrahedral	Tetrahedral to spiny	Tetrahedral to spiny
Neck spines	Strong	Weak	Strong	Strong
GSN	1.14 ± 0.4 (1–2)	2.73 ± 0.5 (1–3)	1.25 ± 0.5 (1–2)	1
Adult colour	Brown with bright transversal bands, diffuse pattern on head, sometimes blackish with white spots and without bands	Brown with bright transversal bands, diffuse pattern on head	Brown with bright transversal bands	Brown to ochre with mosaic of blackish spots and poorly defined transversal bands
Juvenile colour	Brown with broad brighter transversal bands, poor contrast, transversal bands not extending down the flanks, brown to yellowish tail	Brown with broad brighter transversal bands, poor contrast, transversal bands extending down the flanks, brown to yellowish tail	Dark brown with whitish transversal bands, high contrast, bright orange tail, head with pattern of brighter stripes, stripe at occiput without frayings	Blackish with white transversal bands, high contrast, bright orange tail, head with pattern of brighter stripes, stripe at occiput with frayings

SVL, snout–vent length (mm); LTUB, longitudinal count of tubercles on central dorsal row; GSN, granular scales on nose (between supranasals).

LSID for this species. urn:lsid:zoobank.org:act:CD10D431-716A-4CCE-98DA-101C47FA19A2.

Available names No available names. Previously considered conspecific with *P. sanctijohannis* Günther, 1879.

Holotype. ZSM 99/2010 (FGZC 4039), adult male with original tail, collected at Mont Bénara (S12.8802, E45.16428, 624 m a.s.l.), MAYOTTE, Comoros archipelago, on 14 April 2010 by O. Hawlitschek, J. Berger and B. Brückmann.

Paratypes. All from MAYOTTE. ZSM 1694/2008 (no field number), adult male, collected at the summit of Mont Bénara (S12.8801, E45.1623, 653 m a.s.l.), on 23 March 2008 by O. Hawlitschek; ZSM 2/2010 (no field number), adult male, collected at Sazilé (S12.9725, E45.1909, 7 m a.s.l.), on 11 April 2010 by O. Hawlitschek, J. Berger and B. Brückmann; ZSM 96/2010 (FGZC 4033), juvenile, collected at Choungui (S12.9578, E45.1341, 481 m a.s.l.), on 10 April 2010 by O. Hawlitschek, J. Berger and B. Brückmann; ZSM 97/2010 (FGZC 4037), adult male, collected at Sazilé (S12.9687, E45.1814, 15 m a.s.l.), on 11 April 2010 by O. Hawlitschek, J. Berger and B. Brückmann; ZSM 98/2010 (FGZC 4036), adult male, collected at Sazilé (S12.9692, E45.1821, 13 m a.s.l.), on 11 April 2010 by O. Hawlitschek, J. Berger and B. Brückmann; ZSM 100/2010 (FGZC 4040), adult male, collected at Mont Bénara (S12.8779, E45.1599, 553 m a.s.l.), on 14 April 2010 by O. Hawlitschek, J. Berger and B. Brückmann; ZSM 12/2011 (no field number), juvenile, bred in captivity, parents from Choungui, ZSM 51/2011 (no field number), adult female, collected at Choungui (S12.9578, E45.1341, 481 m a.s.l.), on 10 April 2010 by O. Hawlitschek, J. Berger and B. Brückmann; ZSM 339/2012 (no field number), adult female, bred in captivity, hatched on 20 April 2010, inventorized by O. Hawlitschek; MNHN 1994–813; MNHN 1994–814; MNHN 1887–32, MAYOTTE, collected on 1 March 1887 by L. Humblot (no further information is available for the MNHN specimens); and 3 further uncatalogued specimens kept alive in the vivarium that will be catalogued in the ZSM *postmortem* (an adult female collected at Mont Choungui and its offspring, hatched after 27 April 2010 in captivity).

Etymology. The specific name is the feminine form of the Latin adjective "stellatus" and means "set with stars" or "starry." It refers to the bright white dots that are evident (in varying degree) in juveniles and adults of this species.

Diagnosis. Paroedura stellata differs from *P. sanctijohannis* by a lower longitudinal count of tubercles on the central dorsal row from neck to tail base (28–31 in *P. stellata* sp.n. vs. 31–40 in *P. sanctijohannis*, see Table 2), in having only 1 (exceptionally 2) granular scale between the supranasals rather than 2–3 (exceptionally 1) and in the pointed to spiny form of its tubercles at the back of the head, which are flat and smooth in *P. sanctijohannis* (Fig. 5). Adult coloration of the two species is often very similar, but *P. stellata* may express various degrees of the highly characteristic black-and-white "contrasting" colour pattern described below. The bright transversal bands on the dorsum of juveniles of *P. stellata* sp.n. do not extend down the flanks, while in *P. sanctijohannis* juveniles, particularly the second anterior band extends to the ventral side.

Fig. 4 Photographs of Comoran and related Malagasy *Paroedura*. A: Holotype of *P. stellata* sp.n. (ZSM 99/2010). B: *P. stellata* sp.n. (paratype ZSM 97/2010), showing the contrasting colour pattern. C: *P. stellata* sp.n. (paratype ZSM 98/2010), showing the common "cryptic" colour pattern. D: *P. sanctijohannis* (ZSM 110/2010) from Anjouan. E: *P. lohatsara*, Montagne des Francais, Madagascar. F: *P. stumpffi*, Montagne des Francais, Madagascar. G: Juvenile *P. stellata* sp.n. H: Juvenile *P. sanctijohannis*, Grand Comoro. I: Juvenile *P. stumpffi* (left) and *P. lohatsara* (right), bred in captivity. Photographs A–D, G, H by O. Hawlitschek, E, F, I by F. Glaw.

Paroedura stumpffi and *P. lohatsara* are larger than *P. stellata* sp.n., attaining maximum SVL of 70 and 77 mm (vs. 62 mm), respectively. In *P. stumpffi*, the longitudinal count of tubercles on the central dorsal row is lower than in *P. stellata* sp.n. (24–26 vs. 28–31), and adults have a clearly defined brown stripe above the supralabials, which is diffuse or absent in *P. stellata* sp.n. In *P. lohatsara*, the nostril is not in contact with the rostral, the longitudinal count of tubercles on the central dorsal row is lower than in *P. stellata* sp.n. (24–26 vs. 28–31), and adults show a colour pattern of ochre with blackish reticulations. In both species, but not in *P. stellata* sp.n., the anteriormost dorsal transversal band extends down the neck, reaching the ventral side. Juveniles of *P. stumpffi* and *P. lohatsara* display a highly contrasting colour pattern, much brighter than *P. stellata* sp.n., with transversal bands extending down the flanks.

Paroedura stellata sp.n. is distinguished from species of the "*picta* group" by the position of the nostril. In the "*picta* group" species, *P. androyensis*, *P. bastardi*, *P. ibityensis*, *P. picta* and *P. vahiny*, the nostril is excluded from contact with the rostral. *Paroedura vazimba* resembles *P. stellata* sp.n. in the position of the nostril and adult coloration, but has a higher count of supralabial scales (13–19 fide Nussbaum & Raxworthy 2000, vs. 9–10 in *P. stellata* sp.n.). The remaining described species, *P. gracilis*, *P. homalorhina*, *P. karstophila*, *P. maingoka*, *P. masobe*, *P. oviceps* and *P. tanjaka*, do not show adult colour patterns resembling *P. stellata* sp.n. Furthermore, *P. stellata* sp.n. differs from other *Paroedura* species by genetic characters (see Figs 1 and 2, Table 1 and Jackman *et al.* 2008).

Description of the holotype. Specimen well preserved, with complete original tail. Head wider than neck, about as wide as body. Snout angled downward to tip, slight depression between prominent canthal ridges. Ear opening is a vertical slit. Tail about as long as snout–vent length, nearly round in cross section, with sharply pointed tip and prominent, spiny tubercles; ventral pygal section with pair of postcloacal sacs. Digits moderately expanded at tips.

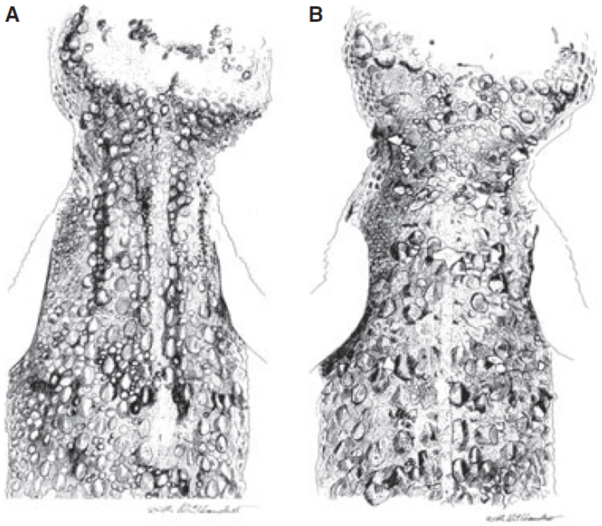

Fig. 5 Drawing of the neck regions of Comoran *Paroedura*. A: *P. sanctijohannis* (ZSM 110/2010) with smaller, mainly oval, tubercles. B: *P. stellata* sp.n. (ZSM 99/2010) with larger, mostly tetrahedral, sometimes spiny, tubercles. Drawings by R. Kühbandner.

Scalation. Rostral scale rectangular, wider than tall, as wide as mental. Nostril in contact with rostral. First supralabial slightly larger than others, labials smooth. Dorsolateral neck and body scales very heterogeneous with about 12 distinct longitudinal rows at mid-body of enlarged, spiny, mainly tetrahedral tubercles separated mostly by small flat scales and smaller tubercles; row along vertebral column with 29 tubercles. Tubercles in occiput and neck regions particularly enlarged and pointed, resembling thorns. Dorsal scales of forelimbs flat or tuberculate and weakly imbricate. Dorsal scales of hindlimbs slightly larger and more strongly tuberculate, much smaller above knee joint. Ventral scales of forelimbs and hindlimbs slightly smaller than surrounding ventral scales of the body. Dorsal pygal scales similar to dorsal body scales; lateroventral pygals tuberculate but less spinous. First 20 postpygal tail segments each with transverse row of spiny tubercles dorsolaterally; first 4 rows with 8 tubercles; after 11 rows, only 2 tubercles remain; posterior tail with flat scales. Mental triangular, bordered posteriorly by a pair of elongate, irregular hexagonal postmentals. Postmentals contact mental, first infralabial, one enlarged lateral gular, one smaller posterolateral gular and one slightly larger central gular. First three infralabials slightly larger than others. Gulars small, granular. Ventrals of chest and abdomen flat, posterior abdominals 2–3 times larger than anterior ones. Subdigitals in rows of 2. Pair of squarish terminal pads, each pad about 1 mm across. Claws curving downwards between terminal pads of digits.

Coloration in life. Base colour of dorsal side brown, head and tail slightly beige to greyish, tail with diffuse bands of lighter grey. Brighter dorsal line along vertebral column from occiput to tail base (Fig. 4A). Four faintly visible brighter transversal bands of 2–4 mm width each one situated in axillar and groin regions, the other two in between, all bordered by slightly darker areas. Overall pattern of grey and brown rather diffuse. Body from occiput to tail base and limbs, with scattered, clearly defined, bright white spots covering a single scale or a small cluster of scales. Spots on the bright line along the vertebral column sometimes elongate. Ventral side whitish, brighter anteriorly and darker posteriorly, mainly due to darker skin between scales. Most scales covered by one to ca. 20 very small dark spots. Ventral side of tail slightly darker than body, gradually darkening towards the tail. Rostral scale complete, surrounding scales partly darker brown, supraorbital regions greyish. Pupil black, in form of vertical slit. Iris silvery to golden with black veins. Tongue dark at the tip.

Variation. Morphometric and meristic variation are summarized in Table 2. No significant differences in colour, scalation and morphometrics could be detected between sexes; however, only one adult female specimen was available for analysis. As in all known species of *Paroedura*, males and females can easily be distinguished by the pair of postcloacal sacs at the tail base of males. The most striking variation within *P. stellata* is the occurrence of two completely different adult colour patterns even within a single population, although intermediate forms between the two colour patterns exist, as represented by the holotype ZSM 99/2010.

The "cryptic" colour pattern resembles the pattern shown by *P. sanctijohannis* and appears to provide better camouflage. We describe this colour pattern based on the specimen ZSM 98/2010: Dorsal base coloration brown, resembling that of the holotype. Bright white spots and patterns absent. Four bright brown to beige bands on the dorsum, partly extending down the flanks, bordered by darker brown. Anteriormost band in axillar region, posteriormost in groin region. Head with bright markings in the supraorbital, labial and temporal regions. Ventral and tail coloration resembling holotype.

The "contrasting" colour pattern is less common than the "cryptic" pattern, but is highly characteristic for this species. We describe this colour pattern based on the specimen ZSM 97/2010, which shows the most striking expression of this pattern. Dorsal base coloration dark brown to blackish. Dorsal side of body, head and limbs scattered with clearly defined, bright white spots comprising one or few scales. Spots along the rows of dorsal tubercles sometimes elongate. Rostral scale light brown. Bright markings on head absent; supralabial, supraorbital and temporal regions all with dorsal colour pattern described above. Ventral coloration as in holotype. Tail missing.

The juvenile colour pattern is not as divergent from the adults as in most other species (Fig. 4G). Dorsal base coloration brown. Four light brown to greyish transversal bands on the back, with an elongate central region and two similarly elongate "wings" reaching the flanks but not extending downwards. Flanks with beige to whitish spots encompassing one single or few tubercles. Limbs dark greyish to brownish, but lighter than dorsum, manus and pes even lighter. Base colour of head slightly lighter than body. Region between rostral and orbitals, as well as occiput region, lighter greyish to brownish, similar to transversal bands on dorsum. Rostrum and labials brown. Supraorbital region dark greyish. White spots, as on flanks of body, present on sides of head posterior to eyes. Ventral side of head and body brown, lighter than dorsal side. Tail yellowish–brown, lighter and darker rings alternating.

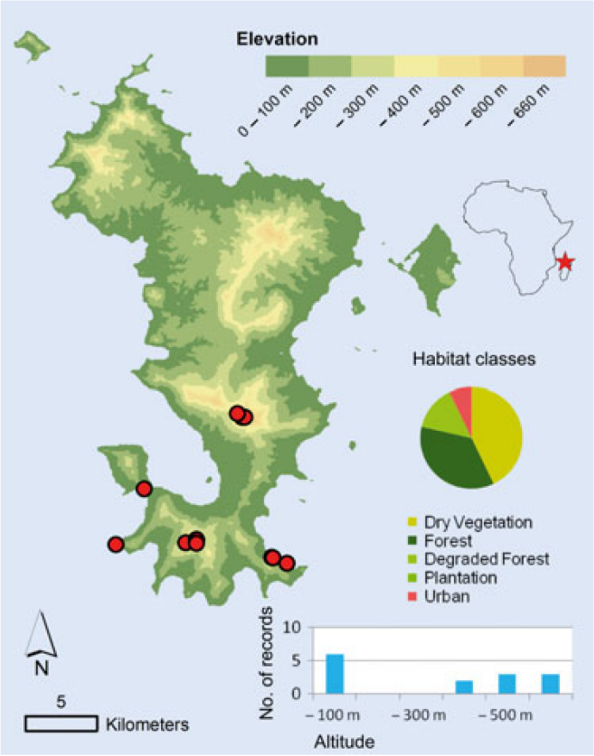

Fig. 6 Map showing the known distribution of *P. stellata* sp.n. on Mayotte Island. Red dots represent localities. The inlays show the position of Mayotte (red star) in relation to the African continent and distribution over habitat classes and altitude levels. The map was created in ArcGIS 10.

Distribution, habitat and habits. Paroedura stellata sp.n. is endemic to Mayotte island. A distribution map of the known localities is given in Fig. 6. So far, no records are known from the northern half of the island. The species has colonized virtually the entire altitudinal range and a variety of habitats on the island (Carretero *et al.* 2005; Hawlitschek *et al.* 2011). However, a concentration in three core distribution areas known so far could be observed. The first is Mont Bénara in the centre of Mayotte, being the island's point of highest elevation, where three individuals were observed on the trunks of trees at altitudes of 553, 624 and 660 m. All specimens were found in pristine humid forest, supporting the earlier hypothesis of Hawlitschek (2008) that *P. sanctijohannis* (including *P. stellata* sp.n.) were closely tied to natural forests. The second is Mont Choungui in the south of the island, where three specimens were discovered at altitudes of 368, 481 and 534 m, two of them on the ground and one on the base of a tree trunk. This mountain is also covered by humid natural forest, with mesic tendencies expressed especially in higher altitudes (Pascal 2002). At these two localities, no other nocturnal geckos could be observed in sympatry, although diurnal *Phelsuma* species were common (Meirte 1999; Brückmann *et al.* unpublished data). The third principal locality is the dry forest of the Saziley peninsula. This forest is rather open and partly degraded by banana plantations and, aside from *P. stellata* sp.n., is inhabited by large numbers of other geckos: the nocturnal invasive *Hemidactylus platycephalus*, the also nocturnal *Geckolepis* cf. *maculata* and the diurnal invasive *Phelsuma laticauda*. We found three specimens close to sea level at 7-, 13- and 15-m elevation, all on the stony forest floor, while other geckos were observed only on vertical vegetative structures. All specimens were observed active at night.

Reproduction. Two female *P. stellata* sp.n., one gravid with two eggs, were captured in April 2010 and kept temporarily in a transport plastic vivarium and subsequently transferred to a glass vivarium. Still in the temporary vivarium, the gravid female buried two eggs in the slightly humid soil consisting mainly of humus and leaf fragments. Eggs had a white calciferous shell and were neither glued to each other nor to the substrate. Both measured 10.0 × 7.9 mm. They were incubated at a constant temperature of 28 °C. After 56 and 63 days, 2 juveniles hatched, measuring 36 and 37 mm. The presence of a gravid female in April, at the beginning of the dry season, does not correspond to the observation by Glaw & Vences (1996) of the peaks in reproduction of many Malagasy squamates corresponding with the beginning of the rainy season. Similar to *P. sanctijohannis*, a year-round reproduction cannot be excluded in *P. stellata* sp.n.

Conservation. *Paroedura sanctijohannis* was included in the Comoros and Madagascar reptile assessment of 2011 and assigned the status of "Endangered (B1ab (iii) + 2ab (iii))" (Hawlitschek & Glaw 2011) based on the data from Hawlitschek *et al.* (2011). A study by El-Yamine (2012) suggests that *P. sanctijohannis* may be more common than previously thought, at least in Anjouan. *Paroedura stellata* sp.n. is limited to a much smaller range, but benefits from conservation management. Based on the data from Hawlitschek *et al.* (2011) and additional locality data by H.-P. Berghof (pers. comm., 26 November 2011: S12.9608, E45.0834), the Area of Occupancy (AOO) according to IUCN (2004) was estimated to be 21.7 sqkm, well below the threshold of 500 sqkm for the assessment as "Vulnerable," but still above the threshold of 10 sqkm for "Critically Endangered." The pristine forest areas are legally protected, and in some places, this species is commonly observed in syntopy with introduced *Hemidactylus* geckos, considered a main threat for *P. sanctijohannis*. Due to ongoing growth of the human population in rural areas, in combination with repeated phases of politic instability, the current legal protection measures may not be sufficient to warrant long-term suitability of the habitats of *P. stellata* sp.n. Due to its already small and fragmented AOO, further declines in the extent and the quality of its habitats may qualify this species for the status "Critically Endangered." Therefore, *P. stellata* sp.n. would qualify for the IUCN status "Vulnerable" under criterion D2. We propose to consider these arguments when assessing *P. stellata* sp.n. for a future update of the IUCN Red List.

Discussion

The close relationship between *P. sanctijohannis*, *P. stumpffi* and partly also *P. lohatsara* has been recognized by previous authors. The first molecular phylogeny of the genus by Jackman *et al.* (2008) confirmed a clade comprising these three species, albeit with *P. lohatsara* as the sister taxon of *P. sanctijohannis* instead of *P. stumpffi*. The polyphyly of Comoran *Paroedura* could not be detected in that study because no samples from Mayotte were available. The previous absence of samples from Mayotte also explains that *P. stellata* sp.n. remained a cryptic species, even though several morphological characters support its distinctiveness from *P. sanctijohannis*. Thus, the initial hypothesis that Comoran *Paroedura* form a monophyletic group with a phylogeny congruent to the assumed age-progressive order of the Comoros Islands is rejected.

This discrepancy between the molecular phylogeny of Comoran *Paroedura*, as suggested by our results, and the suggested geological ages of the islands they inhabit hampers the application of geological calibration points for a molecular clock analysis (Heaney 2007; Papadopoulou *et al.* 2010; Heads 2011). Therefore, we followed an approach by Gómez-Díaz *et al.* (2012) using substitution rates from previous fully calibrated analyses instead of geological calibration points. We acknowledge that the results of this approach should be regarded with particular care: only one of the six markers of our data set could be calibrated by this means, and the substitution rate used was inferred for different, although related, organisms under a different geological setting (lizards, skinks and geckos from the Canary islands; Carranza & Arnold 2012). However, we argue that this approach may yield more reliable results than an analysis that is based on geological calibration points that are obviously in contradiction with the phylogeny.

The age of the initial splitting between the lineages of *P. sanctijohannis* and *P. stellata* sp.n. + *P. stumpffi* + *P. lohatsara* is estimated at 6.8 MA (95% confidence interval 4.1–10.6 MA). According to our phylogenetic analysis, this event has taken place in Madagascar and therefore cannot be correlated with any age estimate for the Comoros Islands (Hajash & Armstrong 1972; Montaggioni & Nougier 1981; Emerick & Duncan 1982; Nougier *et al.* 1986; Bachélery & Coudray 1993). The split between the Mayotte endemic *P. stellata* sp.n. and its closest Malagasy relative *P. stumpffi* is estimated at 3.6 MA (1.9–5.7 MA). Most studies estimate the geological age of Mayotte older than this (e.g. 5.40 ± 0.01 MA, Emerick & Duncan 1982). The most basal split within *P. sanctijohannis* is the split between the populations of Mohéli and those of Grand Comoro + Anjouan. Its age is estimated at 2.7 MA (1.4–4.5 MA), which falls within the range of geological age estimates for Mohéli (1.5–5.0 MA). These two estimates support the correlation of our divergence time estimates with the geological age estimates of the islands.

The divergence time estimates for the Grand Comoro and Anjouan populations of *P. sanctijohannis* are less easily correlated with the geological age estimates for the islands. The most basal split is between the specimen from the Karthala volcano of Grand Comoro and the population of the La Grille volcano of Grand Comoro + all Anjouan populations. It is estimated at 1.5 MA (0.7–2.7 MA). This is within the range of age estimates for Anjouan island (1.2–4.0 MA), but much older than the estimates for Grand Comoro (0.01–0.5 MA). Even excluding the single most divergent specimen from Grand Comoro, the split is estimated at 0.6 MA (0.2–1.1 MA). These estimates suggest that (i) the geologically older island was colonized by a population from the geologically younger island, and (ii) the Grand Comoro population of *P. sanctijohannis* is older than the island they inhabit.

The two most plausible explanations for (i) may be either that Anjouan was initially not colonized by *Paroedura* at all, but only later from the Grand Comoro population, thus long after the origin of Anjouan, or that Anjouan was recolonized after the extinction of the original *Paroedura* population. Although island species are generally attributed a good ability to withstand catastrophic events, such as volcanic eruptions (Heaney 2007), the possibility of the extinction of island populations by such events should not be dismissed completely and may also explain the relative scarcity of endemic species of squamates on Anjouan (Meirte 2004; Hawlitschek *et al.* 2011). The problem described in (ii) of a discrepancy between the estimated geological age of an island and the genetic divergence within an endemic reptile species has previously been discussed, for example, for Galápagos iguanas (Rassmann 1997) and Anolis lizards from Barbados (Thorpe *et al.* 2005). It was also mentioned by Rocha *et al.* (2005b) for the endemic chameleon *Furcifer cephalolepis* from Grand Comoro. Rocha *et al.* (2005b) proposed the tentative explanations that either the evolution of the markers tested in their study might be faster than predicted by the standard mutation rates or that the founder population that colonized Grand Comoro already included haplotypes divergent enough to explain the extant situation. Our data set shows that Grand Comoro is an exceptional case with an age estimate of the oldest divergence within its *Paroedura* population of 1.5 MA (0.7–2.7 MA): the oldest divergences within island populations of other Comoran *Paroedura* are estimated at 0.3 MA (0.1–0.8 MA) for Mayotte, 0.2 MA (0.1–0.3 MA) for Mohéli and 0.2 MA (0.0–0.6 MA) for Anjouan, all far younger than the estimated geological ages of the islands. This is supported by Rocha *et al.* (2005b), where *Furcifer polleni* from Mayotte shows less divergence than *F. cephalolepis* from Grand Comoro despite larger sample size, and by a study on Comoran tree snakes by Hawlitschek *et al.* (2012), where haplotype divergence within the Grand Comoro population is higher than within any other island.

This pattern makes it much harder to apply the tentative explanations proposed by Rocha *et al.* (2005b): There is no obvious reason why the evolution of molecular markers in three independent groups should be accelerated on Grand Comoro, but not on other islands of the archipelago (see also Bromham & Woolfit 2004). Likewise, it is hard to explain why only Grand Comoro should be the target of repeated colonizations by larger founding populations than other islands. An alternative explanation might be that parts of Grand Comoro are much older than currently assumed based on geological dating methods. Many independent studies have attempted to provide age estimates of the islands (Hajash & Armstrong 1972; Montaggioni & Nougier 1981; Emerick & Duncan 1982; Nougier *et al.* 1986; Bachélery & Coudray 1993), yet the "age of the initial volcanism on each island remains more or less speculative" (Nougier *et al.* 1986). Particularly on Grand Comoro, few samples of the oldest lava flows are available, as most of the island surface is covered by younger volcanic series (Emerick & Duncan 1982). Thus, an older origin of Grand Comoro Island should not be excluded and might best explain the patterns displayed by biogeography.

While the populations of Grand Comoro, Anjouan and Mohéli form a monophyletic group belonging to *P. sanctijohannis*, *P. stellata* sp.n. forms an independent clade, as shown by our results. Nevertheless, regarding the adult and juvenile coloration, these two species are more similar to each other than to *P. stumpffi* and *P. lohatsara*, which are in turn more closely related to *P. stellata* sp.n. The Malagasy species show a clear shift between a conspicuous juvenile coloration with brightly contrasting transverse bands and an orange tail, and a cryptic and little contrasting adult coloration, the latter particularly in *P. lohatsara*. No studies have been conducted on the functions of the juvenile coloration of *Paroedura* species, but studies in other lizards have shown that the conspicuously coloured tail may direct attacks away from more vulnerable body parts (Clark & Hall 1970; Hawlena *et al.* 2006), and the contrasting bands may act as camouflage through a 'flicker fusion' effect in moving specimens (Pough 1976; Stevens 2007). Thus, they may be more advantageous for specimens foraging actively than for those adopting a sit-and-wait strategy, which may reflect a change of foraging styles in the life histories of *Paroedura* species due to high competitive and predation pressures. The weakly contrasting juvenile colorations of the Comoran forms, and the overall similarity between juvenile and adult colour patterns in these species, may reflect reduced selection pressure in an insular environment (Mertens 1934). Probably, *Paroedura* species faced little competition from other nocturnal geckos prior to the human-mediated introduction of *Hemidactylus* species (Rocha *et al.* 2007, 2010; Hawlitschek *et al.* 2011). Similar between all islands of the archipelago, but different to Madagascar, these conditions may have provoked convergent responses in phenotypical adaptations in the ancestors both of *P. sanctijohannis* and *P. stellata* sp.n. Studies on various squamate groups showed that similar ecological constraints can produce cases of striking convergence in many morphological traits, leading to the phenomenon of phenotypically very similar species belonging to completely different clades (Carillo de Espinoza *et al.* 1990; Melville *et al.* 2006; Tulli *et al.* 2011). Islands appear to be particularly favourable for convergent adaptations (Losos 1992; Losos *et al.* 2006; Poe *et al.* 2007). These considerations support the view that the similarity of Comoran *Paroedura* is a result of convergent evolution.

As an alternative to convergent evolution, the introgression of mtDNA and nDNA from dispersed *P. stumpffi* individuals might be seen as a possible explanation for the phylogenetic position of *P. stellata* sp.n., but this could probably be traced using molecular methods (Benavides *et al.* 2009; Murphy *et al.* 2009; Cox *et al.* 2010), and our molecular tree (Fig. 1) does not support this interpretation. Another hypothesis to explain this tree topology is to assume the re-colonization of Madagascar from the Comoros and thus a Comoran ancestry of *P. stumpffi* and *P. lohatsara*. While the re-colonization of continents (or larger islands) from smaller islands has been shown to be more common than previously thought (Nicholson *et al.* 2005; Bellemain & Ricklefs 2008), dispersal from the Comoros to Madagascar is an extremely unlikely event under the current setting of ocean currents and winds, going back to the time of the emergence of the Comoros (Crottini *et al.* 2012; Samonds *et al.* 2012).

Most likely, both Comoran *Paroedura* species reached the archipelago in separate overseas dispersal events. This raises the question why the phylogenetically more basal *P. sanctijohannis* is absent from Mayotte, the oldest island of the archipelago. Possibly, the Mayotte population of this species was wiped out by the ancestors of *P. stellata* sp.n. Anthropogenic introductions of foreign species, including geckos, have shown their potentially devastating impact on native species (Cole *et al.* 2005; Cole & Harris 2011). In a scenario of natural dispersal, however, a very small foreign founder population would be confronted with a comparatively very large native population. Because both *Paroedura* species are relatively closely related, cross-matings and the survival of native haplotypes even after outcompetition and extinction of the native population should be considered likely (Podnar *et al.* 2005; McGuire *et al.* 2007), which could probably be traced in our haplotype networks (Fig. 2). On the other hand, the success of the newly arrived ancestor of *P. stellata* sp.n. might be facilitated by geological events. Long after its initial emergence at more than 5.40 MA, Mayotte experienced phases of increased volcanism until about 1.58 MA (Emerick & Duncan 1982). Explosive volcanism on Petite Terre, the northeastern part of Mayotte, may have occurred as late as 7000 years BP (Zinke *et al.* 2003). Such a phase of increased volcanism may have altogether wiped out a former population related to *P. sanctijohannis*, making way for the establishment of the founder population of *P. stellata* sp.n. Alternatively, the ancestors of *P. stellata* sp.n. may have originally colonized one of the smaller islets in the Mayotte lagoon, and later invaded the main island during a phase in which the lagoon had dried up or a land bridge existed (Zinke *et al.* 2003). In the former case, there would have been no chance for the introgression of haplotypes, while in the latter case, the already established population of *P. stellata* sp.n. may have been so large that traces of introgression would no longer be detectable with the methods used here.

Our analysis of the phylogeny of Comoran *Paroedura* shows that the history of dispersal between Madagascar and the Comoros, and the colonization of the islands, is most likely more complex than previously thought. A simple scenario, in which a founder population from Madagascar colonized the geologically oldest island Mayotte in a single dispersal event shortly after its origin, and subsequently the younger islands, is not supported by our results. Rather, these results suggest two separate colonization events of the Comoros. Extinction events of earlier populations of *Paroedura* and following re-colonization might explain the phylogenetic positions of *P. stellata* sp.n. and the Anjouan populations of *P. sanctijohannis*. Our phylogeny also suggests strong differences between the selection regimes of islands and the Malagasy mainland, as two species of *Paroedura* that are not closely related show almost identical juvenile and adult colour patterns as a result of convergent adaptation to the island environment.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Morphological data of specimens of *Paroedura* analyzed for this study. All measurements in mm.

Table S2. Primers and PCR protocols. Forward (F) and reverse (R) primers are given, all in 5′-3′ order.

Table S3. GenBank accession numbers of the sequence data produced for this study.

5.2 PAPER: Pleistocene climate change promoted rapid diversification of aquatic invertebrates in Southeast Australia.

Climate change happened over the entire history of planet Earth and always had a high impact also on island biogeography. For example, through climate change an island in the ocean may lose all its suitable habitats for a certain species that cannot adapt, and the island may thus 'disappear' for this species even though it does not submerge in the ocean. Likewise, it may 're-appear' and be re-colonized if climate changes back to suitable conditions. In terrestrial settings, habitats may change on altitudinal gradients, which becomes particularly obvious on mountains. Habitats may even disappear if their altitudinal bands are elevated to a range higher than the summit of the mountain. An attempt to present evidence for such events in the current phase of climate change has been made by RAXWORTHY et al. (2008). In recent geological history, the Earth's climate changed most drastically in the Pleistocene climate oscillations 2.5 mya to 10,000 years ago that triggered the Ice Ages. It is out of question that these climate changes left massive alterations of habitats and entire biota in their wake. However, today, even such relatively recent events are hard to trace and interpret. Evidence can be found in the fossil and subfossil record, including pollen analyses, and from molecular data (HEWITT 2004, QUENTAL & MARSHALL 2010).

This chapter presents the case of the Australian diving beetle genus *Sternopriscus* SHARP, 1882. Molecular, morphological, and ecological data suggest that, while the genus as a whole is diverse and most likely old, a very recent radiation has taken place that was probably catalyzed by the Pleistocene climate oscillations. No new species were described in this paper. Nevertheless, the attempt to retrospectively delimit the species of the Pleistocene radiation follows an integrative approach sensu MIRALLES et al. (2011), using the lines of evidence of morphology, mitochondrial DNA, nuclear DNA, and ecology. MtDNA and nDNA are not useful for species delimitation in this case, but the two lines of evidence of morphology and ecology support the status of all previously described species.

HAWLITSCHEK O., HENDRICH L., ESPELAND M., TOUSSAINT E.F.A., GENNER M.J., BALKE M. (2012): Pleistocene climate change promoted rapid diversification of aquatic invertebrates in Southeast Australia. BMC Evolutionary Biology 12, 142.

Post-publication comments and errata:

p. 12: According to HEADS (2011, p.7), the maximum diversification rate estimated by MENDELSON & SHAW (2005) may be an overestimate. For calibrating the phylogeny of their study system, the authors assumed that the clades could not be older than the islands they are endemic to. HEADS (2011) argues that, as shown by other examples in his article, the possibility that the clades are older than the island they are endemic to now cannot be easily dismissed, and the extraordinarily high speciation rates may be a methodological artifact. If this were the case, the speciation rate of the *Sternopriscus tarsalis* radiation presented in this chapter would be even more exceptional among insects, provided that the mutation rate of PAPADOPOULOU et al. (2010) used in my work is more reliable than the calibration by MENDELSON & SHAW (2005). See also discussion in Chapter 8.1.3.

p. 12: Last paragraph: "southwestern Australia" is erroneous, replace with "southeastern Australia"

p. 14: Reference [34] is published, Organisms Diversity & Evolution 12, 335–337.

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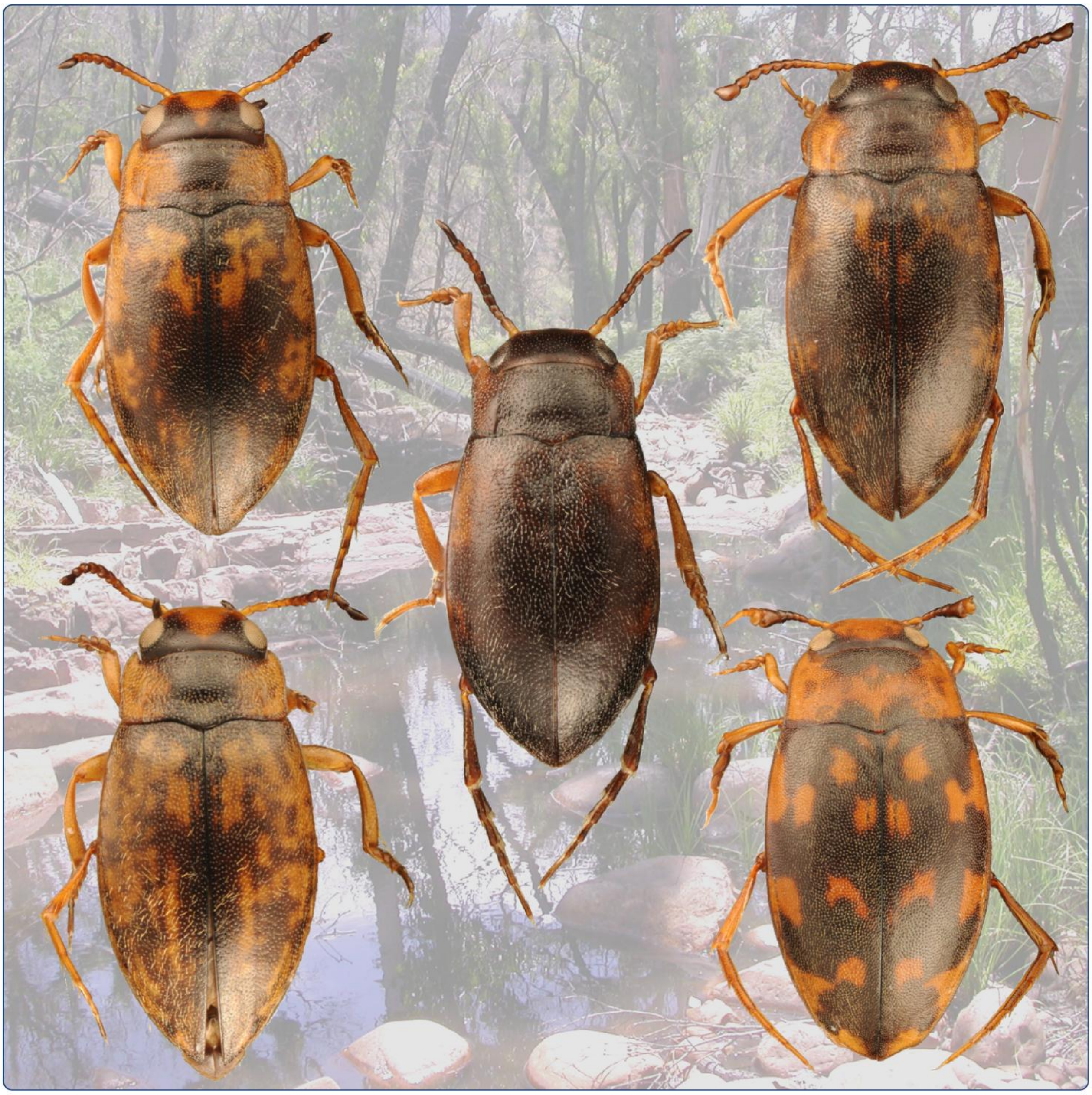

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RESEARCH ARTICLE **Open Access**

Pleistocene climate change promoted rapid diversification of aquatic invertebrates in Southeast Australia

Oliver Hawlitschek[1*], Lars Hendrich[1], Marianne Espeland[2], Emmanuel FA Toussaint[1], Martin J Genner[3] and Michael Balke[1,4]

Abstract

Background: The Pleistocene Ice Ages were the most recent geohistorical event of major global impact, but their consequences for most parts of the Southern hemisphere remain poorly known. We investigate a radiation of ten species of *Sternopriscus*, the most species-rich genus of epigean Australian diving beetles. These species are distinct based on genital morphology but cannot be distinguished readily by mtDNA and nDNA because of genotype sharing caused by incomplete lineage sorting. Their genetic similarity suggests a Pleistocene origin.

Results: We use a dataset of 3858 bp of mitochondrial and nuclear DNA to reconstruct a phylogeny of *Sternopriscus* using gene and species trees. Diversification analyses support the finding of a recent rapid speciation event with estimated speciation rates of up to 2.40 species per MY, which is considerably higher than the proposed average rate of 0.16 species per MY for insects. Additionally, we use ecological niche modeling and analyze data on habitat preferences to test for niche divergence between species of the recent *Sternopriscus* radiation. These analyses show that the species can be characterized by a set of ecological variables referring to habitat, climate and altitude.

Conclusions: Our results suggest that the repeated isolation of populations in glacial refugia might have led to divergent ecological adaptations and the fixation of morphological traits supporting reproductive isolation and therefore may have promoted speciation. The recent *Sternopriscus* radiation fulfills many characteristics of a species flock and would be the first described example of an aquatic insect species flock. We argue that the species of this group may represent a stage in speciation past the species flock condition because of their mostly broad and often non-overlapping ranges and preferences for different habitat types.

Background

Global biodiversity is shaped by the processes of speciation and extinction, whose rates vary depending on region, environment, taxonomic group and geohistorical events [1-3]. Evidence for shifts in the rates of speciation and extinction have been inferred from the fossil record since early paleontology [4], and advances in molecular biology have greatly improved our capabilities to study these processes particularly for taxa with sparse or inconsistent fossil evidence [5,6].

The most recent geohistorical event of major global impact on biodiversity was the Pleistocene glaciations, or Ice Ages, which represent the largest expansion of cold climates since the Permian period 250 million years (MY) earlier. Until 10,000 years ago, temperatures repeatedly oscillated between warm and cold phases. The effects on the environment varied depending on geographical region, but were always accompanied by major biotic shifts. Boreal regions, particularly in the Northern hemisphere, were mostly glaciated and drove species into refugia [7]. In the tropics and subtropics, where glaciations were mostly restricted to high altitudes, a similar effect was attributed to the aridification of formerly humid forest habitats [8]. It has been a matter of discussion whether these cycles of environmental change

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promoted speciation [9] or whether species responded solely by shifting their ranges toward ecologically suitable areas [10]. In Australia, glaciations occurred only at its highest elevations, but biota faced an ongoing process of aridification that was initiated in the Miocene *c.* 15 million years ago (MYA) when Australia drifted northward [11]. During the Ice Ages, the relatively rapid shifts between warm and wet *versus* cold and dry conditions had severe consequences particularly for the fauna [12,13]. Aquatic environments were strongly affected by oscillations between arid and humid conditions [14].

The genesis of the Australian arid zone promoted radiations in various organism groups, e.g., hypogean faunas in the ground waters underneath the spreading deserts, which most likely began with the onset of the aridification *c.* 15 MYA [14]. However, many rapid radiations of insects dating back only 2 MY or less have been described from all around the world. Coyne & Orr [15] proposed an average speciation rate of 0.16 species per MY, which is exceeded by an order of magnitude by the fastest known radiation [16-18]. Phylogenies of such young radiations based on mitochondrial gene trees are often poorly resolved, and species may appear para- or polyphyletic because of shared alleles with other species, which may be the result of incomplete lineage sorting or hybridization [19]. Species trees may cope with these problems: in a method based on a coalescent model and Bayesian inference, all gene trees are co-estimated and embedded in a single species tree whose tips represent species and not single samples [20,21].

Aside from morphological and molecular characters, ecological factors can be useful to distinguish and even delimit species. Many studies have shown that a variety of climate factors often have a profound effect on the distributions of species, and these factors can be combined to project potential distributions of species in an Ecological Niche Modeling (ENM) approach [22,23]. The predictive powers of this method have been demonstrated [24], and it has been successfully applied in species delimitations [22,25]. Naturally, the distinction of species based on differences in their responses to ecological factors is sensible only if there are actual response differences. Evidence of niche conservatism in closely related species, promoting allopatric speciation, is abundant [26]. However, in many examples of rapid radiations in limited geographic areas niche divergence appears to be the more common condition, and closely related species show different responses to ecological factors [2004, 27].

The focus of our study is on the genus *Sternopriscus* (Coleoptera: Dytiscidae: Hydroporini), which is the most species-rich epigean genus of Australian diving beetles and contains 28 species [27,28]. *Sternopriscus* species inhabit a wide variety of lentic and lotic habitats from sea level to high altitudes. 18 species are found in southeastern Australia, of which four species are endemic to Tasmania. The corresponding freshwater ecoregions according to Abell *et al.* [29] are Eastern Coastal Australia, Bass Strait Drainages, Southern Tasmania, and small parts of the Murray-Darling region. Unlike many other aquatic invertebrates, such as crustaceans and gastropods, most species of epigean aquatic beetles use flight to colonize new habitats. Therefore, the presence of suitable habitats most likely has a higher impact on aquatic beetle distribution than the drainage systems defining the biogeographic regions of Abell *et al.* [29]. Nevertheless, only 2 of these 18 species have a wider distribution over mainland Australia (*S. multimaculatus* and *S. clavatus*). 6 species, including some taxonomically and geographically isolated species, are endemic to peaty habitats in the southwest, in an area with cold and humid climate during winter, and 5 species are distributed over the tropical north, including one endemic species in the deep gorges of the Pilbara. None, or only one, species is shared by 2 or more of these areas of endemism. This distribution reflects the restriction of all but the widespread pioneer species *S. multimaculatus* to the more humid coastal areas of Australia. The high level of endemism in the southeast and southwest suggests that the arid barrier between these two regions is long-standing. Another strong pattern is the virtual absence of *S. tarsalis* group members from the north and southwest regions of the continent, whereas members of the *S. hansardii* group, with highly modified male antennae and median lobes, are more widespread [27,28].

Based on male morphological characters, the genus has been divided into 3 groups: the *S. hansardii* group (11 species), the *S. tarsalis* group (13 species), and 4 ‘phylogenetically isolated’ species. The species in the *S. tarsalis* group have been assigned to 3 species complexes: the *S. tarsalis* complex (2 species), the *S. meadfootii* complex (5), and the *S. tasmanicus* complex (3). 3 species have not been assigned to any complex. The 10 species belonging to the *S. tarsalis*, *S. meadfootii* and *S. tasmanicus* complexes in the *S. tarsalis* group are genetically similar and centered in mesic southeastern Australia. Below, we refer to this group of species as the *S. tarsalis* radiation (STR). The STR is supposedly the result of recent diversification; some of these morphologically well-defined species occur in sympatry, and some in syntopy [27,28,30]. Previous genetic studies [30] suggest that species belonging to the STR are not easily delimited using mtDNA and nDNA.

In this study, we attempt to test the following hypotheses: (1) the delimitation of species in the STR, based on morphological characters, can be supported by genetic or ecological data; (2) the STR species originated in a rapid and recent diversification event, most likely in the

Pleistocene; and (3) the radiation of the STR was promoted by the Pleistocene climate oscillations. We use a molecular phylogeny with gene and species trees and diversification rate analyses to investigate how environmental change has affected speciation and extinction rates in the genus *Sternopriscus*. We then discuss which factors might have promoted lineage diversification in the STR and whether the molecular similarities are caused by hybridization or incomplete lineage sorting. Aside from the results of our molecular phylogeny, we use phylogeographic network analyses and ENM paired with empirical ecological data in an attempt to reveal how this diversification was promoted.

Methods

Sampling and laboratory procedures

Specimens were collected by sweeping aquatic dip nets and metal kitchen strainers in shallow water or operating black-light traps [27] and preserved in 96% ethanol. DNA was extracted non-destructively using Qiagen blood and tissue kits (Qiagen, Hilden). Primers are listed in Additional file 1: Table S1. New sequences were submitted to GenBank under accession numbers [EMBL: HE818935] to [EMBL:HE819178]; *cox1* data are [EMBL: FR732513] to [EMBL:FR733591]. The individual beetles from which we extracted and sequenced DNA each bear a green cardboard label that indicates our DNA extraction number (e.g., “DNA 1780 M. Balke”). This number links the DNA sample, the dry mounted voucher specimen and the GenBank entries.

Phylogenetic analyses

The aligned 3858 bp dataset contains three mitochondrial (16 S rRNA, cytochrome oxidase b (*cob*), and cytochrome *c* oxidase subunit I (*cox1*)) and four nuclear gene fragments (18 S rRNA, arginine kinase (ARK), histone 3 (h3), and histone 4 (h4)) for 54 specimens of 25 *Sternopriscus* species and 2 Hydroporini outgroups, *Barretthydrus stepheni* and *Carabhydrus niger*. Among the known species of *Sternopriscus*, only *S. mouchampsi* and *S. pilbaraensis* were not available for sequencing. *S. emmae* was excluded from the phylogenetic analyses because we only had DNA from museum specimens and only obtained a short *cox1* sequence. DNA alignment was performed in MUSCLE 3.7 [31]. We then used jModelTest 0.1.1 [32] to identify appropriate substitution models for each gene separately, assessing lnL, AIC and BIC results and giving preference to BIC. To evaluate different partition schemes, we performed a Bayes factor test with MrBayes 3.1 [33] and Tracer v1.5 [34]. The eleven schemes tested were mitochondrial *versus* nuclear, protein-coding *versus* ribosomal, and according to codon positions (1 + 2 *versus* 3 or one partition for each codon position). We used raxmlGUI 0.93 [35] for maximum likelihood analyses with 1000 fast bootstrap repeats. MrBayes 3.1 [33] was used for Bayesian analyses, with two runs and four chains with 30,000,000 generations (samplefreq = 1,000 and 25% burnin). Runs were checked for convergence and normal distribution in Tracer v1.5 [34]. We then used parsimony analysis to infer phylogenetic relations as implemented in the program TNT v1.1, which we also used to run 500 jackknife replications (removal 36%) to assess node stability [36] (hit the best tree 5 times, keep 10,000 trees in memory). Finally, we used coalescent-based species tree inference models in *BEAST v1.6.1 [21] for comparison with the results of the phylogenetic gene tree. *BEAST requires *a-priori* designation of species, which we performed based on morphological data [27,28]. We conducted two runs over 100,000,000 generations (sample freq = 1,000 and 20% burnin) and checked for convergence and normal distribution in Tracer v1.5 [34]. Additionally, as proposed in Pepper *et al.* [13], we repeated this analysis using simpler substitution models (HKY + G). All analyses in MUSCLE and MrBayes were run on the CIPRES Portal 2.2 [37]. Pairwise distances were calculated in MEGA 5.0 [38].

Lineage diversification and radiation

Analyses were conducted in R with the packages APE [39] and Laser [40]. Based on the phylogenetic tree created in MrBayes, we used the ‘chronopl’ function of APE to create an ultrametric tree in R and cropped all representatives but one of each species. We then constructed Lineage-Through-Time (LTT) plots [41] and calculated γ-statistics [42]. Because new species continue to be discovered in Australia and incomplete taxon sampling might influence γ-statistics, we conducted a Monte Carlo constant rates (mccr) test with 10,000 replicates, assuming 10% missing species. We then tested the fit of two rate-constant [41] and four rate-variable diversification models [43] to our dataset. Finally, we calculated *p*-values by simulating 10,000 trees with original numbers of present and missing species for a pure-birth scenario and for various birth-death rates (b = 0.5 and d = 0.0, 0.25, 0.5, 0.75 and 0.9). To be able to understand the effect of the near-tip radiation in the STR, we also tested γ for a tree in which this group was treated as a single taxon.

Because of a lack of reliable calibration points, we cannot rely on molecular clock analyses to estimate node ages in the *Sternopriscus* phylogeny. However, we attempt to approximate the age of the rapid radiation in the STR using the standard mutation rates of the *cox1* gene [44,45]. We apply the equation presented in Mendelson & Shaw [16] to estimate the relative speed of this radiation for comparison with other known rapid radiations in insects. For young and monophyletic radiations, such as the STR, the equation is $\hat{r} = \ln N/t$, where $\hat{r}$ is the rate of diversification, N is the number of extant species, and t is the divergence time.

Phylogeographic structure analysis

We assembled a matrix of 710 bp of only *cox1* for 79 specimens of STR species to investigate the phylogeographic structure of this group. Additional sequences were obtained from Hendrich *et al.* [30]. The standard population genetic statistics Fu's Fs [46] and Tajima's D [47] were calculated, and mismatch distribution analyses to untangle demographic histories were performed using DnaSP 5.10 [48]. The multiple sequences were collapsed in haplotypes also using DnaSP 5.10. A minimum-spanning network was then inferred in Arlequin 3.5.1.3 [49] and used to create a minimum-spanning tree (MST) using Hapstar 0.5 [50]. The scalable vector graphics editor Inkscape 0.48 was further used to map geographic and taxonomic information on the MST.

Distinguishing incomplete lineage sorting from hybridization

We used an approach developed by Joly *et al.* [51], and employed in Joyce *et al.* [52] and Genner & Turner [53] to test whether the haplotype sharing between STR species was mainly the result of incomplete lineage sorting or influenced by hybridization. In this approach, mtDNA evolution is simulated using a species tree topology that assumes hybridization is absent. If low genetic distances between species pairs are due to incomplete lineage sorting, these similarly low genetic distances should be observed in the simulations. If low genetic distances between species pairs are due to hybridization, then significantly lower genetic distances should be present than observed in the simulations. First, we ran another *BEAST [21] analysis of a subset of the entire multilocus dataset containing only the STR species, using the HKY + G model for 11,000,000 generations (samplefreq = 1,000 and 10% burnin). Second, we used MrModeltest [54] to estimate the parameters of the substitution model for the *cox1* dataset from Hendrich *et al.* [30], which was previously used in the phylogeographic structure analysis. Third, we conducted a run of the JML software [55] using the same *cox1* dataset, the locus rate of *cox1* as yielded by *BEAST, a heredity scalar of 0.5, and the parameters yielded by MrModeltest.

Ecological niche modeling and analyses

In an attempt to detect possible divergence in response to climatic variables in their ranges, we created ecological niche models (ENMs) for the species of the STR. We excluded *S. montanus* and *S. williamsi* from the ENM analyses because of an insufficient number of localities. Our models were based on a total of 215 distribution points [27,28] (Additional file 2: Table S2) and unpublished data by L. Hendrich. With the exception of three records of *S. wehnckei*, all STR species occur in broad sympatry in southeastern Australia including Tasmania.

We preliminarily selected climate variables according to ecological requirements considered critical for the species. Bioclimatic variables [56] represent either annual means or maxima and minima in temperature and precipitation, or variables correlating temperature and precipitation, e.g., "mean temperature of wettest quarter" (BIO8). Such variables are useful for representing the seasonality of habitats [25]. After the preliminary selection, we used the ENMtools software [57] to calculate correlations between the selected climate layers in the area of interest. In our final selection, we removed layers until no two layers had correlation coefficients (r^2) higher than 0.75. ENMs for each species were created in Maxent 3.3.2 [58] (our procedure: Hawlitschek *et al.* [25]). Suitable background areas that were reachable by the species were defined by drawing minimum convex polygons around the species records, as suggested by Phillips *et al.* [59]. We conducted runs with 25% test percentage, 100 bootstrap repeats, jackknifing to measure variable importance and logistic output format. Model validation was performed by calculating the area under the curve (AUC) [60]. To compare ENMs of different *Sternopriscus* species, we measured niche overlap [57] in ENMtools. We also used ENMtools' niche identity test [61] with 500 repeats because the niche overlap values alone do not allow any statements whether the ENMs generated for the two species are identical or exhibit statistically significant differences. In each repeat of this test, pairwise comparisons of species distributions are conducted and their localities pooled, their identities are then randomized and two new random samples are extracted to generate a set of pseudoreplicates. The results are compared with the true calculated niche overlap (see above). The lower the true niche overlap is in comparison to the scores created by the pseudoreplicates of the pooled samples, the more significant the niche difference between the two compared species. Finally, we classified species by altitudinal and habitat preference and compared all data.

Results

Molecular phylogenetics

Bayes factor analyses favored separate partitioning of genes and codon positions (17 partitions in total). This was the most complex partition strategy tested. Substitution models applied were according to jModeltest: the GTR + I + G model (16 S rRNA, mitochondrial non-protein-coding), the GTR + G model (*cox1*, *cob*, mitochrondrial protein-coding), the HKY + I + G model (18 S rRNA, nuclear non-protein-coding), and the HKY + G model (ARK, h3, h4, nuclear protein-coding). Bayesian, maximum likelihood, and maximum parsimony analyses revealed compatible topologies (Figure 1) that were largely congruent with the previously recognized classifications based on morphology. Here, we assign the four species previously supposed to be 'phylogenetically isolated' to either the *S. tarsalis* (*S. browni* and *S. wattsi*), or the *S. hansardii* (*S. eikei* and *S. marginatus*) group. Within the *S. tarsalis* group, all *S. tarsalis* complex species form a strongly supported clade (Figure 1).

The *BEAST species tree is largely congruent to the gene trees. The main difference is that in the gene trees,

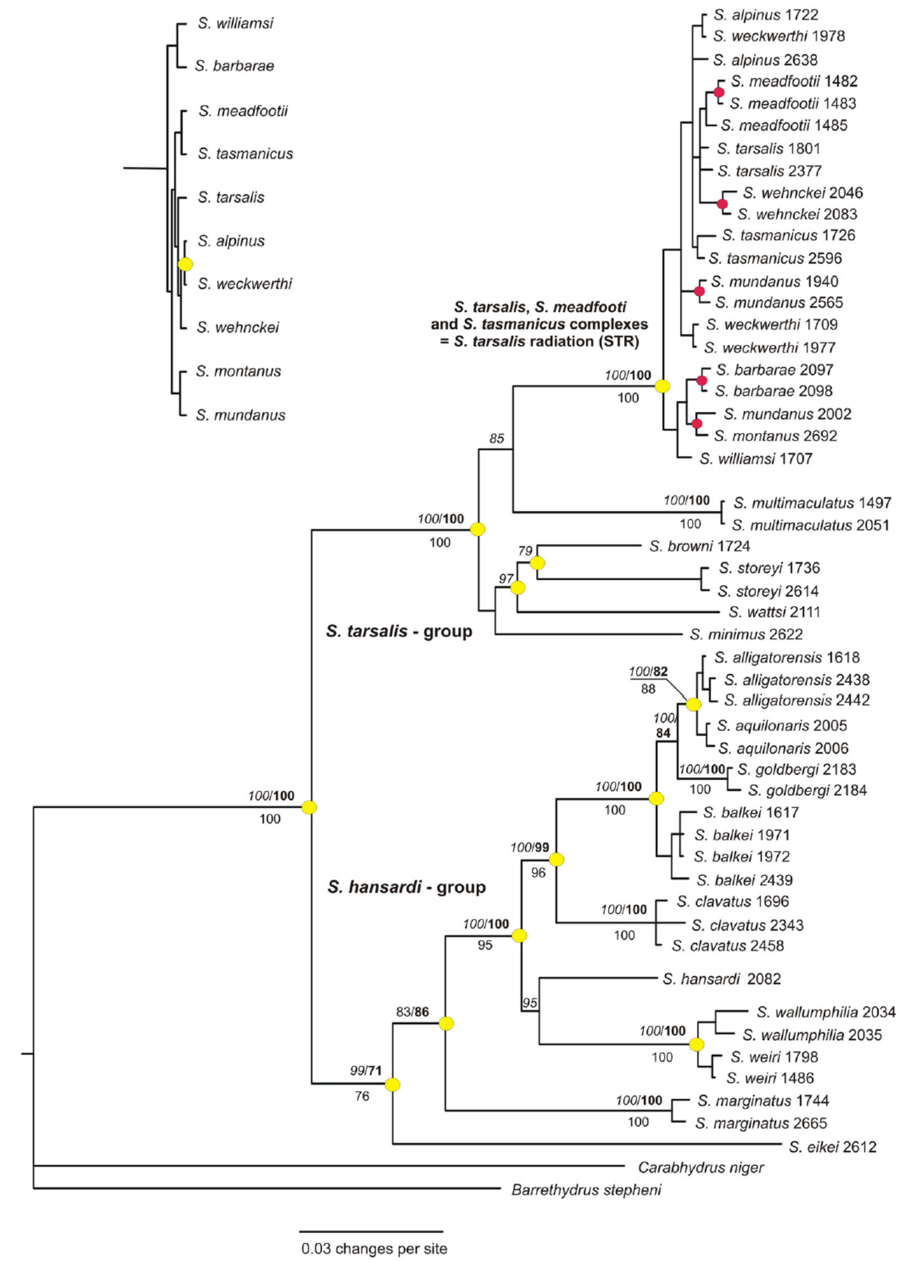

Figure 1 Phylogram of the genus *Sternopriscus*. The phylogram is based on a MrBayes tree with 7 gene loci and 3858 characters. Branch values are: MrBayes posterior probability (italic/above branch), RAxML bootstrap (bold/above branch), and TNT jackknife (below branch). Yellow circles mark nodes with *BEAST species tree posterior probabilities of 75 or more. Red circles mark nodes within the *S. tarsalis* radiation with PP, bootstrap and jackknife values of 75 or more (values not shown for layout reasons). Each tip represents one specimen. Specimen collection numbers are given after the species names. Upper left: *BEAST species tree fragment showing the *S. tarsalis* radiation specimens.

S. multimaculatus is the sister taxon to the STR, whereas in the *BEAST tree *S. minimus* is the sister taxon to the STR and *S. multimaculatus* is the sister taxon to all other members of the *S. tarsalis* group. Almost all species tree nodes within the STR are poorly supported. Notably, the analysis of the *BEAST run log file showed near-critically low posterior and prior effective sample sizes (< 120). This problem could neither be solved by repeating runs with higher sample frequencies nor with the application of simpler substitution models, as proposed in Pepper *et al.* [13], and indicates that the species tree results must be treated with caution.

The largest calculated *cox1 p*-distance between species in the STR was only 3.4% (*S. tarsalis*/*S. barbarae*), but interspecific distances may be as low as 0.3% (e.g., between *S. alpinus*, *S. mundanus* and *S. weckwerthi*, all belonging to different *S. tarsalis* complexes) or 0.2% (*S. alpinus/S. wehnckei*). Thus, no genetic distinction between the three complexes was possible because specimens often cluster with those belonging to other morphologically well-characterized species. This problem could not be solved by inspecting trees based on single or combined nuclear loci; the species *S. mundanus* and *S. weckwerthi* were polyphyletic in single-gene trees of *cob*, *cox1*, and ARK. The STR species shared identical haplotypes in all other nuclear genes studied.

Diversification analyses

Figure 2 shows the LTT plot for *Sternopriscus.* APE yielded a positive γ value of 3.22 (p = 0.0013*). According to the mccr test, the critical value is 1.73 (p = 0.9*10E-3**) and is therefore met by the true value of γ. The test in Laser yielded a Yule-2-rate model as significantly better than the next best model, which was a constant rate birth-death model. The level of significance was highest (p = 0.0073*) for equal rates of b (birth) and d (death) (both 0.5), but all tested combinations of b and d yielded significant test results. In the test run in which the *S. tarsalis*-group was treated as a single clade, γ was negative but not significant at a value of −0.01 (p = 0.4956). This means that for this dataset the null hypothesis that the diversification rates have not decreased over time cannot be rejected.

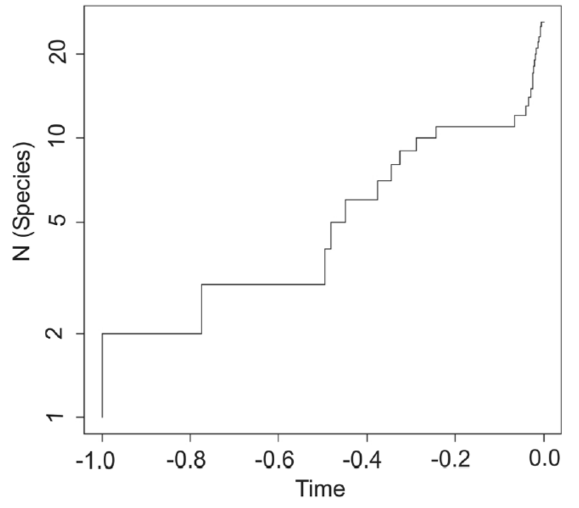

Figure 2 Lineage-through-time (LTT) plot for the genus *Sternopriscus*. Relative time (−1.0 is the time of the initial lineage split within the genus, 0.0 is the present) is given on the x-axis, number of species is given logarithmically on the y-axis.

The STR appears to have a thorough effect on the diversification analysis of the genus *Sternopriscus.* A high positive γ represents a rather unusual condition [6]. While many phylogenies are characterized by a decreasing rate of diversification (logistic growth or impact of extinctions [62]), a γ = 3.22 suggests a diversification rate that is highly increasing over time. This pattern is hard to explain in general. In the case of *Sternopriscus*, it appears appropriate to attribute this pattern to the recent speciation burst of the STR, which comprises 10 of 28 known species. This view is also supported by the test results that indicate a Yule-2-rate model as the most adequate, which fits to a sudden shift in diversification rates.

Papdopoulou *et al.* [44] suggested using substitution rates of 3.54% *cox1* divergence per MY which suggest an origin of the STR *c.* 0.96 MYA, and interspecific distances indicate divergence times as recent as 60,000 to 80,000 years ago. The slower substitution rate (2.3%) suggested by Brower [45] yields an approximate origin of the STR around 1.48 MYA and interspecific divergence times of 87,000 to 130,000 years ago (but see Papadopoulou *et al.* [44] for a discussion of these estimates). The equation by Mendelson & Shaw [16] was used to estimate speciation rates in the STR. Applying the proposed rate of Papdopoulou *et al.* [44], we estimate a speciation rate in the STR of 2.40 species per MY. Applying the proposed rate of Brower [45], we estimate a speciation rate in the STR of of 1.56 species per MY.

Phylogeographic structure

The matrix of 79 *cox1* sequences contained 69 polymorphic sites with a nucleotide diversity of π = 0.0121 and a haplotype diversity of H = 0.9815. We identified 61 distinct and mostly unique haplotypes within the STR with only 8 haplotypes comprising more than one sequence. Neither geographic nor taxonomic (Figure 3) mapping on the star-like MST yielded a comprehensive pattern. More precisely, no geographic structuring could be noticed based on the zoning of Australia, and the haplotypes of individuals of identical species were not systematically gathered in groups. Interestingly, the MST appears to be composed of two central haplotypes of South Australian and Victorian *S. mundanus* from which the rest of the sequences appears to have derived. In addition, even if there is a lack of geographical or taxonomic structuration, one might notice that several haplotypes representing different species are separated from the central network by a deep break of multiple mutation steps. While Tajima's D value does not significantly support a scenario of demographic expansion (D = −1.27773, p-value = 0.06), Fu's Fs significantly support such a demographic history (Fs = −35.731, p-value = 0.01) (see Tajima [47] and Fu [46] regarding the interpretation of Tajima's and Fu's statistics). However, the mismatch distribution analyses yield a multimodal distribution of the pairwise genetic distances, which favors a scenario of demographic equilibrium for the STR even if unimodal distributions are recovered only for recent and fast expansions [63].

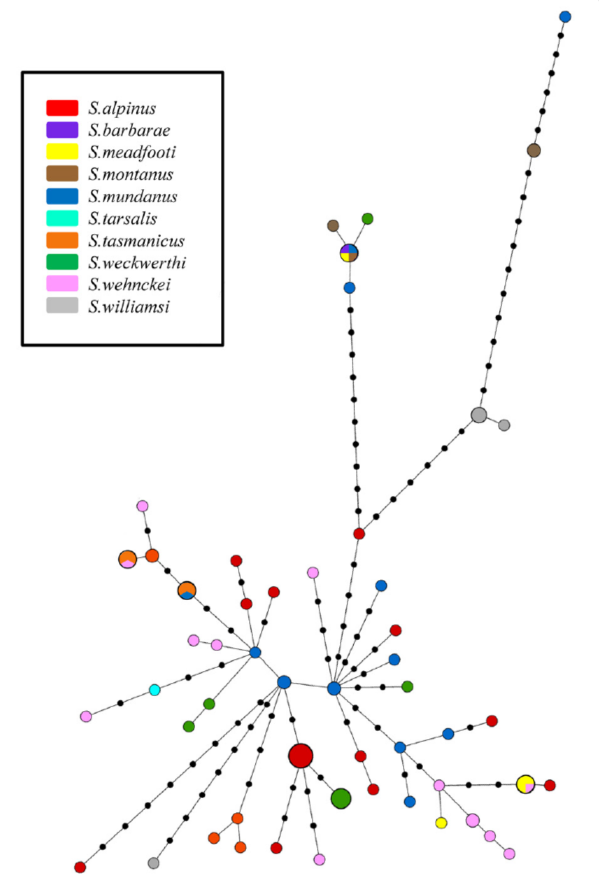

Figure 3 Minimum spanning tree of haplotypes of the *Sternopriscus tarsalis* radiation. The tree was created in Hapstar 0.5. Colors code the species determined according to morphology. Colored circles represent haplotypes, black dots represent mutational steps that are not represented by any haplotype.

Incomplete lineage sorting vs. hybridization

*BEAST yielded a high relative locus rate of 2.332 for *cox1*, which was expected because many other markers included in our multilocus dataset, mainly nuclear markers, are known to evolve slower. The results of the JML run are given in Table 1. All species pairs exhibit genetic distances that are not significantly lower than expected. Thus, we cannot reject the hypothesis of incomplete lineage sorting in any cases.

Ecological niche modeling

Figure 4 summarizes all distribution points for all STR species and Figure 5 summarizes climate variables used for the creation of ENMs. The ENMs for the 8 STR species analyzed, supplemented with other ecological data,

Table 1 Results of the JML run

Distance obs./exp.	*S. alp.*	*S. bar.*	*S. mea.*	*S. mon.*	*S. mun.*	*S. tar.*	*S. tas.*	*S. wec.*	*S. weh.*	*S. wil.*
S. alp.		4.83	2.42	4.83	2.42	2.42	2.42	1.21#	1.21#	4.83
S. bar.	14.81		4.83#	2.42#	4.83#	4.83	4.83	4.83+	4.83	2.42
S. mea.	4.44	0		4.83#	2.42#	2.42	1.21	2.42+	2.42#	4.83
S. mon.	14.81	0	0		4.83#	4.83	4.83	4.83+	4.83	2.42
S. mun.	1.48	0	0	0		2.42	2.42#	2.42+	2.42#	4.83
S. tar.	5.92	23.70	8.89	23.70	4.44		2.42	2.42	2.42+	4.83
S. tas.	5.93	22.22	8.89	22.22	0	5.93		2.42	2.42#	4.83
S. wec.	0	1.48	1.48	1.48	1.48	5.93	4.44		1.21#	4.83
S. weh.	0	19.26	0	19.26	0	1.48	0	0		4.83
S. wil.	10.37	19.26	16.30	16.30	11.85	16.30	14.81	14.81	11.85	

Minimum genetic distance (*1,000), as estimated by JML, of STR species pairs. Lower left: observed minimum genetic distance. Upper right: expected minimum genetic distance (median). Species pairs in which the observed genetic distance is 0 due to the sharing of haplotypes are indicated by #. Species pairs in which the observed minimum genetic distance is higher than the expected distance are indicated by +. There is no case in which the probability that the minimum observed genetic distance is lower than expected is significant ($p \leq 0.05$).

Figure 4 Distribution of species of the *Sternopriscus tarsalis* radiation. Red dots represent specimen localities used for ecological niche modeling.

are given in Figure 6. AUC values for all models range from 0.981 to 0.997. Because all values are > 0.9, the ability to distinguish presence from random background points is considered "very good" for all models according to Swets [60]. We preliminarily selected the climate layers "maximum temperature of the warmest month" (BIO5), "minimum temperature of the coldest month" (BIO6), "mean temperature of the wettest quarter" (BIO8), "mean temperature of the driest quarter" (BIO9), "precipitation of the wettest month" (BIO13), "precipitation of the driest month" (BIO14), "precipitation of the warmest quarter" (BIO18) and "precipitation of the coldest quarter" (BIO19). In our final selection, we omitted BIO13 and BIO14 because of correlation coefficients with other variables of $r^2 > 0.75$. Thus, all models presented here are based on six climate variables. Jackknifing to measure the importance of variables showed that either "maximum temperature of the warmest month" (BIO5: *S. barbarae*, *S. weckwerthi*, *S. wehnckei*), "mean temperature of the wettest quarter" (BIO8: *S. alpinus*, *S. mundanus*), or "precipitation of the coldest quarter" (BIO19: *S. meadfootii*, *S. tarsalis*, *S. tasmanicus*) were the most important variables in creating ENMs. Niche overlap values (I and D) and identity test results are given in Table 2. The results of the identity test are highly significant (Bonferroni corrected) for I in all and for D in nearly all pairwise species comparisons. However, the null hypothesis of identity in the ENMs of two compared species can be rejected only if the true calculated niche overlap is below the 99.9% confidence interval of the values generated in the identity test. In a few cases, the true calculated niche overlap is above this interval, and the null hypothesis of niche identity cannot be rejected [61].

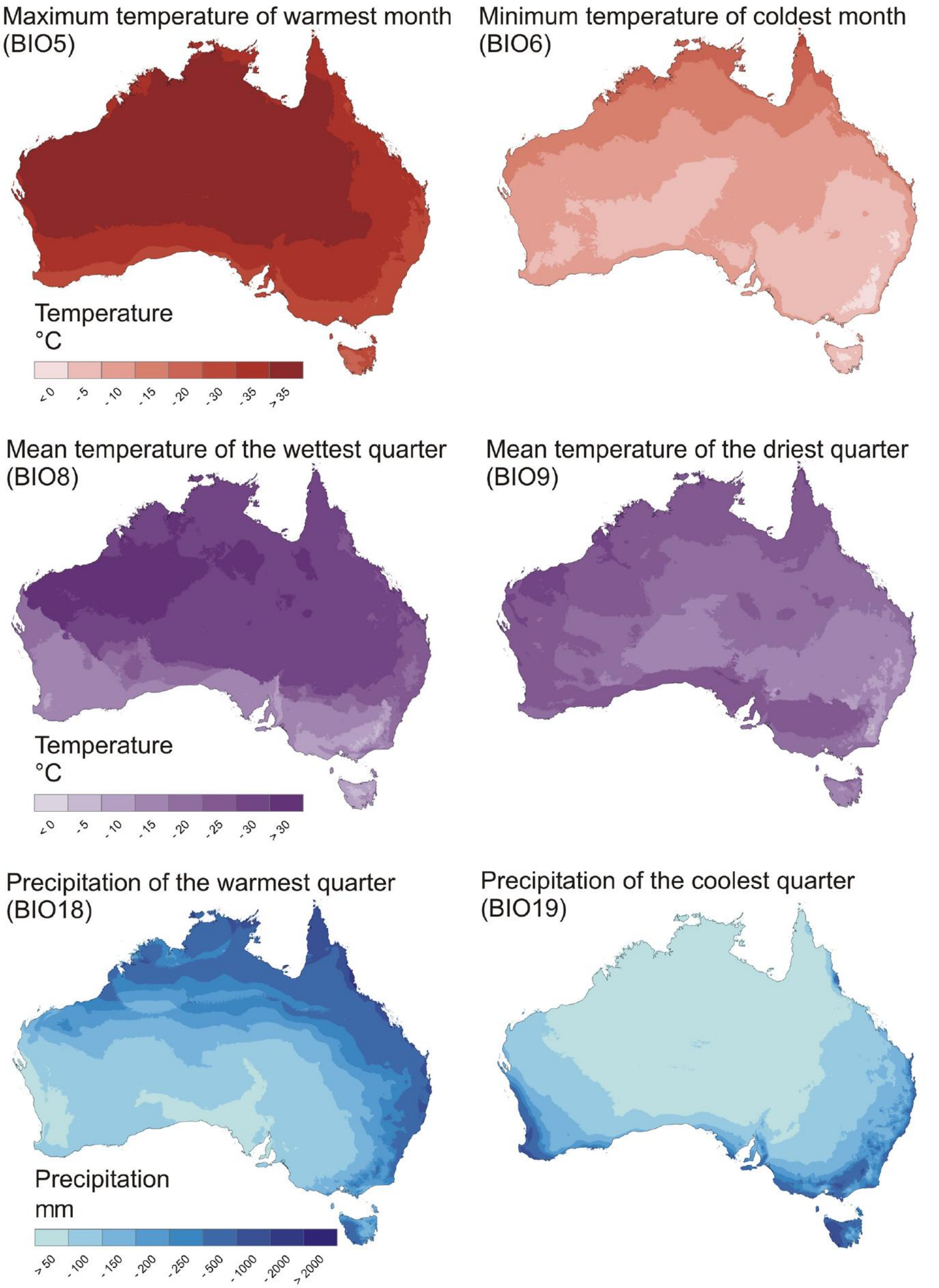

Figure 5 (See legend on next page.)

(See figure on previous page.)
Figure 5 Climate variables used for ENM creation. Variables were selected to represent the effects of temperature, precipitation and seasonality.

Ecological analyses

All species of the STR were compared for their preferences in altitude and habitat and for the most important climate factor in their ENM, which resulted from the jackknifing test in the ENM runs. Table 3 displays these three factors for all species coded by numbers for easy comparison. Only *S. tasmanicus* and *S. tarsalis* are identical in all three factors. *S. montanus* and *S. williamsi* might be identical to *S. alpinus* or *S. weckwerthi* depending on the most important climate factor, but no ENMs could be created. Within each of the three complexes in the *S. tarsalis* group, no two species are identical in all three factors.

Discussion

In the opening section of this article, we suggested three hypotheses: (1) species delimitation in the STR can be supported by genetic or ecological data; (2) the STR species originated in a rapid Pleistocene diversification event; and (3) Pleistocene climate oscillations promoted the radiation of the STR. In the following, we will discuss how our results support these hypotheses.

Our data shows that the molecular methods applied in our study do not serve to unambiguously distinguish and delimit the species of the STR. This is because of the widespread genotype sharing of mitochondrial genes and lack of diversification in nuclear genes between these species. However, the analysis of our ecological data shows that STR species appear to respond differently to ecological variables. Below, we initially discuss whether incomplete lineage sorting or hybridization may have caused the abundance of shared haplotypes in the STR. Then, we discuss the importance of the results of our ecological analyses in the context of the entire genus, and specifically for the STR.

Genotype sharings between species may be explained by incomplete lineage sorting, by hybridization, or a combination of both. Funk & Omland [19] also mention imperfect taxonomy, inadequate phylogenetic information and paralogs as causes for genotype sharing. However, the taxonomy of *Sternopriscus* based on morphological characters is well supported [27,28], and our multi-gene phylogeny is well supported by different analytical approaches. Paralogs can almost certainly be excluded because the patterns of species polyphyly are repeated by different mitochondrial and nuclear markers.

Hybridization, as a reason for genotype sharing in closely related species, has been proposed for various animal groups [64,65], including groups with strong sexual selection (e.g., mating calls [66]), and has been shown to contribute to speciation [64]. However, in the case of *Sternopriscus*, the results of our analyses, the diversity in genital morphology, and the absence of specimens identifiable as hybrids, do not support hybridization [67]. Incomplete lineage sorting, or the retention of ancestral polymorphism, is the more likely explanation for genotype sharing in the case of the STR. Incomplete lineage sorting has often been recognized as a problem in resolving phylogenies of young and closely related taxa [68]. This phenomenon affects nuclear loci more commonly than faster evolving mitochondrial loci, but mitochondrial genes can be equally affected, particularly in closely related taxa where hardly any diversification in nuclear genes is found [19]. Incomplete lineage sorting as an explanation for haplotype sharings in the STR supports the view that the STR is a recent radiation.

A comparison of our ecological findings concerning the STR species with data on other *Sternopriscus* species shows that the STR occupies ecological ranges similar to those of other related species. The currently known altitudinal distribution and ecology of all *Sternopriscus* species in Australia is shown in Additional file 3: Table S3, modified after Hendrich & Watts [27,28]. 10 species of the genus are rheophilic and inhabit rivers and streams that are mainly of intermittent character. 11 species are acidophilic and live in seasonal or permanent swamps, ponds and pools of different types of peatlands. 7 species appear to be more or less eurytopic and occur in various water bodies in open or forested country. The highest species diversity is in lowland or coastal areas and hilly or low mountain ranges from 0 to 500 m. Only 6 species were collected at 1000 m or above (*S. alpinus*, *S. meadfootii*, *S. montanus*, *S. mundanus*, *S. williamsi* and *S. weckwerthi*).

Within the STR, all species inhabit broadly overlapping areas in mesic southeast Australia, except for a few localities of *S. wehnckei* in the northeast (the Eastern Coastal Australia region and small parts of the Murray-Darling region of Abell *et al.* [29]. Many species also inhabit Tasmania, including two endemics (Bass Strait Drainages and Southern Tasmania). ENMs indicate niche diversification within this group of closely related and broadly sympatric species. Aside from the high levels of significance in the identity test, the degree of niche diversification is hard to measure. Therefore, we rely on the importance of the various climate variables used to characterize the species ENMs. The variables of highest importance are

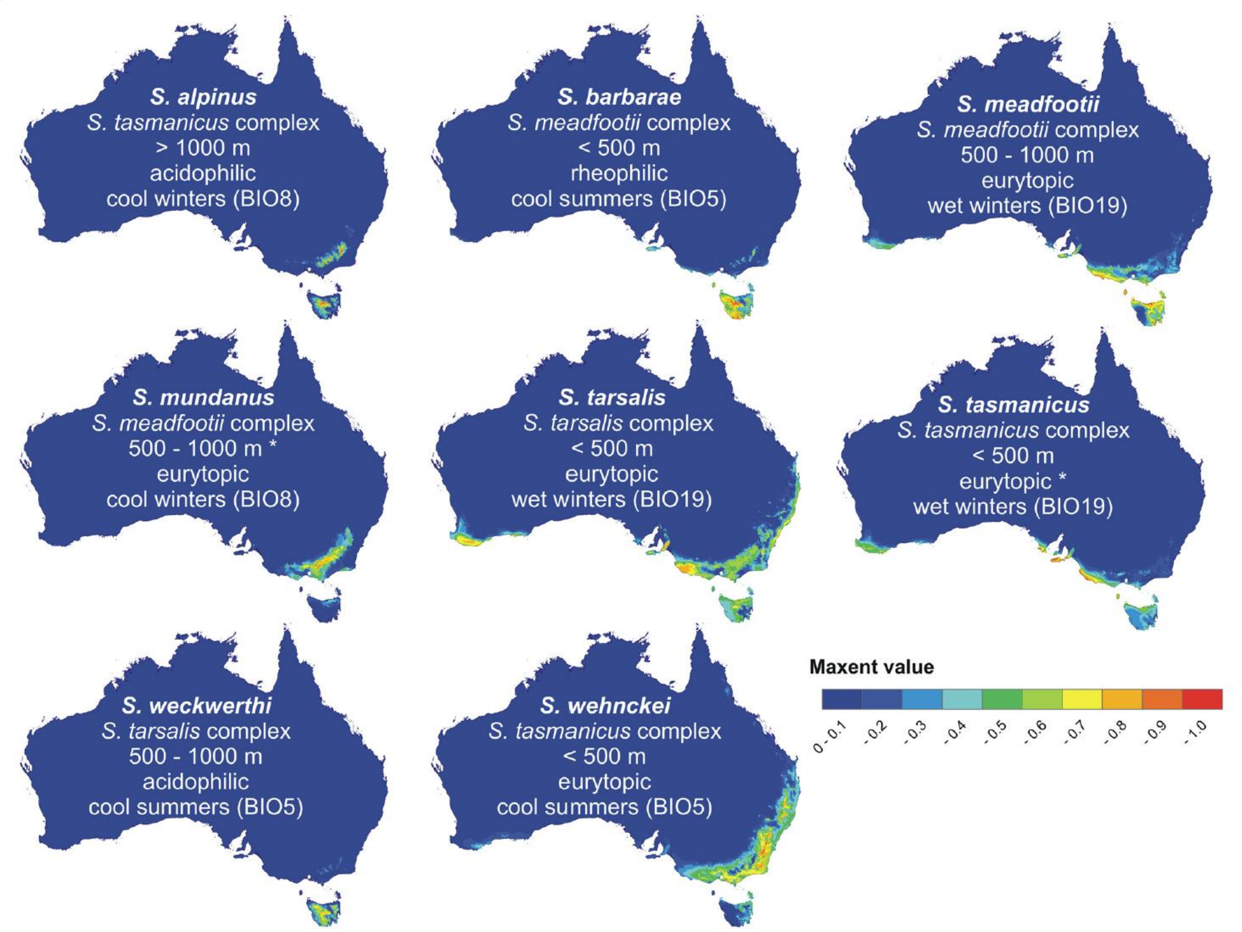

Figure 6 Ecological Niche Models (ENMs) for species of the *Sternopriscus tarsalis* radiation. No ENMs were created for *S. montanus* and *S. williamsi* because of insufficient locality data. High Maxent values indicate high probabilities of occurrence of a species on a raster square (2.5 arc-minutes resolution). Maps include species name, taxonomic affinity, altitudinal range, habitat type and climate variable of highest importance in the ENM.

"maximum temperature of the warmest month" (BIO5), "mean temperature of the wettest quarter" (BIO8), or "precipitation of the coldest quarter" (BIO19). Figure 5 shows that all the species studied inhabit areas with relatively low maximum temperatures, with the lowest on Tasmania. The two species most characterized by this factor are the two Tasmanian endemics, *S. barbarae* and *S. weckwerthi*. A distinction between the two remaining factors is more

Table 2 Results of the niche identity test

Overlap D/I	*S. alp.*	*S. bar.*	*S. mea.*	*S. mun.*	*S. tar.*	*S. tas.*	*S. wec.*	*S. weh.*
S. alp.	0	0.674**	0.682**	0.676**	0.661*#	0.651**#	0.648**	0.571**
S. bar.	0.506**	0	0.733**#	0.569**	0.680**#	0.735**#	0.755**	0.582**
S. mea.	0.481**	0.589**#	0	0.691**	0.801**	0.847**#	0.606**	0.684**
S. mun.	0.474**	0.327**	0.496**	0	0.661**	0.602**	0.520**	0.637**
S. tar.	0.476	0.472	0.711**	0.456**	0	0.759**	0.548**	0.756**
S. tas.	0.433**	0.560	0.762**#	0.378**	0.648**	0	0.583**	0.627**
S. wec.	0.451**	0.642**	0.367**	0.241**	0.282**	0.331**	0	0.459**
S. weh.	0.374**	0.356**	0.523**	0.444**	0.639**	0.419**	0.177**	0

Niche overlap values (D and I), calculated with ENMtools, are given for species pairs and are mostly lower than the randomized overlap levels generated in the identity test at significant (*, $p \leq 0.05$, Bonferroni corrected) or highly significant (**, $p \leq 0.001$, Bonferroni corrected) level. This means that niches are more divergent than expected at random. In some cases, results are not significant, or significantly higher than the randomized overlap (indicated by #). In these cases, niches are not more divergent than expected by random. Note that results yielded by D and I do not accord in all cases.

Table 3 Taxonomic affinities and ecological preferences of species in the *Sternopriscus tarsalis* radiation

Species	Complex	Altitude	Habitat	Climate
S. alpinus	*2*	2	2	1
S. tasmanicus	*2*	0	1*	2
S. wehnckei	*2*	0	1	0
S. barbarae	*1*	0	0	0
S. meadfootii	*1*	1	1	2
S. montanus	*1*	2	2	?
S. mundanus	*1*	1 **	2	1
S. tarsalis	*0*	0	1	2
S. weckwerthi	*0*	2	2	0
S. williamsi	*1*	1	2	?

Complex: 0 = *S. tarsalis*, 1 = *S. meadfootii*, 2 = *S. tasmanicus*. Altitude: preferred altitude range, 0 = < 500 m, 1 = 500 – 1000 m, 2 = > 1000 m. Habitat: 0 = rheophilic, 1 = eurytopic, 2 = acidophilic. Climate: according to the dominating climate variables in the ENM, 0 = cool summers, 1 = cool winters, 2 = wet winters. *: Also occurs in habitats with moderate salinity. **: Actual altitudinal range is 200 – 1550 m.

difficult. Considering Figure 5, "mean temperature of the wettest quarter" is lowest in areas where winters (the wettest quarter in our region of interest) are cold, whereas "precipitation of the coldest quarter" is highest where winters are wet. Some species (e.g., the high-altitude *S. alpinus*) may be tolerant of winter temperatures that are too low for other species, whereas other species are more dependent on sufficient precipitation. Species that require the latter are eurytopic species that also inhabit ephemeral waters, such as ponds at the edge of rivers and creeks, which are only filled after heavy rainfall. The acidophilic species, which inhabit more permanent water bodies with dense vegetation, are often "cold winter" species.

The low divergences between haplotypes in the STR species suggest that these species originated in a recent and rapid radiation. Unfortunately, we could not rely on any calibration points to support our molecular clock approach. Instead, we attempted to estimate the origin of the STR based on standard *cox1* mutation rates [44,45]. We estimated an origin of *c.* 0.96 to 1.48 MYA, which leads to an estimated speciation rate of 2.40 or 1.56 species per MY. Genetic distance might indicate the age of the ancestral species, however divergence time estimates for the extant species should not be considered reliable beyond assumption of a comparably recent origin of the STR. This fact alone, however, suggests that the STR is an exceptional event for what is known of aquatic beetles. For other insect groups, little evidence exists for similarly fast diversification events. The fastest rate (4.17 species per MY) was estimated for a clade of 6 species of Hawaiian crickets over 0.43 MY [16]. However, in the same study, for a related clade comprising 11 species, the estimated rate was much lower at 1.26 species per MY over 1.9 MY. Additional estimates are available for *Galagete* moths in the Galapagos [17] of 0.8 species per MY (n = 12, t = 1.8 MY) and for Japanese *Ohomopertus* ground beetles [18] of 1.92 (n = 15, t = 1.4 MY) to 2.37 species per MY (n = 6, t = 0.76 MY). The average speciation rate in insects was proposed to be 0.16 species per MY [15]. This comparison shows that rapid radiation events, as exemplified in the STR, appear to be exceptional among insects and particularly in continental faunas because all other examples recorded were island radiations.

Species groups that originated from rapid radiation events have been detected in almost all organismic groups and habitats [69]. An overview of many recent and past events suggests three major promoters of rapid radiations: the appearance of a key innovation that allows the exploitation of previously unexploited resources or habitats [70], the availability of new resources [71], and the availability of new habitats, e.g., because of a rare colonization event or drastic environmental changes [72,73]. In the case of the STR, we find no evident key innovation distinguishing this group from other *Sternopriscus* species. We have no data concerning internal morphology or physiology. Additionally, our data show that the observation that STR species have ecological requirements similar to those of other *Sternopriscus* species does not indicate the presence of any key innovations. There is also no indication of any new resource that could be specifically exploited by the STR species. Therefore, we explore the possibility that drastic environmental changes during the Pleistocene climate oscillations mediated the radiation of the STR species.

During most of the Cenozoic, the climate of Australia was hot and humid and currently remains so in the northern rainforest areas [11]. Aridification began in the Miocene (*c.* 15 MYA) and gradually led to the disappearance of forests and to the spread of deserts over much of the present continent. Most of today's sand deserts, however, are geologically younger and appeared only after the final boost of aridification that accompanied the Ice Ages, particularly since the later Pleistocene (*c.* 0.9 MYA). The climate was subjected to large oscillations in temperature and rainfall, which drove many groups of organisms into refugia and also promoted speciation [12,13]. Our results also document a strong and abrupt increase in speciation in the genus *Sternopriscus* about 1 to 1.5 MYA, represented by the STR. This age estimate is congruent with the Pleistocene oscillations. Byrne *et al.* [12] present cases of organisms restricted to mesic habitats that were formerly most likely more widespread, but today occupy relictual areas with suitable climates. However, some of the young species of the STR occupy rather large areas in southwestern Australia. This distribution indicates good dispersal abilities, which are necessary for organisms that inhabit habitats of relatively low persistence [74]. Ribera & Vogler [75] argue that for this reason, beetle species that inhabit lentic aquatic habitats often have better dispersal abilities than those inhabiting lotic habitats. However, it is possible that the STR species of lotic habitats only recently derived from an ancestor adapted to lentic habitats with good dispersal abilities that are maintained in the newly derived species.

Speciation in Pleistocene refugia was previously described for dytiscid beetles on the Iberian Peninsula [9]. During the Pleistocene climate oscillations, the ancestral species of the STR might have been forced into ongoing cycles of retreating into, and the re-expansion from, refugia. Under the recurrent, extremely unsuitable climate conditions, the isolation of small populations over many generations might have promoted speciation and the fixation of morphological traits. This scenario might also explain the lack of clear geographic or taxonomic structuring in the striking haplotypic diversity presented by the STR species. This diversity might be attributed to the cycles of expansion and retreat that repeatedly isolated haplotypes in various geographic locations before newly allowing the expansion and colonization of other areas.

The phenomenon of groups of young and closely related species within a defined distributional range is most familiar in ichthyology, in which it was termed "species flock". Among the most prominent species flocks are the cichlids of the African Great Lakes and other lake ecosystems around the world, the Sailfin Silversides of Sulawesi, and the Notothenioid Antarctic Ice Fishes (see review in Schön & Martens [76]). Schön & Martens [76] summarize the criteria for naming a group of species a species flock as "speciosity [= species-richness], monophyly and endemicity". Compared with the large fish species flocks, the STR is poor in species. Nevertheless, the number of species is "disproportionally high" [77] in relation to the surrounding areas, as no other region in Australia is inhabited by a comparable assemblage of closely related species. In the last decade, an increasing number of less species-rich radiations have been termed species flocks with as little as 3 or 4 species [76,78]. Most other species flocks inhabit lakes, islands or archipelagoes. These are areas more "narrowly circumscribed" [77] than the area of endemism of the STR, which can be broadly termed "the southeast Australian region". Most STR species have relatively large ranges that do not share a common limit and sometimes do not even overlap. Our results show that STR species often occupy different habitat types. Additionally, the clade is not strictly endemic to southeastern Australia, as shown by the northeastern records of *S. wehnckei*. Based on this criterion, other rapid radiations among insects [16,17] are much more adequate examples of species flocks.

Conclusions

Our results provide evidence that STR species are the result of an extremely recent, most likely Pleistocene, radiation. The STR species cannot be distinguished with the molecular methods used in this study, however, the species show clear divergences in their responses to ecological factors of habitat type and climate. We proposed a scenario in which the Pleistocene climate oscillations led to the repeated restriction and expansion of the ranges of the ancestral species of the STR, which may have promoted fixation of ecological adaptations and morphological traits in small and isolated populations restricted to refugia. This suggests that *Sternopriscus* is an example for the hypothesis that Pleistocene refugia promoted speciation.

Taking this scenario into account, the STR does not appear as an evolving or fully evolved species flock but as a radiation based on a species flock. While possibly confined to a narrowly circumscribed area during the Pleistocene, the STR species were able to break the boundaries of their refugia with the end of the Ice Ages and increase their ranges. Today, because the species are no longer confined to a common limited area, the term "species flock" may best fit a stage in speciation the STR has previously passed.

Additional files

Additional file 1: Table S1. Sequences of primers used for PCR and sequencing. Forward (F) and reverse (R) primers are given. Mitochrondrial gene loci: CO1 = cytochrome C oxidase 1, CytB = cytochrome B oxidase, 16 S = 16 S ribosomal RNA. Nuclear gene loci: H3 = histone 3, H4 = histone 4, ARK = arginine kinase,18 S = 18 S ribosomal RNA. I = inosine.

Additional file 2: Table S2. Localities of *Sternopriscus* species used in Ecological Niche Modeling. Coordinates are given in decimal degrees.

Additional file 3: Table S3. Ecological data on all *Sternopriscus* species. Data from Hendrich & Watts [27,28].

Abbreviations

ENM: Ecological niche modeling; MST: Minimum spanning tree; MY: Million years; MYA: Million years ago; STR: *Sternopriscus tarsalis* radiation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

OH performed the laboratory work, the molecular genetic studies, the diversification analyses, the ecological niche modeling and analyses, and drafted the manuscript. LH collected the samples and ecological data and helped to draft the manuscript. ME coordinated the diversification analyses. EFAT conducted the phylogeographic analyses. MJG conducted the analysis of hybridization vs. incomplete lineage sorting. MB conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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5.3 PAPER: Molecular phylogeny of the squeak beetles, a family with disjunct Palearctic – Australian range.

The similarities and differences between island and continental biogeography were discussed in the introduction. At a global scale of space and time, however, continents become somewhat comparable to islands: they disconnected, joined, and were and are subject to climate change, either through changes in global climate or through shifts in the relative positions of the continents (FRISCH et al. 2010).

To study continents as biogeographic islands requires a study organism that, just like the continents, can be studied at a global level and at large geological timescales. One such study system is the genus *Hygrobia* LATREILLE, 1804, the single genus of the aquatic beetle family Hygrobiidae. *Hygrobia* has a very uncommon global distribution, with one species from the western Palearctic, one from China, and the remaining four from Australia. This chapter attempts to clarify whether *Hygrobia* is actually as old as the continents it lives on (after the breakup of Pangea), or whether the genus is younger and its distribution is the result of recent dispersal events.

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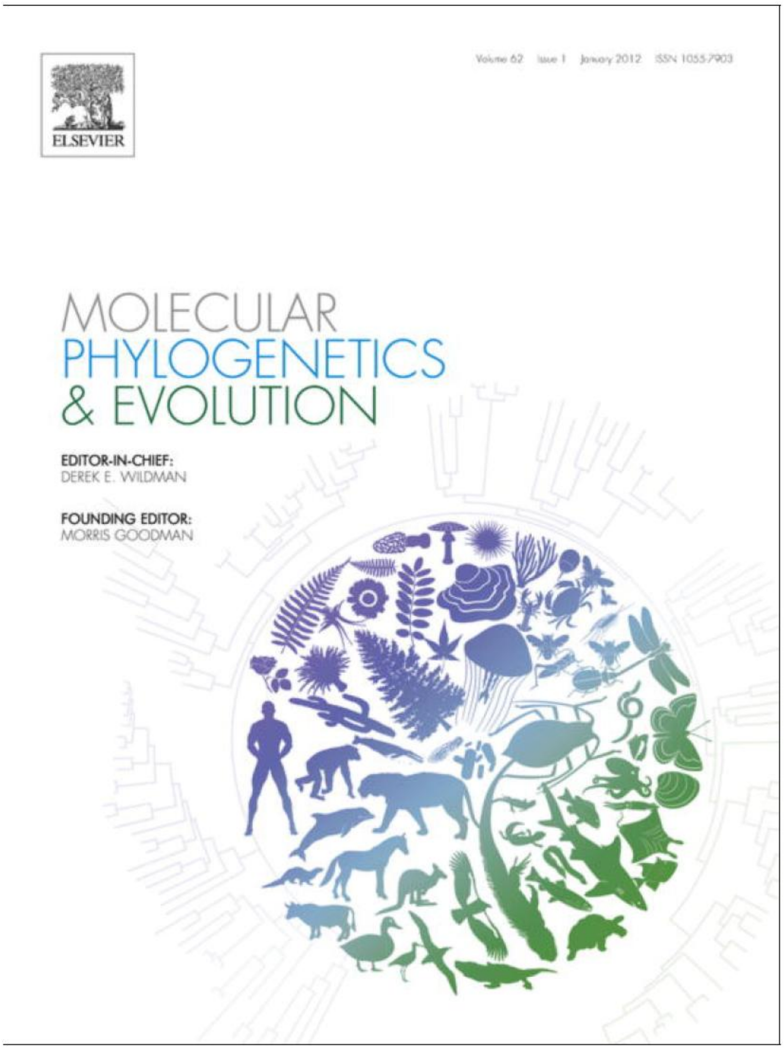
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Short Communication

Molecular phylogeny of the squeak beetles, a family with disjunct Palearctic-Australian range

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A B S T R A C T

Many higher groups of plants and animals show distributional patterns which have been shown or have at some point in time been suggested to be correlated with plate tectonics and the ancient supercontinents Laurasia and Gondwana. Here, we study the family of squeak beetles (Coleoptera: Adephaga: Hygrobiidae) and its enigmatic distribution pattern, with one species in the Western Palearctic, one in China and four in Australia. We present a molecular phylogeny including five of the six extant species, showing the monophyly of the Australian radiation. We use a molecular clock approach, which indicates that Hygrobiidae is an ancient group dating back to the breakup of Pangea and discuss the possibility of vicariance as explanation for its current distribution.

1. Introduction

With the rise of modern biogeography in the second half of the 20th century, biogeographers have increasingly sought to correlate large scale patterns of biodiversity with geological history and the movement of continental plates (Jablonski et al., 1985; Hallam, 1995). The Jurassic breakup of the supercontinent Pangaea split biota into two biogeographic regions, the northern Laurasia and the southern Gondwana, and many present-day taxa are attributed to Laurasian or Gondwanan origins.

A Gondwanan origin is often invoked to explain disjunct distribution patterns (Nilsson et al., 2004). Although transoceanic dispersal may have played a role in many of these cases, the overall distribution is assumed to be mainly a result of vicariance, caused by the breakup of Gondwana and its subsequent separation into the southern continents (Sampson et al., 1998; Cracraft, 2001). The case of Laurasian distributions is simpler, as the parts of Laurasia remained connected during most of their existence. Many Laurasian taxa are distributed throughout most of the northern hemisphere, but they have also colonised areas of Gondwanan origin (Hocknull et al., 2009).

Most current taxa whose origins cannot easily be traced back to Laurasia or Gondwana show a cosmopolitan or subcosmopolitan distribution, or are considered “relictual”, with an ancient origin and disjunctly distributed, monophyletic subtaxa. The beetle family Aspidytidae is known from only two species, one in South Africa and one in central China (Balke et al., 2005). One region characterised by the neighbouring and merging of Laurasian and Gondwanan elements is the Wallacean region, which comprises the central islands of Indonesia east of Java, Bali, and Borneo. Although it acts as a potent barrier, many taxa have been able to cross this region and can now be found in Australia and northern parts of East Asia (Jønsson et al., 2008).

The aquatic beetle family Hygrobiidae (Dytiscoidea) presents yet another pattern of distribution. It contains six extant species in the genus *Hygrobia*, four in Australia, one in China and one in the western Palearctic (Fig. 1). *Hygrobia hermanni* is widely distributed throughout Europe, extending into Turkey and North Africa (Nilsson and Van Vondel, 2005). *Hygrobia davidi* is only known from the holotype from Jiangxi, southeastern China. It can be easily distinguished from the west Palearctic *H. hermanni* by various morphological characters (Jäch, 1995) and is clearly not a synonym of any other species. The remaining four species, *Hygrobia australasiae*, *Hygrobia maculata*, *Hygrobia nigra* and *Hygrobia wattsi*, are Australian (Hendrich, 2001).

Hygrobiidae has been proposed to be the sister group to a clade comprising Dytiscidae (diving beetles, 4000 species), Amphizoidae (trout stream beetles, six species) and Aspidytidae (cliff beetles, two species), based on DNA sequence data (Balke et al., 2005, 2008). However, morphological characters suggest Hygrobiidae is the sister of only the family Dytiscidae (Balke et al., 2005, Beutel et al., 2006). In all cases, the monophyly of the family was confirmed. In using Hygrobiidae as the family name, we follow Bouchard et al. (2011, but see Nilsson and van Vondel, 2005).

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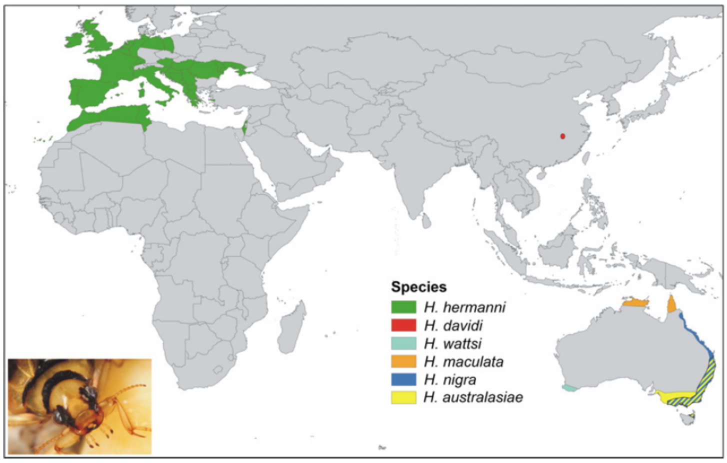

Fig. 1. Global distribution of the Hygrobiidae. The distribution of *Hygrobia davidi* shown here is based on the type locality and has to be confirmed. None of the species occur in sympatry, except for *H. nigra* and *H. australasiae* in south-eastern Australia. The photograph in the lower left corner shows the head of *H. hermanni*.

To our knowledge, Hygrobiidae represents the only metazoan family with a Palearctic-Australian distribution. In this study, we ask whether this highly unusual pattern represents relics of a formerly more widespread distribution or results from more recent dispersal events between Australia, China and the Western Palearctic. We present a molecular phylogeny of Hygrobiidae, testing the monophyly of Australian species. Because *H. davidi* from China is known only from the historical holotype, we use morphological characters to assess its position within the genus. We then use molecular dating techniques to infer the age of relevant nodes and discuss processes that may account for the highly disjunct present-day range of the family.

2. Materials and methods

Adults and larvae were collected with a sturdy aquatic net among submerged sedges, mud, rotten leaves and debris at water depths of 10–60 cm. Beetles were preserved in 96–99% ethanol. Specimens used in this study are listed in Supplement A.1. DNA laboratory protocols follow Balke et al. (2009). Primers are given in Supplementary Table A.2. New data were submitted to GenBank under accession numbers FR873634 to FR873647. Individual beetles from which we extracted and sequenced DNA all bear a green cardboard label that indicates the DNA extraction number of M. Balke (e.g. "DNA 2000 M.Balke"). This number links the DNA sample, the dried mounted voucher specimen and the GenBank entries. Additional sequence data for molecular analyses were obtained from GenBank.

We compiled a morphological dataset after examining all *Hygrobia* species, and we added data from Beutel et al. (2006) for outgroup taxa to identify the position of *H. davidi* within the genus, which is relevant for the interpretation of the results of our molecular phylogeny. We included seven characters of head, pronotum and protarsus in our analysis, which have proven useful in discriminating *Hygrobia* species (see also Supplementary Table A.3).

Our molecular dataset comprises five markers and 2485 aligned base pairs (mitochondrial 16S, *cob* and *cox1*, and nuclear 18S and h3, see Supplementary Table A.2) for all *Hygrobia* species except *H. davidi*, with outgroups representing Dytiscidae, Aspidytidae, Amphizoidae, Noteridae and Haliplidae. Alignment was performed using the program MUSCLE 3.7 (Edgar, 2004) on the CIPRES portal 2.2 (http://www.phylo.org/sub_sections/portal). Pairwise distances were computed using the Kimura-2-parameter model in MEGA 4.0 (Tamura et al., 2007). Model testing was conducted using jModeltest 0.1.1 (Posada, 2008). For the maximum likelihood and Bayesian analyses, we split our dataset into 11 partitions, each one for each ribsosomal marker and one for each codon position of the protein coding markers.

We used raxmlGUI 0.93 (Silvestro and Michalak, 2010) for maximum likelihood analyses with 1000 fast bootstrap repeats. We used MrBayes 3.1 (Huelsenbeck and Ronquist, 2001) on the CIPRES portal for Bayesian analyses, with two runs and four chains with 10,000,000 generations (samplefreq = 1000, conservative 25% burnin) for each partition strategy. Runs were checked for convergence and normal distribution in tracer v1.5 (Rambaut and Drummond, 2005). Finally, we used parsimony searches to infer phylogenetic relations as implemented in the program TNT version 1.1, which we also used to run 500 jackknife (removal 36%) replications to assess node stability (Goloboff et al., 2005) (hit best tree 5 times, keep 10,000 trees in memory).

In our molecular clock approach, we used an ancient fossil and a recent geological calibration point. The former was *Palaeodytes guttata* (Ponomarenko, 1987; Ross and Jarzembowski, 1993), the oldest known dytiscid fossil, from the Karabastau formation in Kazakhstan (160.7–154.8 million years old (Ma) according to Polyanski and Doludenko, 1978 and Walker and Geissman, 2009). The latter were two specimens of *Copelatus* sp. (31k602 and 2vi502, Monaghan et al., 2006) from the Fiji archipelago, endemic to the islands of Kadavu and Viti Levu, whose split is dated to 2.5–1.5 Ma. A possible fossil member of Hygrobiidae is mentioned by Ross and Jarzembowski (1993) from the Miocene (between 23.03 and 5.332 Ma); they are most likely referring to †*Hygrobia cretzschmari* (von Heyden and von Heyden, 1866). Due to the lack of further information on this fossil, we did not use it as a calibration point for our study. However, we assume that the dating yielded by our molecular clock approach would be inappropriate if the origin of the Hygrobiidae was suggested to postdate the Miocene.

We created xml files for use in BEAST 1.5.4 (Drummond and Rambaut, 2007) with BEAUTI 1.5.4. We set the prior for the group containing both *Copelatus* species (representatives of Dytiscidae) to 2.0 Ma (normal distribution, standard deviation 0.2). For the group containing Dytiscidae, Amphizoidae and Aspidytidae, we used a lognormal prior with an offset of 154.8 Ma (mean 2.1, standard deviation 1.0). Thus, we used the dytiscid fossil to infer the minimum age of Dytiscidae after the split from its sister clade, which is composed of Amphizoidae and Aspidytidae. We assumed a relaxed molecular clock approach under the uncorrelated lognormal model, using the partition scheme and substitution models as described for our phylogenetic analyses above, and we chose the Yule process with a constant speciation rate per lineage as the tree prior. We ran BEAST with Markov chains of 30 million generations, sampling every 100 generations. To assess the impact of the prior distributions on the results, we conducted runs with different prior settings to check if changes in standard deviation or use of exponential priors produced significantly different age estimations (Supplementary Table A.3). Additionally, we conducted a "sample from prior only" analysis and conducted test runs (1 million generations, sample every 1000 generations) with only one of the two priors set. Runs were checked for convergence and normal distribution in Tracer v1.5 (Rambaut and Drummond, 2005).

3. Results

Trees produced by RAxML, MrBayes, TNT and BEAST show identical ingroup topologies under all partition strategies (Fig. 2). All analyses were conducted using the GTR + I + G model. In concordance with previous works (Balke et al., 2005, 2008), Hygrobiidae is the sister group of a clade that includes Aspidytidae, Amphizoidae and Dytiscidae. All analyses support the monophyly of the genus *Hygrobia*, with *H. hermanni* as the sister taxon of a clade formed by all its Australian congeners. Among these, *H. maculata* is sister to all other species of the genus, and *H. wattsi* is the sister clade of a clade that includes *H. nigra* and *H. australasiae*.

Our morphological analyses show that *H. hermanni* and *H. davidi* share four derived characters. The Australian *Hygrobia* species share one derived character. Australian and non-Australian Hygrobiidae are not identical in any of the characters checked. Based on these results, *H. davidi* is the sister species of *H. hermanni*. The morphological analysis is presented in detail in Supplementary Table A.4.

Our molecular clock analyses date the split between the Hygrobiidae and the clade composed of Dytiscidae, Amphizoidae and Aspidytidae to 183.91 Ma (95% confidence interval 221.08–162.62 Ma), during the Upper Triassic to Middle Jurassic (Fig. 2). The earliest split within Hygrobiidae, between *H. hermanni* and the Australian Hygrobiidae, is estimated at 126.39 Ma (95% confidence interval 179.23–71.96 Ma), during the Lower Jurassic to Upper Cretaceous. According to our estimates, the earliest split within Australian Hygrobiidae was at 45.46 Ma (95% confidence interval 76.87–23.02 Ma), during the Upper Cretaceous to Eocene. Different prior settings, as described in Supplementary Table A.3, did not result in significantly different age estimates. Test runs show that omitting the recent calibration point (split of Copelatus spp., 2.0 Ma) leads to ages of the nodes of interest that are very similar to those yielded by analyses with both calibration points. By contrast, omitting the older calibration point leads to substantially younger estimates for node ages. The "sample from prior

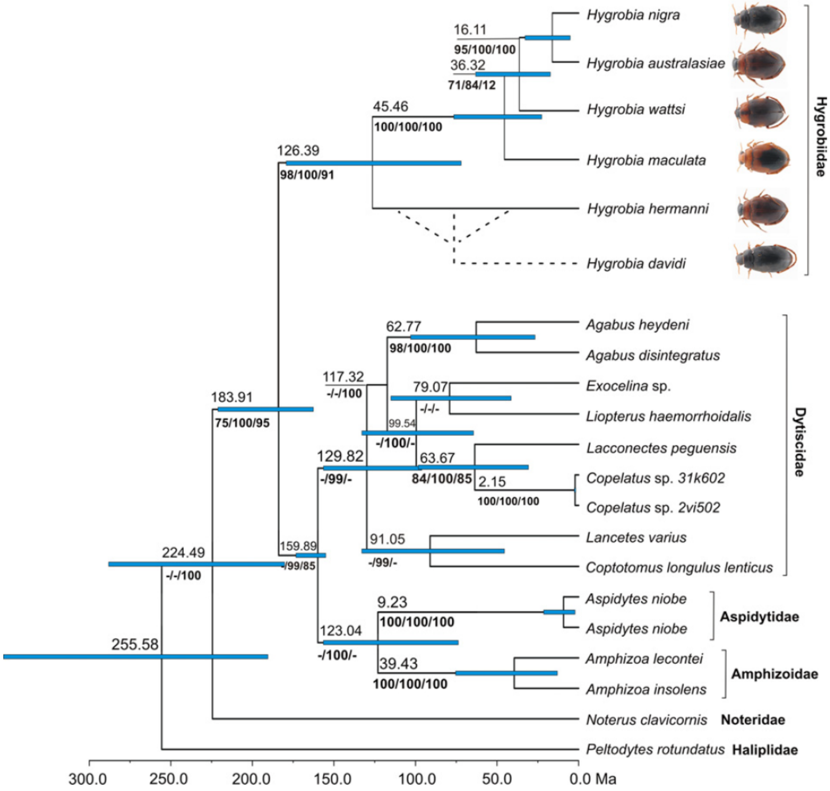

Fig. 2. Chronogram of *Hygrobia* and related families, created with BEAST. This tree was produced using a lognormal prior (offset = 154.8 Ma, mean = 2.1, std. dev. = 1.0) at the node of Amphizoidae + Aspidytidae + Dytiscidae. Node ages are given above nodes in millions of years. Node bars indicate 95% confidence intervals, with ranges in brackets. Support values (maximum likelihood bootstrap/Bayesian posterior probability/parsimony jackknife) are given below nodes. *Hygrobia davidi* was positioned according to the results of the cladistic analysis with uncertain branch length.

only" analysis yields comparatively higher age estimates for uncalibrated nodes.

4. Discussion

An Upper Triassic to Middle Jurassic origin of Hygrobiidae, as proposed here, is much older than the Miocene *Hygrobia* fossil (Ross and Jarzembowski, 1993). However, Hydradephagan beetles are generally scarcely represented in the fossil record, and the absence of earlier fossils does not contradict an older origin of Hygrobiidae, als proposed here. Hunt et al. (2007) date the origin of Hydradephaga to around 219.8 ± 3.89 Ma in the Upper Triassic, with most major lineages splitting already during the Mesozoic, thus supporting the scenario presented here.

Our estimates for the origin of Hygrobiidae, around 183.91 Ma, correspond to a period shortly before the early breakup of the Pangean supercontinent during the Early to Middle Jurassic, around 175 Ma. During this period, Eurasia (as part of Laurasia) and Australia (as part of Gondwana) were already separated by the Tethys and Pacific oceans. At the time of the estimated split between the clade comprising *H. hermanni* and the Australian species in the Lower to Upper Cretaceous, the breakup of Pangea had advanced, and the Antarctic-Australian landmass was moving away from Africa and India. When extant Australian *Hygrobia* species began to diversify in the Upper Cretaceous to Eocene, Australia had moved further away from the other Gondwanan landmasses and was separating from Antarctica.

The ages presented here have to be treated with caution, because only a small dataset and only two reliable calibration points were available to this study. The results of the test runs using only one calibration point and the sample from prior only analysis may be an indication that the sequence data and the recent calibration point (2.0 Ma) constrain the nodes to younger ages than would result from the older calibration point (154.8 Ma) alone. This difference in age estimates may be an effect of the comparatively young age of the recent calibration point and possible effects of island evolution occurring in the *Copelatus* sibling species. However, the hypothesis of a recent origin and subsequent dispersal of Hygrobiidae can be rejected. In our scenario, Hygrobiidae diverged from other Hydradephagan clades around the time of the initial breakup of Pangea. The split between today's Palearctic and Australian clades occurred later. Therefore, one possible explanation for the current distribution might be early colonisation of the already separated landmasses by overseas dispersal. In the middle Mesozoic, distances between the present continents were still relatively short, and many landmasses were connected by smaller continental fragments and islands, allowing for stepping-stone dispersal. Several species of Hydradephagan beetles are known for their good, sometimes exceptional dispersal abilities, e.g., *Rhantus suturalis* (Balke et al., 2009). The dispersal abilities of Hygrobiidae are unknown, but flying specimens have sometimes (though rarely) been caught in light traps and on walls of buildings (specimens of Australian *H. maculata* collected by M. Langer at the Zoologische Staatssammlung München, urn:lsid:biocol.org:col:34660); thus, chance dispersal events seem possible. However, vicariance remains an alternative explanation as neither the molecular dating presented here nor the estimations of paleotectonics are sufficiently precise to exclude the possibility of the origin of Hygrobiidae occurring before or during the Pangean breakup.

In either case, these results make a formerly more widespread distribution of Hygrobiidae seem probable. Whether Hygrobiidae once inhabited Pangea or dispersed shortly after its breakup, Africa and Central Asia must have acted as bridges between the currently known areas of distribution. Two new families of Hydradephagan beetles were discovered during the last decade (Ribera et al., 2002; Balke et al., 2005, 2008). Future field surveys may lead to the discovery of new fossils and extant populations of already known or unknown species also of Hygrobiidae.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.09.015.

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6 A modern view on species concepts

6.1 PAPER: Ecological niche modelling and nDNA sequencing support a new, morphologically cryptic beetle species unveiled by DNA barcoding.

In this chapter I present the case of a cryptic species that had previously not been described because it could not be distinguished by any morphological character studied. In the paper, the species is delimited following an integrative concept, although this is not explicitly formulated so. The lines of evidence – morphology, mitochondrial DNA, nuclear DNA, ecology – correspond (with the exception of ecology) to those described in MIRALLES et al. (2011). Ecology, in the quantifiable form of Ecological Niche Modeling, is introduced as an additional line of evidence. This case underlines that also species that cannot be distinguished from other species by any morphological characters warrant description, if they are supported by other lines of evidence. Not only do they represent separate evolutionary lineages, but – as in the present case – they may also show different responses to environmental factors. The latter is also of importance for applied ecology, conservation biology, and other fields of study that deal not alone with the measuring of biodiversity, but also have an environmental perspective.

HAWLITSCHEK O., PORCH N., HENDRICH L., BALKE M. (2011): Ecological niche modelling and nDNA sequencing support a new, morphologically cryptic beetle species unveiled by DNA barcoding. PloS ONE 6: e16662.

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p. 14: Reference [75] is published, 2011, pp. 243–258 of 544 pp. Reference [76] is published, Global Change Biology 17: 194–205. Reference [77] is published, Biological Journal of the Linnaean Society 101: 155–168. Reference [88] is published, Ecography 33: 607–611.

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Ecological Niche Modelling and nDNA Sequencing Support a New, Morphologically Cryptic Beetle Species Unveiled by DNA Barcoding

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Abstract

Background: DNA sequencing techniques used to estimate biodiversity, such as DNA barcoding, may reveal cryptic species. However, disagreements between barcoding and morphological data have already led to controversy. Species delimitation should therefore not be based on mtDNA alone. Here, we explore the use of nDNA and bioclimatic modelling in a new species of aquatic beetle revealed by mtDNA sequence data.

Methodology/Principal Findings: The aquatic beetle fauna of Australia is characterised by high degrees of endemism, including local radiations such as the genus *Antiporus*. *Antiporus femoralis* was previously considered to exist in two disjunct, but morphologically indistinguishable populations in south-western and south-eastern Australia. We constructed a phylogeny of *Antiporus* and detected a deep split between these populations. Diagnostic characters from the highly variable nuclear protein encoding arginine kinase gene confirmed the presence of two isolated populations. We then used ecological niche modelling to examine the climatic niche characteristics of the two populations. All results support the status of the two populations as distinct species. We describe the south-western species as *Antiporus occidentalis* **sp.n.**

Conclusion/Significance: In addition to nDNA sequence data and extended use of mitochondrial sequences, ecological niche modelling has great potential for delineating morphologically cryptic species.

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

DNA sequencing is an increasingly popular and important tool for the assessment of global species diversity. At present, the mitochondrial cytochrome c oxidase 1 is a standard marker, and "DNA barcoding" or "barcoding" is the name coined for this approach of DNA-based species identification [1–8]. Barcoding is an especially valuable tool for conservation planning, as it provides rapidly releasable quantitative biodiversity data and a glimpse of a level of diversity that extends beyond morphologically delineated entities. Barcoding uses short, standardised sequence segments of the genome, and has proven highly useful when researchers are confronted with high expected species numbers and morphologically cryptic groups ([9–12], see also [13]). As argued in Burns et al. [14], there exist cases in which morphologically and ecologically well distinguishable species exhibit only minimal divergence in their barcodes, and species delimitation by barcoding should not depend on arbitrarily chosen levels of divergence. Similarly, it remains unclear how to deal taxonomically with cases in which morphologically identical populations exhibit certain amounts of divergence in the mitochondrial genome [9,10]. Apparently, the conflict between mtDNA sequence data and morphology requires consideration of other character sources in order to delimit species.

The species concept and the delimitation of species have been a matter of controversy since the early days of systematic biology. Efforts have been made to find a concept which encompasses different approaches to the species problem. DeQueiroz [15] suggested the "Unified Species Concept", which relies on the single definition of species as "existence as a separately evolving metapopulation lineage". Traditional species concepts like the biological [16], ecological [17,18] or genotypic cluster [19] species concepts are "secondary species criteria" or "operational criteria", meaning that not every single criterion must fit every species, but on the other hand more than one of these criteria may be appropriate to a species. They rather act as tools to delimit species.

Among many other possible characters, ecological factors should help delimit species, assuming that each species has formed its own particular niche. However, even for sister-species pairs having detectably distinct niches, the collection of life history data is usually problematic. Ecological Niche Modelling (ENM) is one possible approach to this problem, using widely available environmental data and universally available georeferenced distributional records as a proxy for species ecology. Van Valen

[17] and Andersson [18] argued that species can be understood as groups of individuals occupying the same niche or adaptive zone. Explicit models can be based on species locality data and a raster representing various environmental, mainly climatic, variables (bioclimatic modelling). They have demonstrated their ability in circumscribing species' ecological niches and assessing their potential distributions [20]. Such models always remain restricted to a small selection of environmental variables, but nevertheless have been shown to be capable of predicting potential distributions of species and estimating the impact of the ecological variables studied [21]. Particularly when integrated with phylogenetic studies, ENMs have also proven to be a powerful tool in species delimitation [22–27]. To our knowledge no such studies have yet been conducted for beetles, a group for which generally relatively few analyses using ENM approaches exist [28–30].

Modelling approaches can aid in species delimitation only if the species studied actually diverge in their response to the environmental variables incorporated in the analysis [31]. Evidence suggests that niche conservatism, i.e., the stability of ecological niches over time, is a common pattern in closely related species and that it is a major force driving allopatric speciation [32–37]. Kozak and Wiens [35] postulated that certain North American salamander species are allopatric because of their inability to tolerate the climatic conditions in the lowland areas between their highland habitats, even if these differences appear relatively minor. However, most of these case studies concerned sibling species inhabiting climatically similar areas. Other studies present evidence for niche divergence between sibling species, for distributions of closely related species on environmental axes and for niche divergence as a speciation mechanism [23,38–40]. This apparent contradiction suggests that neither assumption is valid for all groups of organisms and that both cases can occur in closely related species and may contribute to speciation.

We conducted a molecular biodiversity assessment of Australian diving beetles, using 3′ cytochrome c oxidase 1 sequences [41] and found divergence between geographically separated populations of one species, *Antiporus femoralis* (Boheman, 1958). In the absence of morphological differences, we evaluated other data sources and suggest that ecological niche modelling and nDNA characters provide evidence for the presence of a new, cryptic species which we will describe below.

Materials and Methods

Study group: Australian diving beetles

Australia's diverse and highly characteristic diving beetle (Dytiscidae) fauna offers many opportunities to study speciation and radiation events. To date, almost 300 dytiscid species are known, of which approximately 90% are endemic to the continent, belonging to 18 or 19 exclusively Australian radiations [42–47]. Many endemic species of diving beetle are not widespread, but rather restricted to certain climatic regions, river drainage systems or other geographical features. In southern Australia, the arid Nullarbor Plain with a West-East-extent of more than 1200 kilometres acts as a very potent geographical barrier for freshwater organisms due to its arheic conditions and its virtual lack of surface water [48]. Many groups, including the diving beetles, show patterns of disjunct distributions in south-western and south-eastern Australia, excluding the Nullarbor Plain. Geological evidence shows that this situation is a result of rather recent events [49–52]. During the Miocene, vast stretches of southern Australia, including the Nullarbor Plain, were covered by seas during marine intrusions, with lush tropical forests growing in the humid climate along its coast. Only after regression of sea levels, from about 10 to 6.6 million years before present, did the area fall dry and the humid conditions make way for today's arid climate.

The genus *Antiporus* Sharp, 1882 (tribe Hydroporini Aubé, 1836), with 16 described species to date [53–57], is distributed in still or slow-flowing water, mainly in south-eastern and south-western Australia, along the east coast of the continent and with one species in the Northern Territory, north-western Australia and northern Queensland. An additional species is distributed widely across New Zealand in different habitats. Watts [54] described two additional species from Western Australia, *A. pembertoni* Watts, 1997 and *A. hollingsworthi* Watts, 1997. Four additional species (*A. mcraeae* Watts and Pinder, 2000, *A. pennifoldae* Watts and Pinder, 2000, *A. gottwaldi* Hendrich, 2001 and *A. kalbarriensis* Hendrich and Watts, 2010) have been described recently. Most *Antiporus* species are restricted to the southwest, to the eastern coast or to south-eastern Australia, and some show remarkable regional endemism. However, two disjunct populations of *A. femoralis* (Boheman, 1958) have been reported from south-western and south-eastern Australia and were considered conspecific because of the lack of morphological differences (e.g. Watts [53], Brancucci [58]). In this study, we focus on these two *A. femoralis* populations.

DNA sequencing and data analysis

We preserved a part of our collections in pure ethanol in the field and later extracted DNA for sequencing, employing methods explained in detail in Balke et al. [59] and Hendrich and Balke [60].

For a population-level screening of all Australian diving beetles, we sequenced the 3′ end of cytochrome c oxidase 1 (cox1) [41]. In a second step, we sequenced additional genes to infer phylogenetic relations within the present focal clade, *Antiporus.* Genes and primers used for sequencing are given in Table S1. After detection of a possible cryptic species, we sequenced a fragment of the nuclear protein coding gene arginine kinase (ARK).

Sequences were submitted to GenBank and are publicly available under accession numbers FR727264 to FR727325 and as part of a general *cox1* dataset of Australian Dytiscidae (FR 732513 to FR 733591). Individual beetles from which we extracted and sequenced DNA all bear a green cardboard label that indicates the DNA extraction number of M. Balke (e.g. "DNA 2000 M.Balke"). This number links the DNA sample, the dry mounted voucher specimen and GenBank entries.

We ran analyses for two separate datasets. Dataset one included 24 specimens from all available *Antiporus* species and four outgroup taxa, and 2953 characters over all five DNA loci. Dataset 2 included 70 specimens: all available *A. femoralis* specimens (n = 30) and other *Antiporus* species as outgroups. We used 799 characters of cytochrome c oxidase 1 only. The following analyses were all performed on the CIPRES portal 2.2 [61] unless stated otherwise. Both datasets were aligned using the program MUSCLE 3.7 [62]. We used jModeltest 0.1.1 [63] to choose appropriate substitution models.

We ran maximum likelihood analyses using the program GARLI [64] until 10,000 generations revealed no significant improvement of likelihood scores of the topology. We then ran resampling with 250 bootstrap replicates.

We also used Bayesian analyses with the program MrBayes 3.0 [65]. Each of two runs consisted of 4 chains which ran for 1,000,000 generations, with samplefreq = 1,000 and 25% burnin fraction. Convergence between runs and posterior probabilities of the estimates was determined by plotting the log likelihoods in Excel.

Finally, we used parsimony searches to infer phylogenetic relations as implemented in the program TNT version 1.1 (on a local desktop computer), which we also used to run 500 jackknife (removal 36%) replications to assess node stability [66] (hit best tree 5 times, keep 10,000 in memory).

Pairwise distances were computed using the Kimura 2-parameter model in MEGA 4.0 [67].We used the sequence editor Se-Al v2.0a11 (http://tree.bio.ed.ac.uk/software/seal/) to detect diagnostic characters.

Ecological niche modelling

We used the Maxent 3.3.2 [68] software for modelling the potential distribution of the two major clades of *A. femoralis* detected in the phylogenetic analyses. Maxent follows the Maximum Entropy principle [69] and combines presence-only data and environmental layers to create a gridded model of the potential distribution of the target species. Several studies have shown that Maxent produces better results than comparable methods [70,71] and have confirmed its ability to predict a species' distribution outside its known range [72–75]. It has also been frequently used in phylogeographic studies [76,77], some having taxonomic implications [24,27,78]. We obtained a total of 80 distribution points of *A. femoralis* (61 from eastern Australia and 19 from western Australia, Table S2) from our own databases (Hendrich unpublished) and from the ANIC database (http://anic.ento.csiro.au/database/biota_details.aspx). We excluded a single doubtful New Zealand locality that might refer to *A. uncifer* Sharp, 1882. Climate data was obtained from the worldclim database ([79], http://www.worldclim.org). We used the bioclimatic variables at a resolution of 2.5 arc-minutes. These 19 variables likely summarise dimensions of climate of special importance for determining species distributions [80].

As proposed by several authors [81,82], inclusion of too many of these climate variables may cause "over-fitting" problems, as many represent similar and highly correlated dimensions of climate. Furthermore, a specific selection of predictors according to natural history properties of the target species may significantly enhance the reliability of ENMs [37]. Rödder and Lötters [83] also showed that transferability of models across space requires careful attention. To avoid misleading results, Environmental variables should be chosen with special care when models are used to predict species' distributions outside their native range.

A. femoralis inhabits summer-dry wetlands and rest pools of small rivers and creeks having a high seasonal variation in water volume, many of which fall almost completely dry during the dry season (November to March). Thus, precipitation and its seasonal variation is the climatic factor assumed to have the highest impact on the long-term persistence of *A. femoralis* populations. Temperature may also be important as higher insolation and thus higher temperature causes drought. Therefore, aside from "annual mean temperature" and "annual precipitation", we chose factors representing the interaction of precipitation and temperature and the seasonality of these factors, i.e., "precipitation warmest quarter", "precipitation coldest quarter" and "precipitation seasonality". This latter factor gives a direct measurement of the strength of the seasonality, whereas values of precipitation of the warmest and coolest quarters indicate its direction.

We used the default Maxent settings with a random test percentage of 25% of the input localities set aside for model testing. We chose the logistic output format, displaying suitability values from 0 (unsuitable) to 1 (optimal) [84]. Jackknifing was performed to measure the importance of the variables. Model validation was conducted by calculating the area under the curve (AUC), which reflects the model's ability to distinguish between presence records and random background points [68,85]. AUC values range from 0.5 for models without any predictive ability to 1.0 for models with perfect predictive ability. According to Swets [86], AUC values >0.9 are considered to have 'very good', >0.8 'good' and >0.7 'useful' discrimination abilities.

We performed ENMs using locality data of *A. femoralis* from eastern Australia and of *A. femoralis* from Western Australia, restricting background data to areas likely to be colonizable for the species as recommended by Phillips et al. [87] Therefore, we manually delimited areas encompassing the known localities, separating them from completely arid areas away from the coast (Fig. S1). We also performed an ENM using data from all *A. femoralis* individuals pooled together. All runs were performed with 100 bootstrap repeats. Test localities were randomly selected anew for each repeat, and mean output values were used as final results.

For further statistical analysis of the modelling results, we used the ENMtools software [88]. We measured niche overlap of *A. femoralis* from eastern Australia and of *A. femoralis* from Western Australia using Schoener's D [89] and the I statistic, modified from the Hellinger distance [90].

We also used two hypothesis tests included in ENMtools. First, we used the niche identity test to determine whether the ENMs generated for the two species are identical or exhibit statistically significant difference. The test combines the samples of both species into a common pool. Under the assumption that the species behave interchangeably in their use of ecological niche space, their identities are randomized, and two new samples with the same sizes as the original samples are extracted. By repeating this process, a set of pseudoreplicates is generated. The results are compared with the true calculated niche overlap (see above). The lower the true niche overlap in comparison to the scores created by the pseudoreplicates of the pooled samples is, the more significant the niche difference between the two species compared.

Second, we used the background test to evaluate the null hypothesis that all divergence in the ecological niches of two taxa, given that the niches are represented by two sets of localities, can be explained by the differences in their environmental feature spaces. Specifically, we use it to ascertain whether ENMs of *A. femoralis* from eastern Australia and of *A. femoralis* from western Australia are more or less similar than expected based on the environmental differences in their completely disjunct ranges. This test is particularly appropriate for allopatric species because in many cases, distinct geographic spaces provide a different set of environmental conditions. That is, differences in ENMs may result from niche space availability rather than from niche diversification [30]. The test places random occurrence points within the range of one of the two species to be compared and measures niche similarity between these points and the original localities of the second species. If the true measured overlap values are significantly higher (or lower) than the values generated by the background test, the null hypothesis that ENMs are more similar (or divergent) than based on habitat availability is rejected. This test is conducted in both directions, and different directions may yield different results.We performed the identity test, as well as background tests in both directions, with 500 iterations.

Morphology and taxonomy

Specimen depositories:

ANIC	Australian Insect Collection, Canberra, Australia
CFP	Collection Fernando Pederzani, Ravenna, Italy
CLH	Collection Lars Hendrich, Berlin, Germany, property of NMW
CSR	Collection Saverio Rocchi, Firenze, Italy
NMW	Naturhistorisches Museum Wien, Austria

SAMA	South Australian Museum, Adelaide, South Australia, Australia
WAM	Western Australian Museum, Perth, Western Australia, Australia
ZSM	Zoological State Collection, Munich, Germany

Beetles were examined using a Leica MZ 12.5 dissecting scope at 10–100x. Male genitalia were studied and figured in wet conditions. Images of male genitalia were made using incident light and a digital photo imaging system, composed of a Leica DM 2500 M microscope and a Tucsen 5.0 MP camera. The microscope was fitted with Leica HCX PL "Fluotar" 5x and 10x metallurgical grade lenses [91]. Habitus images were taken with a Nikon D700, equipped with a bellows and Leica Photar 2.8/25 mm lens. Image stacks were aligned and assembled in Helicon Focus 4.77TM.

The terminology to denote the orientation of the genitalia follows Miller and Nilsson [92]. Coordinates are given in decimal notation unless cited verbatim from labels. To determine the position of these localities, we used various Australian road maps and Google Earth (http://earth.google.com).

Nomenclatural acts

The electronic version of this document does not represent a published work according to the International Code of Zoological Nomenclature (ICZN), and hence, the nomenclatural acts contained in the electronic version are not available under that Code from the electronic edition. Therefore, a separate edition of this document was produced by a method that ensures numerous identical and durable copies, and those copies were simultaneously obtainable (from the publication date noted on the first page of this article) for the purpose of providing a public and permanent scientific record, in accordance with Article 8.1 of the Code. The separate print-only edition is available on request from PLoS by sending a request to PLoS ONE, 185 Berry Street, Suite 3100, San Francisco, CA 94107, USA along with a cheque for US $10 (to cover printing and postage) payable to "Public Library of Science".

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Zoologische Staatssammlung (Munich, urn:lsid:biocol.org:col:34660).

Results

Molecular phylogenetics

jModeltest selected the GTR+G model for all gene regions but cytochrome c oxidase 1 and histone 3, for which the GTR+I+G model was selected. These models were used for all further analyses. Where partitioning was not possible, the GTR+G model was used.

Maximum likelihood, Bayesian and parsimony analysis of a multigene dataset of Australian *Antiporus* all yielded very similar topologies with generally significant node support values (Fig. 1). Four specimens that we initially identified as *Antiporus femoralis* always formed a monophyletic group, but the single Western Australian specimen diverged from the remaining three specimens, all from the eastern part of Australia, by 6.5%. The sister species of that clade is either *A. interrogationis* or *A. gilbertii*. The Bayesian analysis supported *A. interrogationis* as sister taxon to the *A. femoralis* clade. Maximum likelihood and parsimony analyses yielded a clade comprising *A. interrogationis* and *A. gilbertii* as sister group to the *A. femoralis* clade, albeit with support values of less than 60 in both cases (not shown).

Analysis of cox1 for 30 specimens from the *A. femoralis* clade clearly confirmed a subdivision into a western and an eastern clade (Fig. 2). Within the eastern and western groups of *A. femoralis*, pairwise distances were 0.0% to 2.9% (mean 0.7%±0.7%) for eastern and 0.0% to 1.0% (mean 0.5%±0.3%) for western *A. femoralis*. The divergence between the two clades was 3.5% to 6.6% (mean 4.46%±0.6%).

Within eastern *A. femoralis*, only specimens from South Australia seem to form a monophyletic group, but this clade of three individuals is not significantly supported. The only morphologically divergent specimen, which is larger and darker and originates from Tasmania ("DNA M. Balke 2099"), is nested in a clade comprising specimens from New South Wales and Victoria.

A 510-bp fragment of the nuclear protein coding gene arginine kinase was successfully amplified for specimens from both clades. The sequence divergence was 1.39%, and six parsimony-informative sites were identified.

Ecological Niche Modelling

Ecological niche models are visualised in Fig. 3. According to their AUC values, the ability to distinguish presence from random background points of all models was larger than 0.9 and thus considered 'very good' according to the classification of Swets [86]. AUC values were 0.982 for the ENM of eastern and 0.993 for the ENM of western *A. femoralis*. The ENMs of both species together had a slightly lower AUC of 0.977.

Analysis of the environmental variable contribution showed that for the distributions of eastern as well as western *A. femoralis*, "precipitation coldest quarter" was the variable of highest importance (Table S3). "Annual mean temperature" and "annual precipitation" were the second and third most important predictors in the models of eastern *A. femoralis* and of both groups together. "Annual mean temperature" also provided the highest training gain when used in isolation. For western *A. femoralis*, "precipitation warmest quarter" and "precipitation seasonality" were the second and third most important variables. For the model that included both species, variable importance was similar to that found for eastern *A. femoralis*.

The measured niche overlap between eastern and western *A. femoralis* was $I = 0.454$ and $D = 0.192$. Values close to 0 describe little overlap in ecological niches and values close to 1 describe high similarity. The overlap between the niches of eastern and western *A. femoralis* can therefore be considered low, judging from these values alone. Note that values of D are generally lower than of I.

The results of the identity and background tests are shown in Fig. 4. According to the identity test, the null hypothesis of niche identity is rejected, meaning that the climate envelopes of eastern and western *A. femoralis*, as modelled here, are highly significantly distinct. In the background test, the null hypothesis that differences in the ecological niches can be explained by environmental differences in their areas of occupancy alone is rejected. The

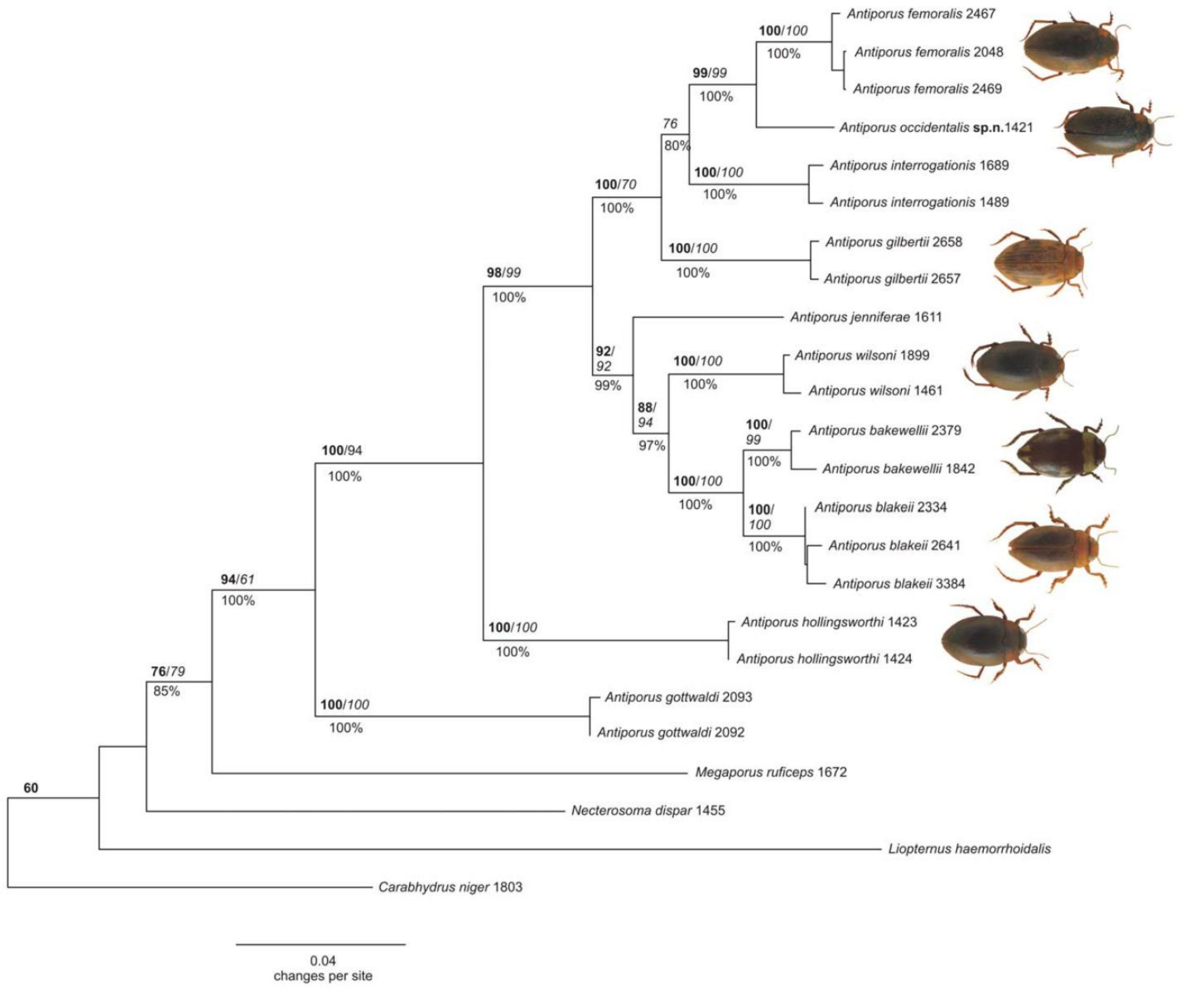


Figure 1. Phylogram of the genus *Antiporus*. The phylogram is based on a maximum likelihood tree with 5 gene loci and 2953 characters made in GARLI. Branch values are: GARLI bootstrap (bold/above branch), TNT jackknife (italic/above branch), and MrBayes posterior probability (below branch). Each tip represents one specimen. Specimen collection numbers are given after the species name.
doi:10.1371/journal.pone.0016662.g001

niches are significantly (I and D) more similar than expected based on the distribution of eastern *A. femoralis* and significantly (I only) more different based on the distribution of western *A. femoralis*.

Taxonomic treatment

Evidence from mtDNA and nDNA sequences, combined with results of ecological niche modelling, suggests presence of two species. *Antiporus femoralis* was described from New South Wales: Sydney, within the geographical range of the eastern clade. Thus, we assign the new species name *A. occidentalis* **sp.n.** to the western clade.

Antiporus occidentalis sp.n.

Fig. 5b.

http://www.species-id.net/w/index.php?title=Antiporus_occidentalis&oldid=2012

urn:lsid:zoobank.org:act:481E4A90-4127-4B33-B878-A35F33A0A35F

Type locality. Australia: Western Australia, Lane Poole Conservation Reserve, Nalyerin Lake.

Type material. Holotype: Male: "AUSTRALIA/WA: Lane Poole Conservation Reserve, Nalyerin Lake, 300 m, 29. & 30.12. 1999, Hendrich leg. (loc.4/151)", "DNA M.Balke 3757", [green printed label], "HOLOTYPE *Antiporus occidentalis* **sp.n.** des. 2010" [red label, printed] (WAM).

Paratypes. 8 specimens with same locality data as holotype (7 specimens with "DNA M.Balke 3750", "3751", "3752", "3753", "3754", "3755", "3756" [green labels, printed]) and "PARATYPE *Antiporus occidentalis* **sp.n.** Hawlitschek, Hendrich & Balke des. 2010" [red label, printed] (SAMA, CLH, ZSM); 2 exs., "AUSTRALIA, WA, 10 Km S Cataby, Brand Highway, Nammegarra Road, 9.9.2002, 30°53′S 115°36′E, Hendrich leg./Loc. 29/193" (1 specimen with "DNA M. Balke 1421" [green label, printed]) (CLH); 1 ex., "Australia, WA/North of Bunbury, Yalgorup N.P., east of Preston Beach, 0 m, 24.11.1996, L. Hendrich leg./Coll. Lok. 30" (CLH); 3 exs., "Australia,WA/Nannup, "Wildflower Walk" n. Nannup 100 m, 25.11.1996, L. Hendrich leg./Coll. Lok. 32" (CLH); 6 exs., "Australia, WA/Nannup, Balingup-Nannup Road, Revelly Bridge, 130 m, 25.11.1996, L. Hendrich leg./Coll. Lok. 33" (CLH); 1 ex., "Australia, WA/5 km S Northcliffe, 10 m, 27.11.1996, L. Hendrich leg./Coll. Lok. 37" (CLH); 1 ex., "Australia, WA/20 km NW Walpole, Interstate Hwy. No. 1, 27.11.1996, L. Hendrich leg./Coll. Lok. 38" (CLH); 2 exs.,

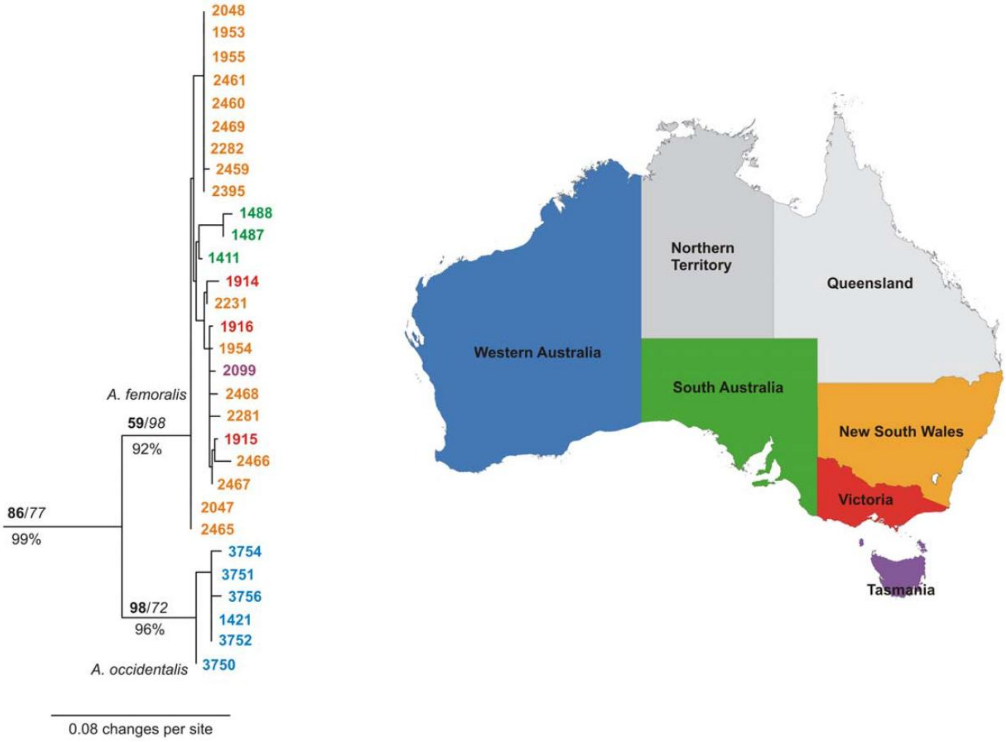


Figure 2. Phylogram of *Antiporus femoralis* and *Antiporus occidentalis* sp.n. Tree based on a cytochrome c oxidase 1 tree with 799 characters made in GARLI. Branch values are: GARLI bootstrap (bold), TNT jackknife (italic), and MrBayes posterior probability (below branch). Each tip represents one specimen. Outgroups (*A. interrogationis*, *A. jenniferae*, *A. wilsoni*, *A. bakewellii*, *A. blakeii*, *A. gilbertii*, *A. hollingsworthi*, *A. gottwaldi* and *Sternopriscus eikei*) are not shown. Colours of specimen numbers represent their state of origin, see map of Australia on the right.
doi:10.1371/journal.pone.0016662.g002

"Australia, WA/Walepole-Nornalup N.P., Peaceful Bay, 0 m, 28.11.1996, L. Hendrich leg./Coll. Lok. 39" (CLH); 3 exs., "Australia, WA/Stirling Range N.P., Stirling Range Drive in Richtung Red Gum Pass, 450 m, 29.11.1996, L. Hendrich leg./ Coll. Lok. 41" (CLH); 1 ex., "Australia (WA), Nannup envir., roadside creeks, 1.12.95 Pederzani" (CFP); 16 exs., "Australia (WA), Pemberton, pond, Della Franca farm, 3.12.98 Pederzani" (CFP, CSR); 2 exs., "AUSTRALIA/WA: Nannup, Balingup-Nannup Road, Revelly Bridge, 130 m, 31.12.1999, Hendrich leg. (loc.6/153)" (CLH); 3 exs., "AUSTRALIA/WA: 5 km S Northcliffe, 50 m, 2.1.2000, Hendrich leg. (loc.10a/156)" (CLH); 2 exs., "AUSTRALIA/WA: D'Entrecasteaux N.P., 15 km S Northcliffe, Windy Harbour Road, 50 m, 3.1.2000, Hendrich leg. (loc. 10c/ 156)" (CLH); 1 ex., "AUSTRALIA/WA: Albany Hwy, Muir Lakes Nature Reserve, SW part of Byenup Lagoon, 4.&5.1.2000, Hendrich leg. (loc. 11/157)" (CLH). 1 ex., "WA Cannington 14/ 08/1924/32°01'00"S 115°57'00"E L. Glauert leg." [40086] (WAM); 1 ex., "WA Cokatea Creek Tenindewa 8/01/1926" [40708] (WAM); 1 ex., "WA Wanneroo Melaleuca Park, 14/08/ 1976 31°40'25"S 115°53'23"E Southwell-Keely leg." [42685] (WAM); 3 exs., "WA Banksiadale 01/05/1969 32°38'S 116°06'E D.S. Adair leg." [42736, 42737, 42738] (WAM); 3 exs., "WA Bullsbrook Tortoise Reserve 10/1963 31°39'S 115°59'E Zoological Honours Class leg." [42739, 42740, 42741] (WAM).

Etymology. A western Australian species.

Description. Body in dorsal view rotundate-oval, convex, widest behind the middle. http://www.species-id.net/o/index.php?title=File:Antiporus_occidentalis_dorsal.jpg&oldid=109760.

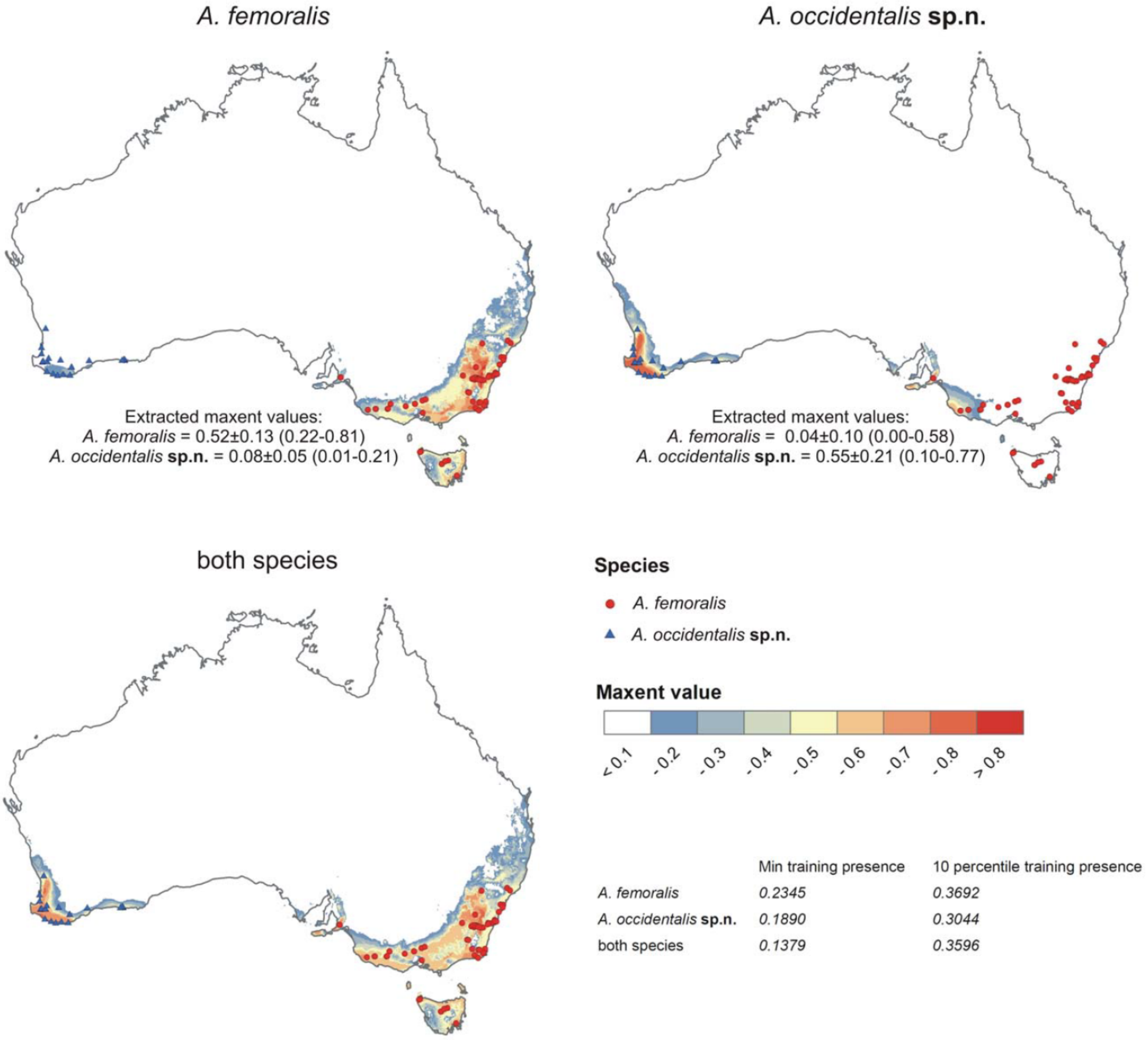
Measurements. Total length of beetle = 4.6–4.9 mm (holotype 4.8 mm); total length without head = 4.4–4.7 mm (holotype 4.6 mm); maximum width = 2.3–2.5 mm (holotype 2.4 mm).

Colour. Upper side reddish brown; some portions with small and less extended dark brown or black patches. Head uniformly black, reddish brown on the anterior part. Antenna testaceous, distal joint apically darkened. Pronotum reddish brown with large patch on middle part which does not reach the anterior border. Elytra reddish brown with small and less extended dark brown or black patches (Fig. 5b). Venter black, including pronotum, epipleuron, metaventrite, metacoxal plate and prosternal process. Legs and abdominal sternites reddish brown.

Sculpture. Head finely microreticulated, regularly and densely punctured, coarser around the clypeal grooves. Interstices between punctures larger than the diameter of the punctures, particularly on the disc.

Pronotum semi-matt, very finely microreticulated. Sides of pronotum regularly and gently curved. Puncturation regular on the whole surface, except on a round area situated on both sides of the disc where the punctures are more sparse and on the lateral border where they are coarser and very close. Pronoto-elytral angles obtuse.

Puncturation on elytra regular and very dense, covering the whole surface. The interstices between punctures are narrower than the diameter of punctures, but less so on the apical half. Ground sculpture finely microreticulated, semi-matt on the basal half, shagreened on the apical half.



	Min training presence	10 percentile training presence
A. femoralis	0.2345	0.3692
A. occidentalis **sp.n.**	0.1890	0.3044
both species	0.1379	0.3596

Figure 3. Ecological niche models. Localities of *Antiporus femoralis* (blue triangles) and *Antiporus occidentalis* **sp.n.** (red circles), displayed on the backgrounds of Maxent-created ecological niche models. Higher Maxent values (yellow and red colours) represent areas more suitable for the species according to the Maxent models, lower values (green and blue or white colours) represent areas less suitable.
doi:10.1371/journal.pone.0016662.g003

Ventral surface; prosternal process narrowly lanceolate, rounded tip, weakly carinate in cross section, slightly narrowed between procoxae. Metacoxal lines raised, moderately separated, subparallel in posterior half, diverging to about twice their narrowest width in anterior half. Metacoxae and sternites very strongly punctured.

Male. Pro- and mesotarsi moderately expanded, robust; single proclaw thickened, sharply curved and with a small tooth near base. Metafemora slightly incised into a triangular process near apex. Last abdominal sternite rounded in middle. Parameres broad and rounded. Median lobe of aedeagus in ventral view very broad, strongly bilobed towards tip (Fig. 5d), in lateral view rather thin and elongated. Minor differences between median lobi of *A. femoralis* and *A. occidentalis* **sp.n.** (Fig. 5) are attributed to individual variability.

Female. Pro- and mesotarsi narrower than in males, not expanded. Proclaws simple. Mesotibia narrow.

Affinities. The new species is the sister species of *A. femoralis* and cannot be separated using morphological characters such as size, colour and form of median lobe (Fig. 5). However, the species are allopatric: *Antiporus occidentalis* **sp.n.** occurs in south-western Australia, and *A. femoralis* in south-eastern Australia, south of Brisbane, along the east coast to Victoria, South Australia and Tasmania.

Distribution. South-western Australia. South of a line from Carnavon to the Stirling Ranges (Fig. 3).

Habitat. *Antiporus occidentalis* **sp.n.** was collected from shaded or at least half-shaded pools, peatland swamps and lakes, overgrown roadside ditches and rest pools of intermittent creeks (Fig. S2), from the coast (Preston Beach near sea level) up to 450 m in the Stirling Ranges. In contrast to the south-eastern Australian *A. femoralis*, it seems that the species prefers more peaty water with a dark bottom consisting of mud, peat and plant debris.

Antiporus femoralis (Boheman, 1858)

Fig. 5a.

Hydroporus femoralis Boheman, 1858: 19.

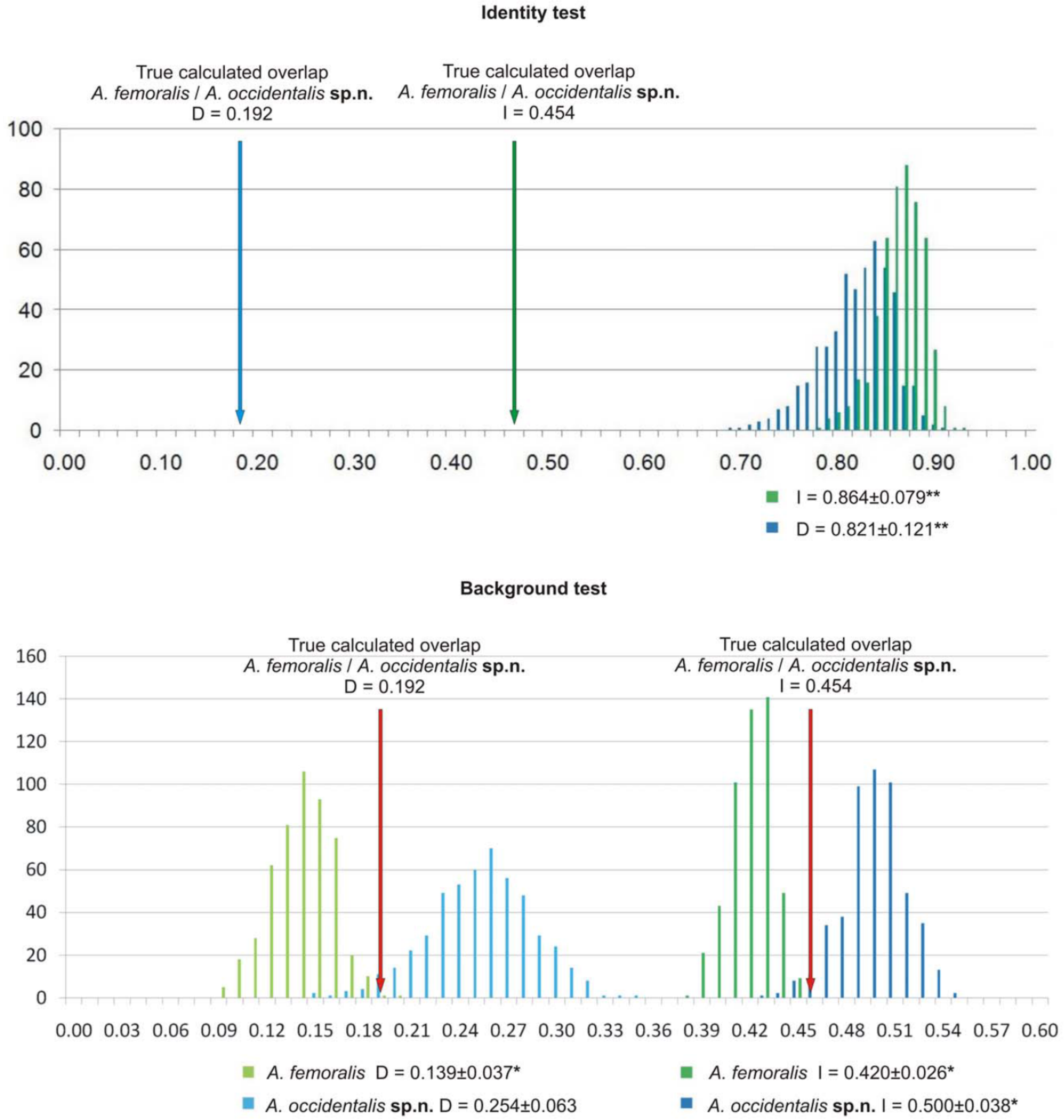


Figure 4. Results of the identity and background tests. Arrows indicate the results of ENMtools' niche overlap test representing the true calculated niche overlap. Columns represent the niche overlap values created in the replicates of the identity and background tests. The true calculated overlap values (I and D) are far outside the 99.9% confidence intervals of the identity test results and thus highly significant (indicated by two asterisks **). For the background tests, results are given for *A. femoralis* (compared to the background of *A. occidentalis* **sp.n.**) and for *A. occidentalis* **sp.n.** (compared to the background of *A. femoralis*). If marked with an asterisk *, the true calculated niche overlaps are outside the 95% confidence intervals but not outside the 99.9% confidence intervals of the background test results and are therefore significant.
doi:10.1371/journal.pone.0016662.g004

Antiporus femoralis (Boheman, 1858): Watts 1978: 67; Brancucci 1984: 151; Watts 1997: 36.

Type locality. Australia: New South Wales, Sydney.

Material examined. New South Wales: 2 exs., C NSW, 25 km N Wollongong, Darkes Forest, Maddens Fall Lookout, 480 m, 29.X.2006, 34.13.335S 150.54.465E, L. & E. Hendrich leg. (NSW 86); 2 exs., C NSW, 17 km SE Nowra, Jerwis Bay NP, Coonemia Road, 54 m, 31.X.2006, 34.58.156S 150.43.045E, L. & E. Hendrich leg. (NSW 90); 2 exs., C NSW, 1 km N Nowra, Bomaderry, Bomaderry Creek, 71 m, 31.X.2006, 34.50.383S 150.35.509E, L. & E. Hendrich leg. (NSW 91); 3 exs., C NSW, 10 km S Nowra at Falls Creek, Parma Creek, 27 m, 1.XI.2006, 34.58.104S 150.35.415E, L. & E. Hendrich leg. (NSW 92); 2 exs., C NSW, 40 km SW Nowra, Braidwood Road, Tianjara Creek, 498 m, 1.XI.2006, 35.06.382S 150.20.037E, L. & E. Hendrich leg. (NSW 93); 2 exs., C NSW, Endrick River at Braidwood Road,

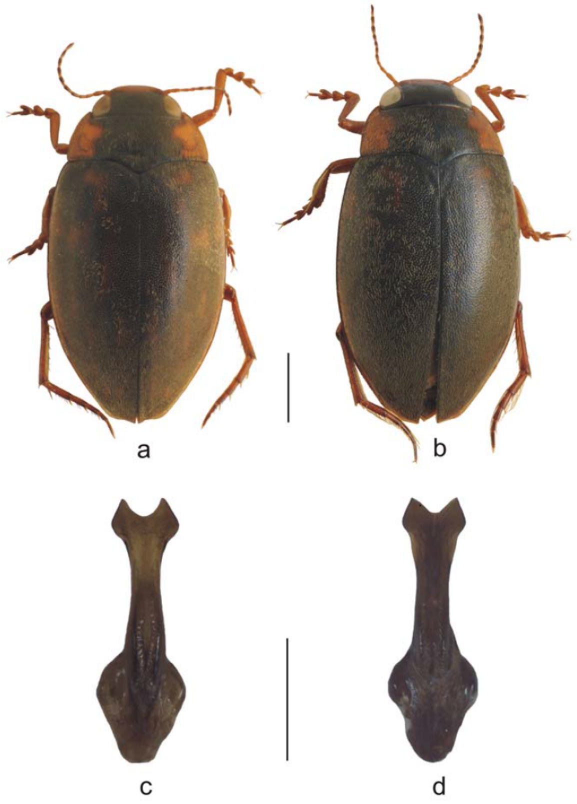


Figure 5. Habitus photographs. Habitus of a) *Antiporus femoralis* (male, SE Australia), b) *A. occidentalis* **sp.n.** (male, SW Australia) (scale bar 1 mm). Ventral views of median lobes of aedeagi of c) *A. femoralis* and d) *A. occidentalis* **sp.n.** (scale bar 0.4 mm). Minor differences between median lobi of *A. femoralis* and *A. occidentalis* **sp.n.** (c, d) are attributed to individual variability. Photos: L. Hendrich.
doi:10.1371/journal.pone.0016662.g005

554 m, 1.XI.2006, 35.05.193S 150.07.182E, L. & E. Hendrich leg. (NSW 94); 8 exs., C NSW, 48 km NE Braidwood, Corang Creek, 589 m, 1.XI.2006, 35.10.488S 150.04.101E, L. & E. Hendrich leg. (NSW 95); 2 exs., C NSW, 10 km W Braidwood, Shoalhaven River at Bombay Bridge, 628 m, 2.XI.2006, 35.25.419S 149.42.582E, L. & E. Hendrich leg. (NSW 96); 10 exs., S NSW, 2 km NE Queanbeyan, Molonglo Gorge, 584 m, 12.XI.2006, 35.19.313S 149.15.029E, L. & E. Hendrich leg. (NSW 101); 2 exs., S NSW, 3 km N Jindabyne, Wollondibby Creek, 928 m, 13.XI.2006, 36.23.406S 148.35.533E, L. & E. Hendrich leg. (NSW 102); 2 exs., S NSW, Mt. Kosciusko NP, Diggers Creek (Alpine lake), 1517 m, 14.XI.2006, 36.21.357S 148.29.281E, L. & E. Hendrich leg. (NSW 106); 4 exs., S NSW, 12 km SW Delegate, Bog Road, 812 m, 15.XI.2006, 37.04.356S 148.53.260E, L. & E. Hendrich leg. (NSW 108); 1 ex., S NSW, Imlay Road, White Rock Picnic Area, 497 m, 15.XI.2006, 37.08.039S 149.21.324E, L. & E. Hendrich leg. (NSW 109); 1 ex., S NSW, Imlay Road, 8.5 km E from Monaro Hwy to Eden, 564 m, 15.XI.2006, 37.08.029S 149.25.028E, L. & E. Hendrich leg. (NSW 110); 2 exs., S NSW, 6.5 km SW Eden, Towamba Road 2 km N Nullica, 556 m, 16.XI.2006, 37.04.412S 149.51.200E, L. & E. Hendrich leg. (NSW 111); 1 ex., S NSW, Wallagaraugh River Picnic Area, 43 km SW Eden, 54 m, 17.XI.2006, 37.22.079S 149.43.073E, L. & E. Hendrich leg. (NSW 112). Victoria: 2 exs., E VIC, Tonghi River at Hwy 1 3–5 km SW Cann River, 126 m, 17.XI.2006, 37.33.503S 149.03.546E, L. & E. Hendrich leg. (VIC 115); 1 ex., S VIC, Simpsons Creek 12 km SW Orbost at Princess Hwy, 31 m, 18.XI.2006, 37.45.095S 149.20.436E, L. & E. Hendrich leg. (VIC 116); 2 exs., C VIC, Hughes Creek at Avenel, 161 m, 25.XI.2006, 36.54.221S 145.14.191E, L. & E. Hendrich leg. (VIC 120); 2 exs., C VIC, 5–7 km W Puckapunyal, street to Tooborac, 205 m, 25.XI.2006, 37.00.282S 144.58.415E, L. & E. Hendrich leg. (VIC 122); 3 exs., C VIC, Kyneton, Boggi Creek, Mineral Springs Picnic Area, 485 m, 26.XI.2006, 37.14.094S 144.25.259E, L. & E. Hendrich leg. (VIC 123); 3 exs., W VIC, Grampians, 7 km NW Dunkeld, street to Lavendish, 229 m, 27.XI.2006, 37.38.510S 142.17.507E, L. & E. Hendrich leg. (VIC 125); 3 exs., W VIC, Grampians, Wannon River, 5 km N Dunkeld, 236 m, 27.XI.2006, 37.37.494S 142.20.226E, L. & E. Hendrich leg. (VIC 126); 2 exs., W VIC, Grampians, Fyans Creek, 15 km S Halls Gap, 363 m, 27.XI.2006, 37.14.595S 142.32.240E, L. & E. Hendrich leg. (VIC 128). South Australia: 1 ex., SE SA, 10–12 km N Mt. Gambier, Mt. Gambier Forest Reserve, 77 m, 29.XI.2006, 37.42.308S 140.47.523E, L. & E. Hendrich leg. (SA 135); 3 exs., SA, Meadows Creek at Kuitpo Forest, 286 m, 3.XII.2006, 35.12.367S 138.42.004E, L. & E. Hendrich leg. (SA 137). Tasmania: 1 ex., NW TAS, Montagu River at Togari, 41 m, 12.XII.2006, 40.54.545S 144.52.399E, L. & E. Hendrich leg. (TAS 145); 3 exs., NW TAS, Welcome River at Hwy A 2, 44 m, 12.XII.2006, 40.57.004S 144.48.325E, L. & E. Hendrich leg. (TAS 146); 10 exs., C TAS, CPCA, 500 m E Lake Ada, pools, 1154 m, 14.XII.2006, 41.52.575S 146.28.432E, L. & E. Hendrich leg. (TAS 149).

Description. Morphology and size as in the above species. Minor differences between median lobi of *A. femoralis* and *A. occidentalis* **sp.n.** (Fig. 5) are attributed to individual variability.

Remarks. Specimens from Tasmania are larger and darker than specimens from the mainland.

Distribution. South-eastern Australia. From around Sydney along the east coast south to Victoria, Tasmania and South Australia, including Port Lincoln and Kangaroo Island (Watts 1978, 1997) (Fig. 3).

Habitat. The species inhabits a wide variety of freshwater habitats and can be found in slow-flowing creeks, rest pools of intermittent streams and rivers, ponds, old farm dams, ditches, and seasonal or permanent sedge swamps from near sea level up to an altitude of 1154 m. The ideal habitat should be rich in rotten leaves or plant debris and overgrown with sedges or reed (Fig. S2).

Discussion

Taxonomy

We strongly support the utilisation of Internet technology to enhance dissemination of taxonomic knowledge (e.g., Knapp [93] for an example from this journal), like SpeciesID (http://www.species-id.net/w/index.php?title=Antiporus_occidentalis&oldid=2012). WikiSpecies pages, in our opinion the best taxonomic information facility on the web (see also Page [94] on WikiPedia), are given in Text S1. The species pages have links to GenBank entries and additional material such as habitat photos. Where necessary, they will be updated to provide further data as they become available.

Phylogeny

We inferred a fully resolved phylogeny for 10 of the 16 species of the genus *Antiporus*, with four major lineages. *Antiporus gottwaldi* is the sister taxon to all other species. Next, *A. hollingsworthi* is the sister taxon to all remaining species except *A. gottwaldi*. The remaining species are divided into two clades. Species from the clade including *A. femoralis* are distributed in the southern parts of Australia (from southern New South Wales, Victoria, Tasmania, South Australia and the southern part of Western Australia), while members of the clade including *A. bakewellii* range from Northern Territory to northern Queensland (*A. jenniferae*) and down the east coast to Tasmania (*A. blakeii*). Although *A. blakeii* and *A. femoralis* belong to separate clades, their distribution is almost congruent. These broadly sympatric species belong to different clades. Occasionally, both species can be found in the same habitat (e.g., Tasmania, Victoria).

Ecological niche modelling of *A. femoralis* and *A. occidentalis* sp.n.

Our results indicate that *A. femoralis* and *A. occidentalis* **sp.n.** differ in their realised ecological niches, represented here by their modelled climate envelopes. As suggested by the results shown in Fig. 6, the distributions of both species depend heavily on winter rain. However, the variables representing a high level of seasonal

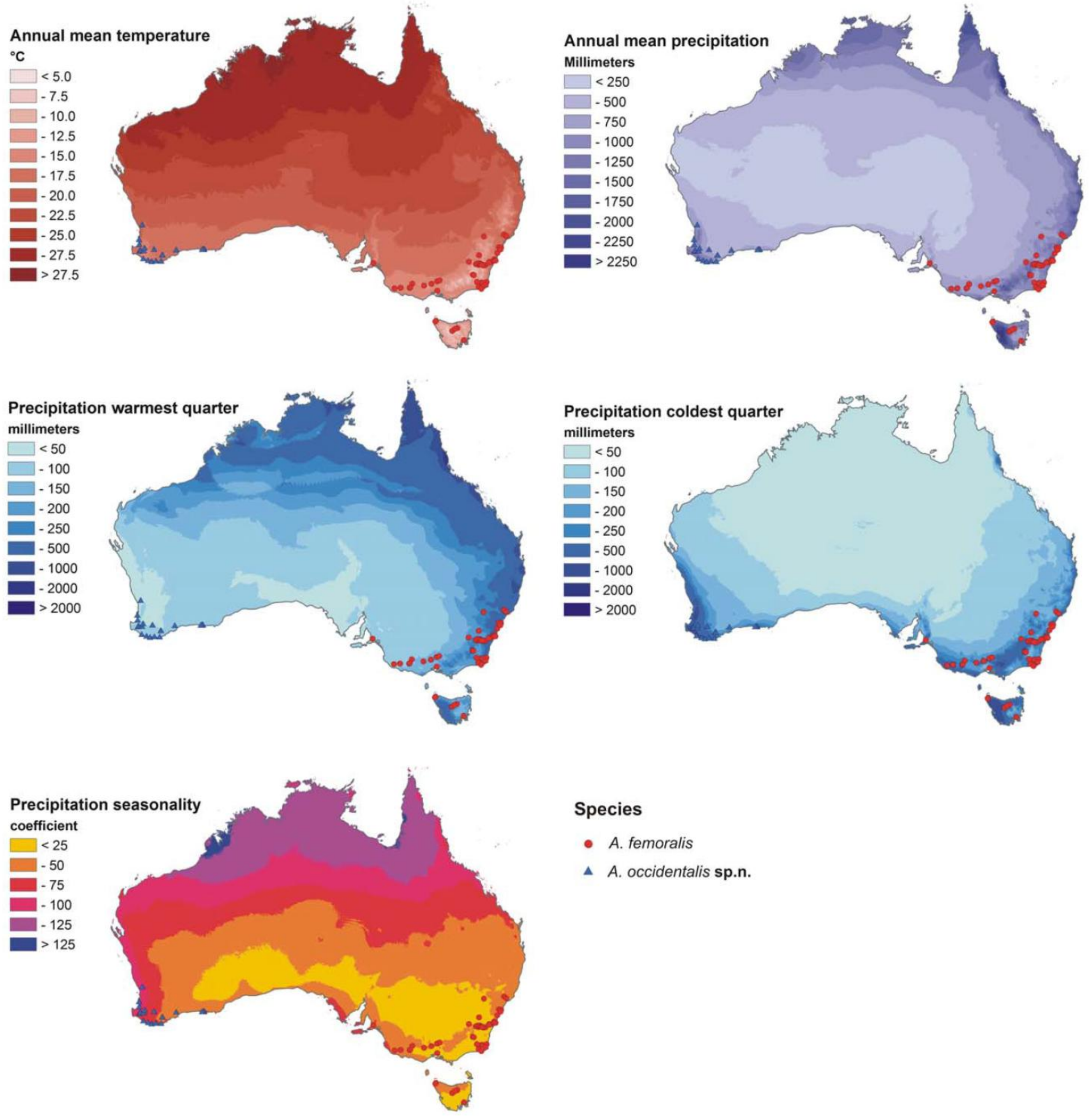


Figure 6. Climate variables. A projection of *Antiporus femoralis* (blue triangles) and *Antiporus occidentalis* **sp.n.** (red circles) localities on climate variables. Note that localities of both taxa are situated in areas with relatively high precipitation in the coldest quarter. In the warmest quarter, most localities of *A. femoralis* also receive high precipitation, while localities of *A. occidentalis* **sp.n.** are predominantly dry in this season. This effect is also visualised as precipitation seasonality, where *A. femoralis* inhabit areas with relatively low precipitation seasonality, and *A occidentalis* **sp.n.** inhabit areas with moderate precipitation seasonality.
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variation in climate ("precipitation warmest quarter" and "precipitation seasonality") are more important in the *A. occidentalis* **sp.n.** model, while in the *A. femoralis* model they are of lower relative importance than "annual mean temperature" and "annual precipitation".

As shown in Fig. 3, the distributions both of *A. femoralis* and *A. occidentalis* **sp.n.** cover areas highly suitable for both taxa according to the ENMs. However, *A. femoralis* is commonly found at localities that are less suitable for *A. occidentalis* **sp.n.** and vice versa.

Fig. 5 shows that areas of occupancy of both *A. femoralis* and *A. occidentalis* **sp.n.** correspond to relatively high precipitation in the Southern Hemisphere winter (coldest quarter). However, *A. femoralis* lives in areas where summer (warmest quarter) precipitation is at a level similar to that in winter, whereas *A. occidentalis* **sp.n.** inhabits areas with very dry summers. Apparently, the main difference between the climatic envelopes of these two *Antiporus* species is the summer drought in the area of *A. occidentalis* **sp.n.** The ecological validity of this difference is also confirmed by the decrease in regularized training gain if "precipitation warmest quarter" is omitted from the model of *A. occidentalis* **sp.n.** This pattern suggests a possible niche divergence between the two taxa, with *A. occidentalis* **sp.n.** showing a preference for areas with lower summer precipitation and *A. femoralis* preferring areas with relatively wet summers and low seasonal differences in precipitation. Given the nature of ENMs, especially the restricted set of abiotic variables and the complete exclusion of biotic variables from the analyses, such results must be treated with caution [33,80]. The apparent divergence in climatic envelopes might be due to abiotic factors not included in the analysis, such as differences in microhabitat structures, soil or water chemistry. It might also be influenced by biotic factors. Thise might, for example, be predators or competing species present in the area of *A. occidentalis* **sp.n.** Both species occur in syntopy with several other species of dytiscid beetles with similar ecology, but none of these syntopic similar species are present in the ranges of both *Antiporus* species [53]. The presence of these other species might keep *A. occidentalis* **sp.n.** from occupying the niches of its Western sibling taxon, *A. femoralis*.

We used two approaches to model validation to address these possible problems. First, the hypothesis that *A. femoralis* and *A. occidentalis* **sp.n.** occupy different environmental niches was tested by comparing ENMs of the two species to a model based on both species together. As described in Raxworthy [24], species delimitation by ecological niche modelling is most reliable if models of each split clade alone are superior (according to better fit and more significant statistical model validation) to models of all clades lumped together. Models in which this is not the case might also be validated by including negative locality data (but see [95]), which in the present case has not been available. As all models have fits considered "very good", this criterion does not contribute to the verification of species delimitation in the case of *A. occidentalis* **sp.n.**

Another method of model validation is the use of various statistical tests, as implemented in the ENMtools software [88,90]. According to the results of the identity test, niche diversification between these two sibling species must be considered highly significant. The climate envelope of *A. occidentalis* **sp.n.** is very different from that of *A. femoralis*. The background test yields results in which the significance is much smaller in magnitude than that of the identity test results. Nevertheless, the background test results indicate that this divergence cannot be attributed to the ecological difference in the species' allopatric ranges alone. This suggests that in the area of occupancy of *A. occidentalis* **sp.n.**, a different climate space is available than in the range of *A. femoralis*.

However, the results of the two test runs seem to contradict each other. The climate envelopes of both species are more divergent than expected based on localities of *A. occidentalis* **sp.n.** (and on random test samples drawn from the background of *A. femoralis*), but they are more similar than expected based on the reverse comparison (Fig. 4, explained in Fig. S3). Nakazato et al. [96] performed background tests for species distribution models of four sibling species pairs and obtained a variety of outcomes. Whereas identity tests yielded highly significant results, sibling species were either ecologically more divergent, less divergent or not significantly divergent according to the background tests. One case resembled that of *Antiporus*: species were either more or less divergent depending on the direction of the test. The authors explain this counterintuitive result by differences in the heterogeneity of the species' environmental backgrounds.

In our view, the identity tests clearly indicates that *A. femoralis* and *A. occidentalis* **sp.n.** are ecologically divergent. This divergence may result from their exposure to different environmental backgrounds alone, but it may also be result from evolutionary niche diversification. The results of the background test do not contradict the latter assumption. They simply state that this diversification is higher than expected if tested one way and lower than expected if tested the other way.

Speciation/species delimitation in *A. femoralis* and *A. occidentalis* sp.n.

In our view, *A. occidentalis* **sp.n.** constitutes a valid species according to the unified species concept, as it represents a metapopulation lineage evolving separately from other metapopulation lineages, including that represented by its closest relative, *A. femoralis*. In this paper, we used two different approaches to validate this hypothesis. First, a taxonomic/phylogenetic approach using morphological and molecular genetic data was employed. The morphological analysis showed that *A. occidentalis* **sp.n.** is indistinguishable from *A. femoralis*. Genetic data, however, unambiguously supported presence of two clades, and the relatively high cox1 divergence (>6%) clearly suggested further investigation into the possible presence of a cryptic species [97]. The operational criterion applicable to this result is the genotypic cluster of Mallet [19]. This criterion defines species as identifiable clusters having no intermediates.

In our second approach, we used ecological data to test the ecological species concept, as proposed in Van Valen [17] and Andersson [18], as operational criterion. According to this concept, individuals occupying the same niche or adaptive zone constitute a species. The results of our modelling suggest that *A. femoralis* and *A. occidentalis* **sp.n.** do not occupy the same niche. The difference in their niches can be attributed largely, but not completely to the different environmental conditions prevailing in their distributional ranges. The distributional range of *A. occidentalis* **sp.n.** features drier summers and generally higher seasonal variation in precipitation than those experienced by *A. femoralis*. In our view, these two operational criteria support the assessment of *A. occidentalis* **sp.n.** as a separately evolving metapopulation lineage.

Precise estimation of the age of separation using a molecular clock approach is difficult due to the lack of reliable calibration points. Other pairs of dytiscid species (*Hyderodes shuckardi* Hope, 1838 and *H. crassus* Sharp, 1882, *Spencerhydrus latecinctus* Sharp, 1882 and *S. pulchellus* Sharp, 1882) are known to exhibit a distribution pattern similar to *A. femoralis* and *A occidentalis* **sp.n.**, but no studies on molecular dating have yet been performed. The observed intraspecific distance suggests that *A. femoralis* and *A. occidentalis* **sp.n.** have remained in evolutionary separation for a long time. Applying the "molecular clock" evolution rates of about 3.54% divergence per million years (myr) of Papadopoulou et al. [98] to the minimum interspecific cytochrome c oxidase 1 distance suggests that the two lines have split around 1.0 to 1.9 myr ago. As shown by various studies, age estimations using standard mutation rates must be viewed with great caution [99–102]. Nevertheless, this result supports the view that speciation between *A. femoralis* and *A. occidentalis* **sp.n.** took place well after the Miocene transgression period, when the Nullarbor plain had already fallen dry. In this scenario, speciation probably followed a colonization event across the arid plain, possibly during a temporary phase of less arid conditions.

The scenario presented here attempts to connect present biodiversity with evidence from the geological record. It is based on several assumptions, for some of which evidence is scarce, but offers one possible explanation for the two morphologically indistinguishable, but genetically and ecologically divergent sibling species *A. femoralis* and *A. occidentalis* **sp.n.** It may be supported by future studies on similar speciation events, especially if more accurate age estimations are possible. We believe that the results of such studies may help elucidate the implications of geological history and past environmental changes for Australia's present biogeography.

Supporting Information

Table S1 Sequences of primers used for PCR and sequencing. Forward (F) and reverse (R) primers are given. Mitochrondrial gene loci: coi = cytochrome C oxidase 1, cob = cytochrome B oxidase, 16S = 16S ribosomal RNA. Nuclear gene loci: H3 = histone 3, 18S = 18S ribosomal RNA, ArK = arginine kinase.
(DOC)

Table S2 Coordinates of *Antiporus femoralis* and *A. occidentalis* used for modeling. Geographic latitude and longitude are given in decimal degrees.
(DOC)

Table S3 A heuristic estimate of the contributions of the bioclimatic variables used for modelling. Results of the jackknife analysis of variable importance are given as ranks (1 to 5) for all variables. Isolation: rank of the variable's training gain when used in isolation. Omission: rank of the variable in decreasing the total regularised training gain when omitted.
(DOC)

Figure S1 Background selection in ecological niche modelling. This picture shows each two ecological niche models for *Antiporus femoralis*, *A. occidentalis* **sp.n.** and both species together. For each set of locality data, one model was created using a manually specified background, as indicated by the green frame, and another one using no specified background. Both models were tested for niche overlap. All resulting values of I and D are close to 1 and thus indicate high overlap between models, confirming the similarity apparent from visual comparison.
(TIF)

Figure S2 Habitat of *Antiporus occidentalis* sp.n. a) Pond near Preston Beach, Western Australia (Loc. 30) and b) seasonal swamp at "Nannup Wildflower Walk" near Nannup, Western Australia (Loc. 32,).
(TIF)

Figure S3 Apparent contradiction in the background test results. This picture (modified from Nakazato et al. [96]) shows the environmental spaces available to (red and blue lines) and occupied by (shaded areas) both allopatric *Antiporus* species. In the niche overlap test, true localities of both species are compared. In the background test, the true localities of each one species are compared to random samples points drawn from the background areas (i.e., available environmental spaces) of the other species. Here, background test (1) yields relatively more divergent results than the true calculated overlap because, although the same overlap exists, it includes much more non-overlapping environmental space. Background test (2) yields more similar results than the true calculated overlap because it includes far more overlap than non-overlap between niche spaces. See Fig. 3.
(TIF)

Text S1 Web links. *Antiporus femoralis* and *A. occidentalis* **sp.n.** on Wikispecies.
(DOC)

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Author Contributions

Conceived and designed the experiments: OH NP LH MB. Performed the experiments: OH LH. Analyzed the data: OH MB. Contributed reagents/materials/analysis tools: MB. Wrote the paper: OH NP LH MB. Specimen acquisition: LH. DNA lab work and phylogenetic analyses: OH. Ecological Niche Modelling: OH. Morphological analyses and species description: LH.

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6.2 PAPER: Island evolution and systematic revision of Comoran snakes: why and when subspecies still make sense.

This chapter presents the case of a radiation of Comoran snakes in which the different degrees of diversification are taken into account by assigning specific and subspecific rank to the detected taxonomical units. The concept of the 'lines of evidence' approach in integrative taxonomy (MIRALLES et al. 2011) explicitly states the possibility of assigning subspecific rank to taxa. However, whether the rank of subspecies should be maintained at all or not is a matter of debate in systematic zoology, especially in herpetology.

> […] a perfect example of why subspecies do NOT make sense, and why they are NEVER appropriate! […] The authors present […] evidence for the existence of four distinct taxa. Why on earth would one not want to describe these as species!? That is like getting a full scholarship to Harvard, and deciding to go to a community college instead. […] Once again, I implore the authors to simply elevate these taxa to species […].
>
> Anonymous, reviewer comment on this paper

> "While I may wail on Linnaean Systematics and the issues with ranks, and the use and potential loss of the concept of the 'genus' as an entity separate from that of the species, some authors out there are going at it in the opposite direction. That is, rather than concerning themselves with the issue of supraspecific ranks, they are looking as [*sic*] subspecific ranks. Subspecies, in fact."
>
> Jaime A. Headden in the blog "The Bite Stuff" at WordPress.com, on the following article

The following article is one of the first publications including the descriptions of new taxa that were made after the amendment to the ICZN code of 4th September, 2012 (ICZN 2012).

Figure 14: Specimens of Lycodryas maculatus comorensis *with dark crossbands. A: Juvenile (male) specimen observed at Lac Karehani, Mayotte, on 2013-03-02. B: Adult male specimen observed at Pointe Handréma, Mayotte, on 2013-03-06. Photograph (B) by* F*RANK* G*LAW.*

HAWLITSCHEK O., NAGY Z.T., GLAW F. (2012): Island evolution and systematic revision of Comoran snakes: why and when subspecies still make sense. PloS ONE 7(8): e42970.

Post-publication comments and errata:

p. 6: In a field survey on Mayotte in 2013, several specimens of *Lycodryas* with dark crossbands were observed (Fig. 14). All were identified as *L. maculatus comorensis*, supporting the validity of this taxon.

p. 12: The paratype ZSM 43/2010, collected 02.03.2010, is erroneous. ZSM 43/2010 correctly designates the holotype. The paratype mentioned here is correctly referenced as ZSM 44/2010, collected on 08.03.2010.

p. 17: References: reference numbers [42] and [43] are reversed.

Island Evolution and Systematic Revision of Comoran Snakes: Why and When Subspecies Still Make Sense

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Abstract

Species delimitation and species concepts have been a matter of debate among biodiversity researchers in the last decades, resulting in integrative taxonomy approaches and the use of modern species concepts, such as the phylogenetic, evolutionary or general lineage species concepts. The discussion of subspecies status and concepts has been addressed much less extensively, with some researchers completely refraining from recognizing subspecies. However, allopatric insular populations that are particularly differentiated have traditionally been assigned subspecies status. We studied the molecular phylogeny and morphology of endemic Comoran tree snakes of the genus *Lycodryas*. Taking an integrative taxonomic approach, we used the concept of independent lines of evidence to discriminate between evidence for specific and subspecific status. Molecular (mtDNA) and morphological data provided sufficient evidence to support four different taxa within Comoran *Lycodryas*. In a revision of this group, we propose two species, each with two subspecies. We present a discussion of the strong sexual dichromatism unique to Comoran *Lycodryas* within the genus and related genera that may be explained by sexual selection in combination with the absence of major predators. Then, we discuss the effects of insular evolution and the "island rule" on morphological traits in Comoran *Lycodryas* and in *Liophidium mayottensis*, another snake endemic to the Comoros. The absence of larger-bodied snakes may have promoted an increase in body size and the number of dorsal scale rows in these species. Finally, we discuss the subspecies concept, its applications and its significance for integrative taxonomy and for limiting taxonomic inflation. We emphasize that taxon descriptions should be based on an integrative approach using several lines of evidence, preferably in combination with statements on the underlying species concepts or operational criteria, to increase the objectivity and comparability of descriptions.

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Introduction

Islands have been regarded as model systems for speciation even by the earliest evolutionary biologists [1,2]. Particular attention has been given to groups of oceanic islands that have never been connected to other land masses since their (usually volcanic) origins [3–10]. In contrast, the Comoros archipelago in the Western Indian Ocean (WIO) has received relatively little attention, possibly because it contains no conspicuous or "odd" endemics, such as the now extinct Dodo (*Raphus cucullatus*) of Mauritius or the Marine Iguana (*Amblyrhynchus cristatus*) of the Galápagos or extensive and characteristic radiations such as drosophilid flies in Hawaii [11] or *Echium* plants in the Canaries [12].

The degree of endemism on the Comoros is not as high as that of comparable island biota although it is considerable: 17 out of 60 species of breeding birds are endemic (28.3%) [13,14], and Pascal [15] assumes 15% endemism of native plants on the Comoros. The Galápagos have 60.4% endemic birds and 45% endemic plants [16], and the Mascarenes have 66.7% birds and 72% plants [17]. Among terrestrial Comoran reptiles, 13 out of 28 (46.4%) recognized species are endemic, but most of the non-endemics are introduced; if only native species are taken into account, endemism rises to 76.5% according to the current state of taxonomy [18,19]. All native terrestrial reptiles on the Canaries and the Galápagos are endemic. On Hawaii, there are no native reptile species [20].

For island biota in particular, the question of endemism strongly depends on the underlying taxonomy. A number of both widespread and endemic bird species are present on the Comoros, in addition to island-endemic subspecies. If all these subspecies were to be elevated to species rank, endemism would more than double from 28.3% to 70.0% [14]. In reptiles, the degree of endemism would increase from 46.4% to 60.6% [19]. The degree of endemism, especially of island biota that are generally considered vulnerable [21–23], is of high importance in general biodiversity research and conservation planning [24,25]. It is therefore essential to adopt clear and preferably objective criteria for the delimitation of species and subspecies.

While systematic biology has many categories by which to classify organisms, the rank of species alone is subject to a scientific definition, or rather, ongoing attempts to agree on such a definition. Until the late 20th century, the most widely accepted definition of a species was provided by the biological species concept of Mayr [26] (see also Dobzhansky [27]), who described species as reproductively isolated lineages. Obviously, in most studies on speciation, reproductive isolation was assumed rather than empirically tested. Later, several new definitions of species were proposed based on ecological [28,29] or phenetic [30] differences between species. Although these definitions would prove useful in many cases, many authors realized that no single set of characters could serve as a universal tool to delimit species. The newly developed species concepts shifted the focus of species delimitation to the questions of recognizing lineages and their degrees of separation. In the evolutionary species concept, species are considered to be independent lineages with own evolutionary tendencies and own historical fates [31–33]. Various phylogenetic species concepts exist, sharing the idea that species are clusters of individuals with a parental pattern of ancestry and descent [34–37] and a minium diagnosable monophyletic unit [38]. In the general lineage concept, de Queiroz [39] (see also Mayden [33]) defines species as separately evolving metapopulation lineages. These and other [40–42] modern species concepts considerably improved our understanding of how to define species. However, testing the proposed criteria in practice and applying them to species delimitations and descriptions has proved to be difficult.

De Queiroz [43] argued that all species concepts established thus far agree that species exist as separately evolving metapopulation lineages, as previously formulated in de Queiroz [39]. In his unified species concept [43], he proposed that the numerous criteria traditionally applied in species delimitation are maintained as "operational criteria". Thus, lineage separation could be inferred from evidence for intrinsic reproductive isolation, ecological divergence, or differences in molecular genetic characters, among other phenomena. With the advances of molecular techniques in phylogenetics and taxonomy, molecular characters (mtDNA and nDNA) have been increasingly used in species delimitation because they often provide information regarding the degree of lineage sorting, haplotype sharing and hybridization [44–47]. Integrative taxonomy [48–50] considers these different factors, such as morphological, molecular or ecological ones, to be separate lines of evidence when assigning species status.

Universal definitions, or attempts thereof, do not exist for any taxonomic rank of a higher or lower level than species. Higher ranks are assigned to taxa arbitrarily based on the knowledge and opinion of the taxonomist in question. This practice is also widely adopted for taxa below the rank of species, in which a clear lower limit for recognizing any two entities as distinct does not exist. Therefore, some authors rejected the subspecies category and trinomials already in the mid-20th century [51,52]. Most species concepts following the biological species concept, including the more theoretical evolutionary, phylogenetic and general lineage species concepts, have not included any recommendations for how to handle subspecies (but see Cracraft [38]). However, various authors proposed subspecies concepts, most of which share the notion that subspecies differ "taxonomically" (by one or more distinctive features) and by their geographical ranges [53–55]. Some authors sought to improve this definition. The 75% rule proposed by Amadon [56] states that for a diagnostic character or a set of characters, 75% of the population of a proposed subspecies must lie outside 99% of the range of other populations. According to Böhme's [57] review of the ecological work of Kühnelt [58], geographically separated populations should also show differences in ecological preferences to warrant subspecies status. Despite these proposals, subspecies have never been very popular among invertebrate zoologists [59]. In vertebrate zoology, many herpetologists in particular argued against describing new subspecies or even maintaining existing subspecies [60–62] (but see Mulcahy [63]). Most arguments in favor of subspecies were advanced by ornithologists, who argued that the rank of subspecies is helpful in studies of evolutionary divergence and conservation [64], useful for identifying distinct populations within biological species [65], and convenient for managing taxonomic entities that do not warrant species status [66]. Allopatric island forms of species with some degree of morphological variation have been traditionally recognized as subspecies, and in modern herpetology, new subspecies are also described [67–70]. Recently, Miralles et al. [71] applied the concept of integrative taxonomy, as discussed in Padial et al. [49], to delimit species and subspecies alike.

Morphological differences between insular taxa and related mainland taxa may result from different selection regimes, from founder effects, or from interactions between these two factors [72,73]. Most commonly, insular taxa particularly of vertebrates have been recorded as divergent in size, with small animals showing a tendency towards gigantism and large ones towards dwarfism. This phenomenon was observed so commonly that it was termed the "island rule" [28,74]. While this rule has been confirmed in various studies of mammals [74–76], reptiles seem to follow a less clearly directed pattern. Often, both giant and dwarf insular forms of the same taxonomic group are known [77–79], suggesting that the mechanisms influencing morphological traits on islands in reptiles warrant further study.

In this paper, we investigate the snakes of the genus *Lycodryas* (Lamprophiidae) in the Comoros archipelago, which have thus far been recognized as one endemic species, *Lycodryas sanctijohannis* Günther, 1879 [80]. These predominantly arboreal and nocturnal snakes are present on all four major islands and several smaller islets and are the only advanced snake species throughout most of their range [19,81]. They are distinguished among snakes and unique among their congeners in that they display striking sexual dichromatism. In an integrative taxonomic approach, we use morphological and molecular data to revise the taxonomic status of Comoran *Lycodryas*. We discuss the effects of island evolution in *Lycodryas* compared with the second Comoran endemic snake, *Liophidium mayottensis*, and the sexual dimorphism in Comoran *Lycodryas*. Finally, we discuss the designation of the subspecies rank for insular populations.

Methods

Sampling

Morphological data and tissue samples from *Lycodryas* and *Liophidium* species were obtained from specimens stored at the Zoologische Staatssammlung München, Germany (ZSM) [19,82]. To gain additional morphological data on the Comoran taxa, all available historical museum specimens were examined. We use the following abbreviations for zoological collections: Natural History Museum, London, United Kingdom (BMNH); Muséum national d'Histoire naturelle, Paris, France (MNHN); Senckenberg Naturmuseum Frankfurt, Germany (SMF); Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK); Museum für Naturkunde Berlin, Germany (ZMB); Zoologische Staatssammlung München, Germany (ZSM). A list of all specimens studied is given in Table S1.

Collection and transport of specimens was conducted with the following permits: (1) Issued by the Direction Générale de l'Environnement, Moroni, Union des Comores: research and export permit (no permit number, 1st March 2000), research permit (02/121/MPE/DGE, 12th April 2002), export permit (02/141/MPE/DGE, 2002), research and export permit (no permit number, 12th March 2008), research permit (CNDRS/08/2010, 22nd January 2010), export permit (CNDRS/030/2010, 5th April 2010). (2) Issued by the Direction de l'Agriculture et de la Forêt, Mayotte, France: research and export permit (no permit number, 23rd February 2000), research and export permit (24/DAF/SEF/2008, 19th March 2008), research and export permit (2010-13/DAF/SEF, 30th March 2010). None of the species concerned are listed on CITES appendices. Permission for import to the EU and Germany is not required.

Morphology

To detect morphological variation between the Comoran *Lycodryas* populations and differences compared with the Malagasy *Lycodryas* species, we studied six morphometrical characters (and five ratios between these characters), thirteen meristic characters, and the colors of 43 specimens. Snout-vent length and tail length were measured to the nearest millimeter and other morphometric characters to the nearest 0.1 millimeter using a digital steel caliper. Meristic characters were examined visually, if necessary using a binocular microscope. Color was considered a reliable character only if photographs or descriptions of living or freshly dead specimens were available. A list of all characters studied, with abbreviations, is given in Table S1. The sex of specimens was determined via dissection of the tail base and inspection of the reproductive organs, if possible.

To detect significant differences among the *Lycodryas* populations of the four Comoro islands, we used Multivariate Analysis of Variance (MANOVA) in PAST version 1.55 [83]. A MANOVA allows testing for equality of the means among several multivariate samples. Given the overall significance of the MANOVA result, pairwise significance tests with Hotelling's p for every pair of island populations are provided. A Canonical Variates Analysis (CVA) is employed to visualize the results.

We also studied six morphometrical characters (and five ratios between these characters) and ten meristic characters in eleven specimens of *Liophidium mayottensis* for comparison with data from the Malagasy *Liophidium* species. Data on the Malagasy species of *Lycodryas* and *Liophidium* were obtained from Glaw & Vences [84] and Nagy et al. [82].

Laboratory protocols

DNA was extracted from tissue samples of *Lycodryas* using the standard protocols of the Macherey & Nagel NucleoSpin® 96 Tissue kit. We amplified four mitochondrial markers, 16S rRNA (16S), cytochrome *b* (cyt *b*), cytochrome C oxidase subunit 1 (COI), and NADH dehydrogenase 4 (ND4), and one nuclear locus, the proto-oncogene *mos* (c-mos). To test for nuclear divergence below the species level, we also amplified recombination activation gene 2 (Rag2) and the prolactin receptor (PRLR) for the Comoran samples, but these sequences were not included in the overall dataset. The standard PCR protocol used 25 µl reactions with 1 µl of template DNA and the following steps: initial denaturation for 3 min at 94°C, followed by denaturation with 35 cycles of 30 sec each at 94°C, 30 sec of annealing at 47°C and 60 sec of elongation at 72°C, and a final elongation step of 10 min at 72°C. Primer sequences and modifications of the standard PCR protocol are described in Table S2. Sequencing was conducted using the BigDye® Terminator v1.1 Cycle Sequencing Kit on ABI 3730 and ABI 3130xl capillary sequencers. Sequence data were deposited in GenBank and are available under accession numbers HE798386 to HE798447.

Phylogenetic analyses

We analysed a dataset of 16S, cyt *b*, COI, ND4 and c-mos with a total of 3498 basepairs. The dataset contained 22 specimens belonging to 9 currently recognized species of the genus *Lycodryas*. We aligned our data with MAFFT 6 [85,86]. With respect to the different evolutionary characteristics of our molecular markers, we split our dataset into 10 partitions, treating all codon positions of each protein-coding gene and the 16S gene as separate partitions. To identify appropriate substitution models for each partition, we used jModeltest 0.1.1 [87]. We assessed AIC and BIC results, giving BIC preference over AIC.

We conducted maximum likelihood analyses with 1000 fast bootstrap repeats in raxmlGUI 0.93 [88,89] and Bayesian analyses in MrBayes 3.1.2 [90] on the CIPRES portal 2.2 [91], with two runs and four chains of 30,000,000 generations (samplefreq = 1,000, 25% burnin). MrBayes runs were checked for convergence and normal distribution in Tracer v1.5 [92]. Finally, we conducted parsimony analyses in TNT 1.1 with 1,000 jackknife (removal 36%) replications [93] (hit best tree 5 times, keep 10,000 trees in memory). Pairwise distances were calculated in MEGA 5.0 [94].

We also constructed haplotype networks of all mtDNA markers for Comoran *Lycodryas* using statistical parsimony [95] with a connection limit of 95% in the software TCS v1.21 [96] and manually constructed haplotype networks of all nuclear markers.

Integrative taxonomy

Currently, the name *Lycodryas sanctijohannis* Günther, 1879 [80] is applied to all Comoran *Lycodryas*. We explore the distinction of all four island populations of this snake as distinct taxonomic units. Vieites et al. [97] distinguish between Unconfirmed Candidate Species (UCS), Confirmed Candidate Species (CCS) and Deep Conspecific Lineages (DCS). UCS are lineages that can be distinguished by molecular characters but that cannot be confirmed by any other means. A CCS is characterized by a detectable genetic differentiation and distinctiveness in at least one character that mediates a reproductive barrier or is known to be of value for species discrimination in the taxonomic group concerned and/or sympatric occurrence with other lineages without admixture. In contrast, DCL are characterized by the absence or only slight expression of differences in characters that mediate a reproductive barrier or are known to be of value for species discrimination and/or indications of admixture with other species.

After testing these criteria, we follow Miralles et al. [71] (see also [98]) in using three different lines of evidence, based on independent datasets to clarify the taxonomy of Comoran tree snakes and assign specific or subspecific status. Each candidate species may qualify for the following lines of evidence: (1) mtDNA: representation by an independent cyt *b* parsimony network with a connection limit of 95% [99]; (2) nDNA: absence of shared Rag2 haplotypes with any other clade in question [100]; (3) morphology: at least one fixed diagnostic character state (qualitative or significant quantitative) [101]. In congruence with Miralles et al. [71], we apply subspecies status if a candidate species qualifies for only one line of evidence and species status if a candidate species qualifies for two or all lines of evidence. For better comparability, we also use the same molecular markers (cyt *b* and Rag2) as Miralles et al. [71]. After formally applying these criteria, we compare the results with those of other molecular markers and the complete phylogenetic results, we compare the three lines of evidence, and we check for taxonomic plausibility.

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Results and Discussion

Phylogeny

The tree resulting from our phylogenetic analyses is presented in Fig. 1. The monophyly of all four island populations of Comoran *Lycodryas* is highly supported by all analyses. The sister-group relationships of the Anjouan and Mayotte populations vs. the Grand Comoro and Mohéli populations are equally well supported. All Comoran populations of *Lycodryas* are displayed as a monophyletic group, but with moderate support only. Preliminary analyses with a smaller set of molecular markers presented the taxon *Lycodryas gaimardii*, which had not been included in previous studies, nested within Comoran *Lycodryas*. The multi-gene phylogeny presented in Fig. 1 shows *L. gaimardii* as a sister taxon to all Comoran *Lycodryas*. This view is supported by morphological characters, which clearly distinguish *L. gaimardii* from Comoran *Lycodryas* [82,102]. Furthermore, the clade including all Comoran *Lycodryas* plus *L. gaimardii* is highly supported. This relationship confirms that *Stenophis* Boulenger, 1896 [103] (type species *Lycodryas gaimardii*) is a junior synonym of *Lycodryas* Günther, 1879 [80] (type species *Lycodryas sanctijohannis*).

Under a connection limit of 95%, all four clades of Comoran *Lycodryas* were retrieved as independent parsimony networks in TCS v1.21 for the protein-coding mtDNA markers (Fig. 2). Haplotypes from Mayotte and Anjouan form a common network in 16S. Genetic divergences, measured as uncorrected p-distances of the cytochrome *b* gene, within island populations of Comoran *Lycodryas* are 1.2% or less (maximum in the Grand Comoro population). The divergences between the groups of individuals from Anjouan and Mayotte are 5.3% to 5.5%, between Grand Comoro and Mohéli 7.6% to 7.8%. The distances between these two major Comoran clades are 8.6% to 10.4%. Notably, the distances between samples of *L. granuliceps* and *L. pseudogranuliceps* are 7.1% to 7.2%, and can be as low as 6.1% according to Nagy et al. [82]. These two taxa are poorly differentiated by morphological characters according to Vences et al. [102]. The next shortest distance of 8.5% was measured between *L. citrinus* and *L.* sp. aff. *gaimardii*, which are highly distinct from each other by morphology and coloration. All other interspecific divergences measured within *Lycodryas* were comparable to or higher than the level expressed within Comoran *Lycodryas*.

P-distances in the COI gene are 5.8% between the Anjouan and Mayotte populations, 5.4% to 5.9% between the Grand Comoro and Mohéli populations, and 7.7% to 9.1% between these two clades (Fig. 1). Nagy et al. [104], in a DNA barcoding study of Malagasy reptiles, found thresholds for COI divergences between sister species to be specific for various reptile groups. For lamprophiid snakes, including the genus *Lycodryas*, they found a threshold of 8.3%. A comparison shows that the distance within both major clades of Comoran *Lycodryas* is well below this threshold and thus lower than between other closely related species of *Lycodryas*. The distance between these major clades, however, matches this threshold.

Three nuclear markers were analyzed for possible differentiation between the insular populations of Comoran *Lycodryas*. An inspection of chromatogram data did not indicate the presence of heterozygotes, so separation of alleles was not necessary. A single substitution was detected in each 468 bp of PRLR (Mayotte+Anjouan clade vs. Grand Comoro+Mohéli clade), 626 bp of c-mos (Grand Comoro clade vs. other clades), and 613 bp of Rag2 (Anjouan clade vs. other clades). These data support the clades produced by analyzing the entire molecular dataset.

Morphological variation within Comoran Lycodryas

Comoran *Lycodryas* show an overall relatively high morphological variability. However, this variation extends throughout Comoran *Lycodryas* and is not suited for distinguishing insular populations. This is in contrast to the relatively large genetic distance described above. MANOVA results for an overall comparison of all Comoran *Lycodryas* are not significant. However, pairwise comparisons of meristic characters show significant differences ($p<0.039$*) for Anjouan-Mayotte, Anjouan-Mohéli, Grand Comoro-Mayotte and Grand Comoro-Mohéli, but not for Anjouan-Grand Comoro and Mayotte-Mohéli. This contradicts the results of the molecular phylogeny, according to which the populations of Grand Comoro and of Mohéli and those of Anjouan and Mayotte form sister groups.

The CVA scatterplot (Fig. 3) displayed only partial separation between the insular populations. The characters with the highest loadings on both axes displayed were the number of subcaudal scales (axis 1: −1.84; axis 2: +3.06) and the number of ventral scales (axis 1: +0.85). Table 1 lists these and other characters useful for discriminating between island populations of Comoran *Lycodryas*. Specimens from Grand Comoro are shorter than those from other islands, with a maximum snout-vent length of 650 mm (867 for Anjouan, 757 for Mayotte and 835 for Mohéli). Male specimens from Mayotte are distinguished by a dark ventral line (see also Boettger [105]). The dark dorsal pattern is a character that may be useful in discriminating among males from all islands (Table 1 and Fig. 4), but it appears that this pattern is graded between insular populations. It often fades in preserved specimens. Females on all islands share uniform reddish to yellowish color. Notably, we found specimens on all islands, except for Anjouan,

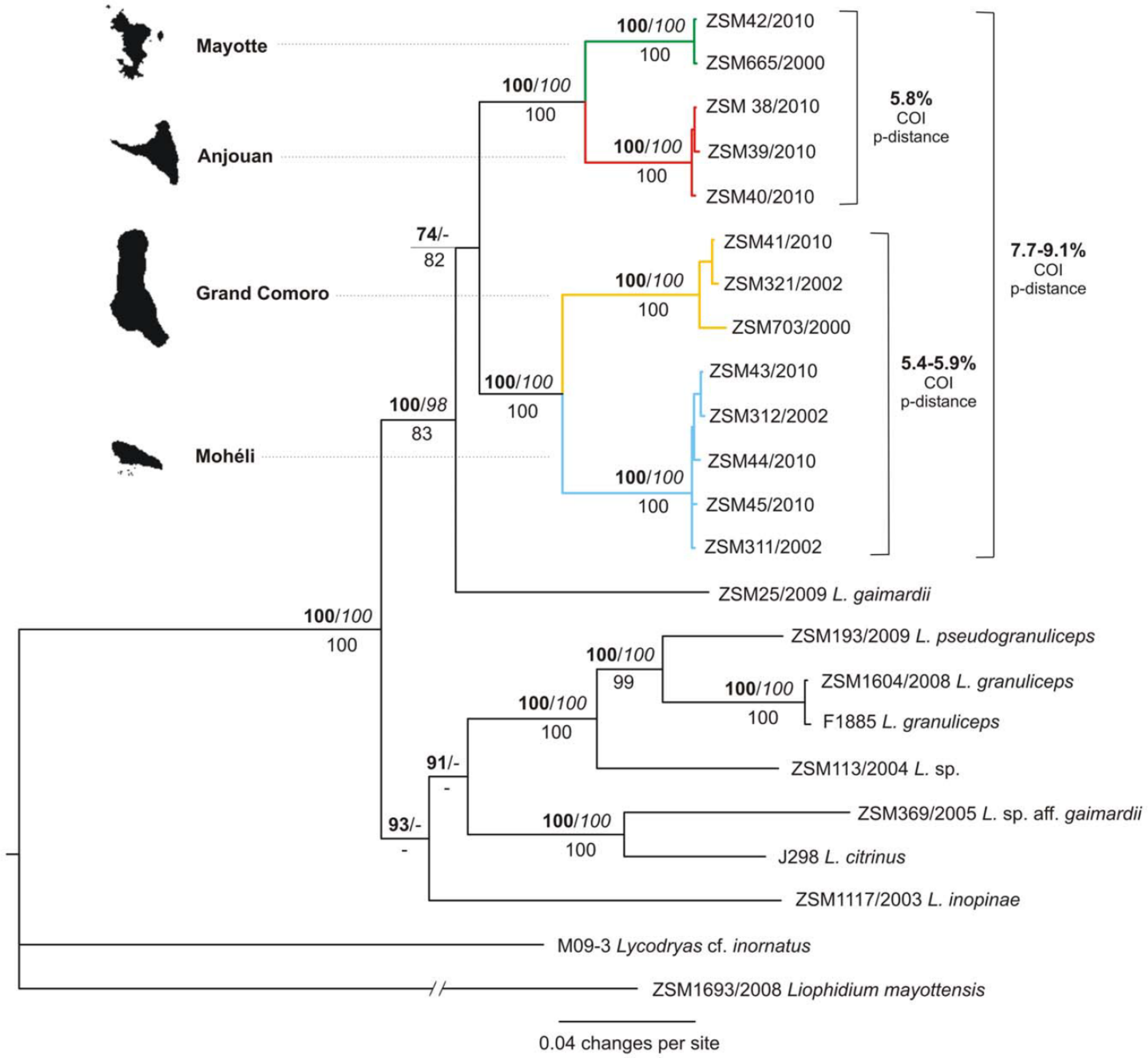

Figure 1. Phylogram of Comoran and Malagasy *Lycodryas*. The tree is based on a Bayesian analysis of a 3498 bp dataset. MrBayes posterior probabilities (*100, bold) and RAxML bootstrap support values (italic) are given above the nodes, TNT jackknife support values are given below the nodes. P-distances of the COI marker, as calculated in MEGA, are given for the entire clade of Comoran *Lycodryas* and for the two pairs of sister taxa. Note that the threshold for species delimitation in lamprophiid snakes, including *Lycodryas*, determined in the barcoding of Malagasy reptiles [104] was 8.3% of COI p-distance.
doi:10.1371/journal.pone.0042970.g001

that had only 17 dorsal scale rows at midbody in contrast to the regular 19. No significant morphometrical or meristic differences were detected between sexes in any of the insular populations.

One morphological character supports the two sister-clade relationships shown in the phylogram (Anjouan+Mayotte and Grand Comoro+Mohéli). In specimens from Mayotte and Anjouan the loreal scale is in contact with supralabials 2 and 3, whereas in specimens from Grand Comoro and Mohéli it is in contact only with supralabial 2 (Fig. 5).

As already mentioned by Domergue [106], distinguishing sexes by dissection of the tail base and inspection of the hemipenes is difficult in Comoran *Lycodryas* due to well-developed hemiclitores of the females. The hemiclitores of these snakes are very similar in size and aspect to the hemipenes, and the size of both organs seems to vary with reproductive state. Hemipenes have a bifurcate tip, although it is not always easy to identify.

Taxonomic status of Lycodryas sanctijohannis, discussion of Dipsadoboa maculata and Lycodryas gaimardii comorensis

The taxon *Lycodryas sanctijohannis* was established by Günther [80] and has since been used to refer to Comoran *Lycodryas*. We discuss two further taxa, *Lycodryas gaimardii comorensis* (Peters, 1874) [107] and *Dipsadoboa* (*Lycodryas/Stenophis*) *maculata* Günther, 1858 [108], which most likely also refer to Comoran tree snakes.

Peters [107] briefly described a taxon "*Dipsas (Heterurus) gaimardii* Schlegel var. *comorensis*" from Mayotte based on a juvenile

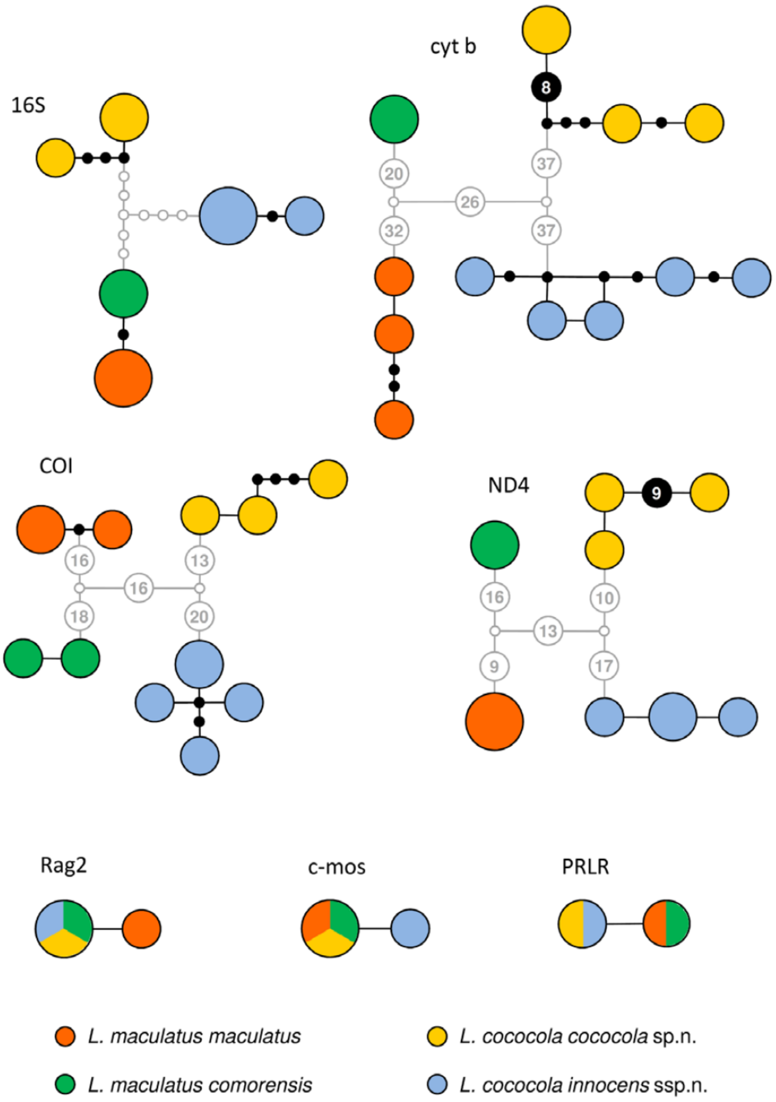

Figure 2. Haplotype networks of all molecular markers analyzed. Colored circles represent haplotypes; larger circles represent haplotypes that are shared by more than one specimen. MtDNA haplotypes that are situated in a common TCS network are connected by black lines, with black dots representing interlying mutation steps. Higher counts of mutation steps are given in numbers. Separate TCS networks are connected by grey lines according to MEGA distance trees. NDNA ''networks'' were constructed manually.
doi:10.1371/journal.pone.0042970.g002

specimen that is distinguished from *Lycodryas gaimardii* by a black line over its supralabials, below the eye to the corner of the mouth (often found in *L. sanctijohannis*), and dark crossbands that are narrower than in the typical form. *L. sanctijohannis* often shows patterns of dark blotches and dots but no clearly defined crossbands. It was also described as having 17 dorsal scale rows at midbody, like *L. gaimardii* and unlike *L. sanctijohannis*, which normally has 19. Despite extensive efforts, this specimen could not be traced in ZMB or any other museums that were inspected (R. Günther, M.-O. Rödel, F. Tillack, C. Kucharzewski, pers. comm.). However, the collection at SMF holds a clutch of four hatchlings and three translucent eggs claimed to be collected on Mayotte (SMF 19620 to 19627) and mentioned in Boettger's report on Voeltzkow's travels to the Comoros (in Boettger [105], p. 343) that have been assigned to ''*Stenophis* cf. *gaimardi*''. Meirte [81] described *L. sanctijohannis* as ovoviviparous, which may explain the translucence of the eggs. All hatchlings in this clutch have 17 dorsal scale rows and dark dorsal crossbands.

While 19 dorsal scale rows are the rule, we recorded a small number of specimens with 17 dorsal scale rows on all Comoro islands but Anjouan (Table S1). We know of no adult specimens of *L. sanctijohannis* with clear dark crossbands, but no other hatchling or small juvenile specimens were available for examination. Therefore, it is possible that hatchlings (and juveniles) of *L. sanctijohannis* have distinct dark crossbands that become less distinct in adult specimens, similar to other *Lycodryas* species. This leads us to the conclusion that *L. gaimardii comorensis* refers to the *L. sanctijohannis* population from Mayotte.

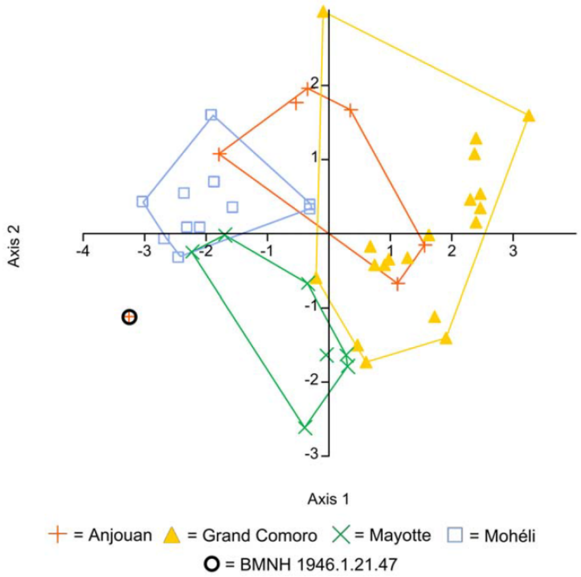

Figure 3. CVA plot of meristic data of Comoran *Lycodryas* species. Characters included are: V (number of ventral scales), MD (number of middorsal scale rows), SC (number of subcaudal scale rows), SLAB (number of supralabial scales – mean of left and right side), ILAB (number of infralabial scales – mean of left and right side), SLCE (number of supralabials in contact with the eye – mean of left and right side). Note that BMNH 1946.1.21.47, the type specimen of *L. maculatus*, represents an outlier of the Anjouan sample due to its uniquely low SLAB and uniquely high SC.
doi:10.1371/journal.pone.0042970.g003

The taxon *Dipsadoboa maculata* was originally described by Günther (1858, p. 183) based on a single male specimen (BMNH 1946.1.21.47) with "Central America" listed as its type locality. Boulenger [103] (p. 43) placed this taxon in the genus *Stenophis*, provided a redescription and a head drawing and changed the locality to "unknown". The reasons for rejecting this type locality remain speculative. Günther [107] noted that the specimen was "From M. Parzudaki's Collection"; Parzudaki collected in Central and South America [109–111]. Boulenger [103], however, placed the specimen in the genus *Stenophis* based mainly on dental morphology (maxillary teeth "13 or 14, equal", p. 39–40 vs. "12 or 13, anterior longest"p. 44) and the presence of well-developed hypapophyses throughout the vertebral column. The latter character is used by Boulenger to characterize a very small number of snake genera, including *Stenophis* and *Lycodryas*. Because all other *Stenophis* spp. were from Madagascar, Boulenger most likely doubted the reported origin of the specimen. Notably, the two descriptions of the type specimen disagree in that according to Günther [108], the anal shield is "bifid", whereas according to Boulenger [103] it is "entire". Our examination supports the view of Günther.

Nearly all characters in the description of *Dipsadoboa maculata* in Günther [108], as well as in Boulenger [103], fit with *Lycodryas sanctijohannis*. According to C. Kucharzewski (pers. comm., 18 April 2012), the type specimens of *Dipsadoboa maculata* and *Lycodryas sanctijohannis* both have enlarged anterior maxillary teeth and hypapophyses developed throughout the vertebral column and thus show no differences in these characters. In the case of conspecifity, *L. maculatus* (Günther, 1858) [108] would be the oldest and therefore valid name. Consequently, Domergue [106] reported two specimens of *L. maculatus* from the Comoros. The descriptions of the color, pholidosis and morphometrics of these specimens can also be applied to *L. sanctijohannis*. While both Günther [108] and Boulenger [108] state that the holotype has only undivided subcaudal scales, Domergue found that both specimens available to him had anterior undivided and posterior divided subcaudal scales. Our examination showed that the holotype actually has posterior divided subcaudals. Another difference is that both specimens examined by Domergue [106] had "white" supralabials, a common feature of male *L. sanctijohannis* also visible on Boulenger's [103] drawing of this species (plate III), but not on that of *L. maculatus* (plate IV). Our examination of the *L. maculatus* holotype confirmed the absence of white supralabials, but this may be the result of color loss from

Table 1. Morphological data and colors of *Lycodryas* from the four islands of the Comoro archipelago.

	Anjouan (N = 6)	**Mayotte (N = 7)**	**Grand Comoro (N = 19)**	**Mohéli (N = 11)**
SVL	653.83±130.60 (516–867)	602.57±87.60 (504–757)	569.68±80.02 (402–650)	682.64±126.11 (491–835)
MD	19	19 (17)	19 (17)	19 (17)
V	241.83±10.70 (233–259)	238.29±10.92 (227–261)	244.16±7.73 (232–260)	242.18±8.12 (231–255)
SC	95.50±11.78 (85–126*)	95.50±15.59 (84–117)	97.05±9.33 (84–116)	104.11±20.08 (77–140)
SC divided	partly (posterior)	partly (posterior)	partly (posterior)	partly (posterior)
Anal shield	divided	Divided	Divided	divided
SLAB	8–9	8–9	7–10	8
ILAB	8–10**	9	9–11	9–11
SLAB in contact with loreal	2+3	2+3	2	2
Dark ventral line	absent	present (males)	Absent	absent
Male dorsal color	grey with mostly clearly defined dorsal black blotches	grey to olive with pattern of dark spots (sometimes diffuse)	uniformly grey	grey with diffuse pattern of dark spots

SVL = snout-vent length [mm], MD = number of middorsal scale rows at midbody, V = number of ventral scales, SC = number of subcaudal scales, SLAB = number of supralabial scales, ILAB = infralabial scales. In MD, most specimens have 19, but exceptions of 17 were recorded (2 on Grand Comoro, 1 on Mayotte, 2 on Mohéli). No significant morphometrical or meristic differences were detected between sexes on any island.
*: 126 in BMNH 1946.1.21.47, type specimen.
**: 8 only in BMNH 1946.1.21.47.
doi:10.1371/journal.pone.0042970.t001

Figure 4. Photographs of Comoran *Lycodryas* specimens. A: ZSM 38/2010, male, Anjouan. B: ZSM 40/2010, female, Anjouan. C: ZSM 42/2010, male, Mayotte. D: female specimen observed at Boungoundranavi, Mayotte. E: ZSM 43/2010, male, Mohéli. F: ZSM 1682/2008, female, Mohéli. G: ZSM 41/2010, male, Grand Comoro. H: ZSM 703/2000, female, Grand Comoro. Photographs A, B, C, E, G by O. Hawlitschek, D by G. Viscardi, F by B. Brenzinger, H by F. Glaw.
doi:10.1371/journal.pone.0042970.g004

preservation. Additionally, the holotype has 8/8 infralabial scales, which is less than in all examined specimens of *L. sanctijohannis* in which at least 9/9 infralabials are present.

L. maculatus and *L. sanctijohannis* are distinguished from all Malagasy species of *Lycodryas* by having 19 instead of 17 dorsal scale rows. Among related genera of Malagasy tree snakes, the only species exhibiting 19 dorsal scale rows is *Phisalixella iarakaensis* [112]. According to Vences et al. [102], this snake is clearly distinguished from *L. maculatus* by several other characters.

Despite minor differences that may be attributed to intraspecific variation or a poor state of preservation, we conclude that all morphological characters examined suggest conspecifity of *L. maculatus* and *L. sanctijohannis*. We consider the possibility that there is a second species of *Lycodryas* on the Comoros (living in sympatry with the *L. sanctijohannis* complex) to be extremely unlikely, as we could not find any other indication of this despite extensive surveys in the field and of the museum material. The possibility that *L. maculatus* represents a Malagasy species of *Lycodryas* awaiting rediscovery cannot be excluded. Because no such species is known, we tentatively assign the name *maculatus* to Comoran *Lycodryas*. We also cannot entirely exclude that *L. maculatus* actually represents a snake from America or elsewhere in the world, but consider this very unlikely. Examination of the head scalation allows assigning the type specimen to the Anjouan+Mayotte clade, as the loreal scale is in contact with supralabials 2 and 3. Even in poorly preserved male specimens from Mayotte, the dark ventral line was often partly visible, which is not the case in the *L. maculatus* specimen. Therefore, we assign this specimen to the Anjouan population.

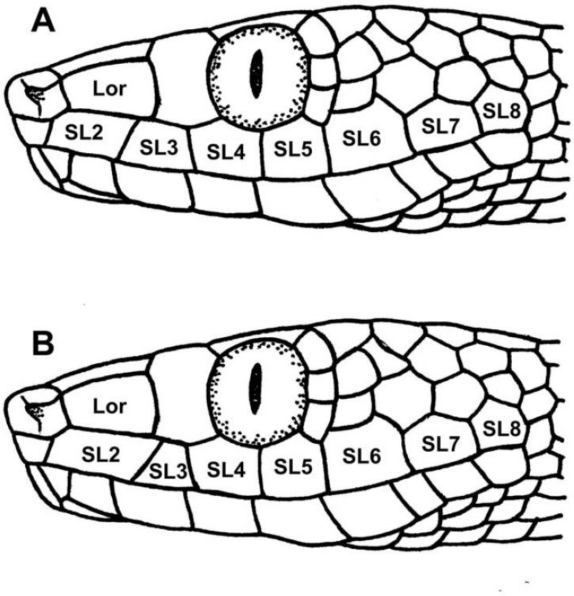

Figure 5. Position of the loreal scale in Comoran *Lycodryas*. Drawings represent specimens from Anjouan and Mayotte (A) or from Grand Comoro and Mohéli (B). In (A), the loreal scale (Lor) is in contact with supralabials (SL) 2 and 3, whereas in (B) it is in contact only with supralabial 2.
doi:10.1371/journal.pone.0042970.g005

Lycodryas maculatus (Günther, 1858) [108] is the oldest available name for Comoran tree snakes and thus has nomenclatural priority over *L. sanctijohannis* Günther, 1879 [80]. *Lycodryas gaimardii comorensis* (Peters, 1874) [107] is the oldest available name for the Mayotte population of Comoran tree snakes. We conclude that *Lycodryas maculatus maculatus* (Günther, 1858) [108] is the valid name for Comoran *Lycodryas* from Anjouan and that *Lycodryas maculatus comorensis* (Peters, 1874) [107] is the valid name for *Lycodryas* from Mayotte.

Lines of evidence for assigning species and subspecies rank to populations of Comoran tree snakes

All four Comoran *Lycodryas* populations are here considered to be candidate species and can be confirmed as distinct taxonomic units (CCS sensu Vieites et al. [97]) according to genetic and morphological data; the status of UCS is not maintained for any clade. The criterion of sympatric occurrence does not apply due to the insular distribution of these clades. We rule out the possibility of DCL because there are no indications of admixture with other lineages.

An inspection of the three lines of evidence (mtDNA, nDNA and morphology) yields the following results: (1) mtDNA: All clades are clearly differentiated by mtDNA markers and are represented by independent cyt *b* parsimony networks. This is confirmed by the parsimony networks based on other markers and by the phylogenetic tree. (2) nDNA: All clades are characterized by an unique combination of nDNA haplotypes. However, the divergence is always limited to a single substitution per marker. Additionally, no single nDNA marker serves to distinguish all four clades. Although all mtDNA markers provide results in accordance with one another, the results provided by the nDNA markers differ from one another, and we cannot exclude the possibility that further study of nDNA markers will yield other results. We therefore assume that these nDNA differences are only slight, not unequivocal and not distinctive enough to be recognized as a line of evidence. (3) Morphology: The position of the loreal scale is a fixed character state distinguishing the Anjouan+Mayotte clades from the Grand Comoro+Mohéli clades. All four clades are distinguished to some degree by differences in male coloration, but these are considered insufficient for completing a line of evidence because they are graded. The smaller size of specimens from the Grand Comoro clade may be a response to ecological constraints rather than a genetically fixed trait.

Strict application of the lines of evidence concept could lead to a view favoring four separate species of Comoran *Lycodryas*: all four candidate species are supported not only by mtDNA but also by differences in nDNA and morphology, albeit only slight differences. In the present case study, we argue that a line of evidence should not be confirmed by such slight differences alone, and we appeal for taxonomic plausibility: Vieites et al. [97] argued that characters to confirm candidate species should be of high value for discriminating species in the respective groups of animals. This is the case for nDNA at a higher level of divergence and for coloration if differences are more significant and possibly quantifiable, but not at levels detected in Comoran *Lycodryas*.

Based on this argument, we resurrect and redescribe *Lycodryas maculatus maculatus* (Günther, 1858) [108] from Anjouan and *Lycodryas maculatus comorensis* (Peters, 1874) [107] from Mayotte. We newly describe *Lycodryas cococola cococola* sp. n. from Grand Comoro and *Lycodryas cococola innocens* sp. n. from Mohéli.

Redescription of Lycodryas maculatus

Genus *Lycodryas* Günther, 1879 [80]

Lycodryas maculatus (Günther, 1858) [108]

Fig. 4: A, B.

http://species-id.net/wiki/Lycodryas_maculatus

Original name. *Dipsadoboa maculata* Günther, 1858 [108].

Holotype. BMNH 1946.1.21.47; adult male; type locality "Central America", changed to "unknown" by Boulenger [103].

Synonym. *Lycodryas sanctijohannis* Günther, 1879 [80].

Holotype. BMNH 1946.1.5.20, adult male; type locality "Anjouan island".

Diagnosis. Largest subspecies of Comoran tree snakes, snout-vent length max. 867 mm; max. snout-vent length for *L. cococola cococola* sp. n. 650 mm, for *L. maculatus comorensis* 757 mm and for *L. cococola innocens* ssp. n. 835 mm. 19 middorsal scale rows, no specimens with 17 known (see Table 1 and Fig. 3 for other taxa of Comoran *Lycodryas*). 233 to 259 ventral scales, 85 to 126 subcaudal scales, posterior ones divided. Loreal in contact with supralabials 2 and 3, as in *L. maculatus comorensis*, but unlike the other two subspecies. Anal shield divided. BMNH 1946.1.21.47 is the only specimen studied with 8/8 infralabial scales, while all other Comoran *Lycodryas* specimens have at least 9/9. Males dorsally grey, head with marbled pattern of darker spots and dots. Lower part of supralabials in males white, upper part brown or black. Body with regular middorsal band of dark brown blotches, scales between these blotches sometimes appearing brighter. Dark ventral stripe always absent. Females with typical pattern of reddish, brownish or yellowish dorsal and yellowish ventral side.

Redescription of BMNH 1946.1.21.47 (Holotype of *Lycodryas maculatus*). Specimen in good condition. No DNA for molecular studies available. Hemipenes not everted. Body slender, approximately as wide as high, snout-vent length 497 mm. Tail complete, length 168 mm. Head clearly distinct from neck, length 14.6 mm. Eye diameter 2.6 mm, pupil vertical, distance between eye and snout-tip 4.2 mm, distance between eyes 2.6 mm.

Scalation: Rostral concave, much wider than tall, hardly visible in dorsal view. Nostrils bordering prenasals, postnasals and supranasals. Loreals each 1 left and right, wider than tall, bordering postnasals, supralabials 2 and 3, preoculars and prefrontals. Supranasals 2, prefrontals 2, frontal 1, preoculars 1/1 (left/right), supraoculars 1/1, postoculars 3/3, parietals 2, supralabials 8/8, 4th and 5th in contact with eye. Mental rhombic, broader than tall. Mental groove separating first infralabials and chin shields, extending to the mental. Infralabials 8/8. Dorsal scales smooth, in 19 rows along the body, ventrals 243, anal shield divided, subcaudals 126, 41st and the posterior 49 divided.

Coloration in preservative. Coloration probably poorly preserved; live coloration unknown. Dorsal and lateral ground color light grayish with irregular brownish shadings, which are probably a result of preservation. Darker grayish or brownish spots smaller than 1 scale distributed over dorsal and lateral sides of body. No regular band of blotches or crossbands visible. The specific name "maculatus" might suggest that distinct spots were originally visible. Head of rather uniformly grayish color; supralabial scales not significantly brighter than rest of head, possibly as a result of poor preservation. Irregular dark blotch extending over the anterior parts of both parietals, bordering frontal, second smaller blotch on posterior part of right parietal. Irregular and asymmetrical shape of blotches suggests they may have resulted from injury. Iris olive-grey. Ventral side of body uniformly brightly grayish without any dark lines or markings.

Redescription of BMNH 1946.1.5.20 (Holotype of *Lycodryas sanctijohannis*). Adult male. Specimen in good condition. No DNA for molecular studies available. Hemipenes not everted. Body slender, approximately as wide as high, snout-vent length 621 mm. Tail complete, length 146 mm. Head clearly distinct from neck, length 16.0 mm. Eye diameter 3.0 mm, pupil vertical, distance between eye and snout-tip 5.5 mm, distance between eyes 5.7 mm.

Scalation: Rostral concave, much wider than tall, hardly visible in dorsal view. Nostrils bordering prenasals, postnasals and supranasals. Loreals each 1 left and right, wider than tall, bordering postnasals, supralabials 2 and 3, preoculars and prefrontals. Supranasals 2, prefrontals 2, frontal 1, preoculars 1/1 (left/right), supraoculars 1/1, postoculars 3/3, parietals 2, supralabials 9/9, 4th and 5th in contact with eye. Mental rhombic to triangular, broader than tall. Mental groove separating first infralabials and chin shields, extending to the mental. Infralabials 10/10. Dorsal scales smooth, in 19 rows along the body, ventrals 259, anal shield divided, subcaudals 85, the posterior 62 divided.

Coloration in preservative. Dorsal and lateral ground color beige to light brownish. Dark brown color elements forming marbled pattern of spots on head and body. Poorly defined row of dark brown blotches visible along vertebral column in anterior third of body, sometimes extending diffusely down the flanks, gradually converting to a diffuse pattern of dark brown spots posteriorly. Flanks with diffuse pattern of smaller dark spots. Supralabial scales beige to whitish, dorsally bordered by dark brown line. Parietals brighter than surrounding scales. Iris olive-brown. Ventral side anteriorly beige with pattern of brown dots, whose density increases posteriorly, turning ventral side almost totally brown towards anal shield (but always lighter grey than dorsal side).

Description of ZSM 38/2010. For better comparability with other taxa of Comoran *Lycodryas*, we include the description of an adult male specimen with sequence data and photographs available. Specimen in good condition. Tongue was removed and separately stored as tissue sample for DNA extractions. Hemipenes not everted. Body slender, approximately as wide as high, snout-vent length 688 mm. Tail complete, length 180 mm. Head clearly distinct from neck, length 22.4 mm. Eye diameter 3.3 mm, pupil vertical, distance between eye and snout-tip 6.9 mm, distance between eyes 6.4 mm.

Scalation: Rostral concave, much wider than tall, hardly visible in dorsal view. Nostrils bordering prenasals, postnasals and supranasals. Loreals each 1 left and right, wider than tall, bordering postnasals, supralabials 2 and 3, preoculars and prefrontals. Supranasals 2, prefrontals 2, frontal 1, preoculars 1/1 (left/right), supraoculars 1/1, postoculars 3/3, parietals 2, supralabials 9/8, 4th and 5th in contact with eye. Mental rhombic to triangular, broader than tall. Mental groove separating first infralabials and chin shields, extending to the mental. Infralabials 10/10. Dorsal scales smooth, in 19 rows along the body, ventrals 233, anal shield divided, subcaudals 87, the posterior 60 divided.

Coloration in life. Dorsal and lateral base color grey. Dark brown color elements forming marbled pattern of spots on head. Well-defined row of dark brown blotches visible along vertebral column; scales between these blotches sometimes appearing brighter, especially in anterior body half. Blotches in anterior body half extending diffusely down the flanks, but with sharp contrast between blotches and their lateral extensions. Flanks with diffuse pattern of smaller dark spots. Loreal and prefrontal beige. Lower parts of rostral and supralabials bright beige, similar to ventral color, upper part dark brown, almost black. Frontal and parietals appearing marbled in beige and brown. Iris silvery grey. Ventral side anteriorly beige with pattern of grey dots, whose density increases posteriorly, turning ventral side totally grey towards anal shield (but always lighter grey than dorsal side). Mental with dark median bar connecting to mental groove.

Variation. Morphological and chromatic variation is summarized in Table 1 and Fig. 4, respectively.

Distribution, natural history and conservation. Endemic to the island of Anjouan. All living specimens for which exact locality data is available were found on trees in plantations and near-natural forests. A distribution map is given in Fig. 6. For geographic coordinates of localities and further comments on habitat and conservation, see Hawlitschek et al. [19].

Resurrection of *Lycodryas maculatus* comorensis with designation of a neotype

Genus *Lycodryas* Günther, 1879 [80]

Lycodryas maculatus comorensis (Peters, 1874) [107]

Fig. 4: C, D.

http://species-id.net/wiki/Lycodryas_maculatus

Original name. *Dipsas* (*Heterurus*) *gaimardii* Schlegel var. *comorensis* Peters, 1874 [107].

Holotype. Not traced and considered lost, juvenile; type locality "Mayotte island".

Synonym. *Lycodryas sanctijohannis* var. *mayottensis* Boettger, 1913

Neotype of Dipsas (Heterurus) Gaimardii Schlegel var. comorensis Peters, 1874. ZSM 42/2010, adult male; collected 7 February 2010; Comoros Archipelago, MAYOTTE, Petite-Terre, near Moya, under bark on a tree; by O. Hawlitschek, J. Berger, B. Brückmann. Justification: The taxon *Dipsas* (*Heterurus*) *gaimardii* Schlegel var. *comorensis* Peters, 1874 [107], has been considered a record of *L. gaimardii* from Mayotte [13]. According to our results, all *Lycodryas* from Mayotte belong to a single taxon that is not conspecific with *L. gaimardii*. Therefore, the designation of a neotype is necessary to stabilize the nomenclatural identity of *Lycodryas* populations from Mayotte.

Diagnosis. Subspecies of Comoran *Lycodryas* of intermediate snout-vent length (max. 757 mm); longer than *L. cococola cococola* sp. n. (max. 650 mm), shorter than *L. maculatus maculatus* (max. 867 mm) and *L. cococola innocens* ssp. n. (max. 835 mm). 19 middorsal scale rows, 17 in MNHN 1884-518 (see Table 1 for comparison with other taxa of Comoran *Lycodryas*). 227 to 261 ventral scales, 84 to 117 subcaudal scales, posterior ones divided. Loreal in contact with supralabials 2 and 3, like in *L. maculatus maculatus*, but unlike other taxa of Comoran *Lycodryas*. Anal shield divided. Males can be distinguished from other taxa of Comoran *Lycodryas* by a mostly clearly defined, sometimes diffuse dark stripe extending from the gular region to the tail tip along the row of ventral scales. Dorsal ground color of males grey or olive-grey, the latter being recorded in no other taxa of Comoran *Lycodryas*. Lower part of supralabials in males white, upper part brown or black; pattern of more or less diffuse dark spots extending over the dorsal side. Dark dorsal crossbands were never recorded in any adult specimen. Females show the typical pattern of reddish, brownish or yellowish dorsal and yellowish ventral side.

Description of the Neotype. Specimen in good condition. Tongue removed and separately stored as tissue sample for DNA extractions. Hemipenes not everted. Body slender, approximately as wide as high, snout-vent length 757 mm. Tail complete, length 218 mm. Head clearly distinct from neck, length 18.0 mm. Eye diameter 3.8 mm, pupil vertical, distance between eye and snout-tip 7.4 mm, distance between eyes 7.1 mm.

Scalation: Rostral concave, much wider than tall, hardly visible in dorsal view. Nostrils bordering prenasals, postnasals and supranasals. Loreals each 1 left and right, wider than tall, bordering postnasals, supralabials 2 and 3, preoculars and prefrontals. Supranasals 2, prefrontals 2, frontal 1, preoculars 1/1 (left/right), supraoculars 1/1, postoculars 3/3, parietals 2, supralabials 8/8, 3rd, 4th and 5th in contact with eye. Mental rhombic, broader than tall. Mental groove separating first infralabials and chin shields, extending to the mental. Infralabials 9/9. Dorsal scales smooth, in 19 rows along the body, ventrals 231, anal shield divided, subcaudals 84, the posterior 36 divided.

Coloration in life. Dorsal and lateral base color olive-grey. Beige, light brown, and dark brown color elements forming diffuse pattern of spots on head and body. , Ill-defined row of brown blotches is visible along the vertebral column in anterior half of body. Blotches extending diffusely down the flanks posterior to head. They may be remnants of dark crossbands, as visible in the hatchlings of SMF 19620 to 19627, and decribed for a juvenile specimen by Peters [107]. On head, prefrontals and supranasals dark, interrupted by beige half-circle connecting to loreals and preoculars, whose upper half is also beige. Lower parts of rostral and supralabials bright beige, similar to ventral color, upper part dark brown, almost black. Frontal and parietals appearing marbled in beige and brown. Iris silvery grey. Ventral side beige, becoming grey posteriorly. Mental with dark median bar connecting to mental groove, then disappearing and reappearing as dark ventral line on first ventral scale. This line, first less than half as broad as the ventral scales, becomes broader and more diffuse towards the tail tip.

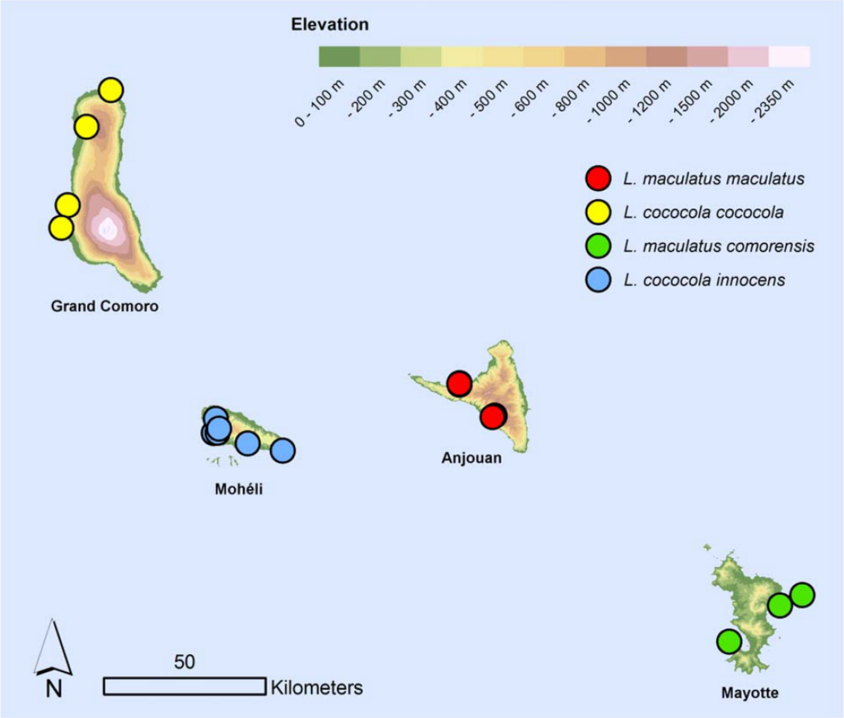

Figure 6. Map of the Comoro archipelago. The circles show records of Comoran specimens of *Lycodryas*.
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Variation. Morphological and chromatic variation is summarized in Table 1 and Fig. 4, respectively.

Distribution, natural history and conservation. Endemic to Mayotte island and adjacent small islands; recorded on Grande-Terre, Petite-Terre, and Chissioua Mbouzi. All living specimens for which exact locality data is available were found on trees in at least near-natural forests, dry forests or mangrove. However, we know of observations in gardens and plantations. A distribution map is given in Fig. 6. For geographic coordinates of localities and further comments on habitat and conservation, see Hawlitschek et al. [19].

Description of *Lycodryas cococola* sp. n.

Genus *Lycodryas* Günther, 1879 [80]

Lycodryas cococola sp. n.

Fig. 4: G, H.

http://species-id.net/wiki/Lycodryas_cococola

urn:lsid:zoobank.org:pub: urn:lsid:zoobank.org:act:70362EFB-B959-439F-9448-6BC3A8B11CEE

Holotype. ZSM 41/2010 (field number FGZC 1512); adult male; collected 13 February 2010; Comoros Archipelago, COMOROS, Grand Comoro, Lac Salé; volcanic rocks around lake (11.37375°S; 43.37306°E, 72 m above sea level); by O. Hawlitschek, J. Berger, B. Brückmann.

Paratypes. All from Comoros Archipelago, COMOROS, Grand Comoro. ZSM 703/2000; adult female; collected February 2000; exact locality uncertain, said to be from near Chindini; by local collector. ZSM 321/2002; adult female; collected 09 April 2002; Moroni, garden of hotel "La Grillade"; by F. Glaw, M. Hiermeier, M. Kotrba. ZSM 1679/2008; adult male; collected 25 February 2008; plantation near Mbachilé, under stone near mango tree; by O. Hawlitschek, B. Brenzinger. BMNH 1985.338; adult male; near Moroni; by M. Pinchon. MNHN 1890-31; adult male; collected 25 January 1890; by Humblot. MNHN 1899/213; adult female; collected 1899; by Pobeguin. MNHN 1899/214; adult male; collected 1899; by Pobeguin. MNHN 1902-392; adult male; collected 1902. MNHN 1902-393; adult male; collected 1902. MNHN 1902-394; sex undetermined; collected 1902. MNHN 1902-395; sex undetermined; collected 1902. MNHN 1957-732; adult female; collected 1957. MNHN 1961-657; adult female; collected 1961; by Millot. MNHN 1978-2925; adult male; collected 1978; by Domergue, Lt. Plassant. ZFMK 45944; adult female. ZMB 19266; 3 specimens, 2 adult males, 1 adult female; coast; by Voeltzkow.

Etymology. Derived from "*Cocos*", genus name of the coconut palm, and the Latin lexical suffix "-cola", meaning "inhabiting". This taxon is commonly found on and near coconut palms, as also reflected by the French vernacular name "serpent des cocotiers" (Coconut palm snake). The species epithet is used as an invariable noun in apposition.

Diagnosis. Smallest subspecies of Comoran *Lycodryas*, snout-vent length max. 650 mm; max. snout-vent length for *L. maculatus comorensis* 757 mm, for *L. cococola innocens* ssp. n. 835 mm and for *L. maculatus maculatus* 867 mm. 19 middorsal scale rows, 17 in BMNH 1985.338 and MNHN 1961-657 (see Table 1 for comparison with other taxa of Comoran *Lycodryas*). 232 to 260 ventral scales, 84 to 116 subcaudal scales, posterior ones divided. Loreal in contact with supralabial 2, like in *L. cococola innocens* sp. n., but unlike the other two taxa of Comoran *Lycodryas*. Anal shield divided. Males dorsally almost uniform grey to brownish. Lower part of posterior supralabials in males whitish, 3 anterior supralabials shareing general head coloration. No dark color elements on supralabials as in other taxa of Comoran *Lycodryas*; whitish supralabials may have darker margins. Dark ventral stripe always absent. Females show the typical pattern of reddish, brownish or yellowish dorsal and yellowish ventral side.

Description of the Holotype. Specimen in good condition. Tongue removed and separately stored as tissue sample for DNA extractions. Hemipenes not everted. Body slender, approximately as wide as high, snout-vent length 535 mm. Tail complete, length 137 mm. Head clearly distinct from neck, length 18.0 mm. Eye diameter 2.7 mm, pupil vertical, distance between eye and snout-tip 5.1 mm, distance between eyes 5.4 mm.

Scalation: Rostral concave, much wider than tall, hardly visible in dorsal view. Nostrils bordering prenasals, postnasals and supranasals. Loreals each 1 left and right, wider than tall, bordering postnasals, supralabial 2, preoculars and prefrontals. Supranasals 2, prefrontals 2, frontal 1, parietals 2, preoculars 1/1 (left/right), supraoculars 1/1, postoculars 3/3, parietals 2, supralabials 8/8, 3rd, 4th and 5th in contact with eye. Mental triangular, broader than tall. Mental groove separating first infralabials and chin shields, extending to mental. Infralabials 10/10. Dorsal scales smooth, in 19 rows along the body, ventrals 233, anal shield divided, subcaudals 87, posterior 77 divided.

Coloration in life. Dorsal and lateral base color of head and body rather uniform grey to brownish. Patterns of darker spots absent. Lower parts of rostral brighter. Anterior 3 supralabials shareing general head color, others whitish, posterior 2 with darker margins. Iris silvery grey. Ventral side yellowish beige without spots or dots. Margins between ventral scales grayish. Upper part of mental darker, no clear stripe visible.

Variation. Morphological and chromatic variation is summarized in Table 1 and Fig. 4, respectively.

Distribution, natural history and conservation. Endemic to Grand Comoro island. Living specimens for which exact locality data is available were found on trees in plantations and degraded forests, but also on the ground. A distribution map is given in Fig. 6. For additional geographic coordinates of localities and further comments on habitat and conservation, see Hawlitschek et al. [19].

Description of *Lycodryas cococola innocens* ssp. n.

Genus *Lycodryas* Günther, 1879 [80]

Lycodryas cococola innocens ssp. n.

Fig. 4: E, F.

http://species-id.net/wiki/Lycodryas_cococola

urn:lsid:zoobank.org:pub: urn:lsid:zoobank.org:act:70362EFB-B959-439F-9448-6BC3A8B11CEE

Holotype. ZSM 43/2010 (field number FGZC 1537); adult male; collected 2 March 2010; Comoro Archipelago, COMOROS, Mohéli, near Ouallah (12.32706°S; 43.66918°E, 12 m above sea level); on tree in degraded forest; by O. Hawlitschek, J. Berger, B. Brückmann.

Paratypes. All from Comoros archipelago, COMOROS, Mohéli island. ZSM 311/2002; adult female; collected 18 April 2002; East of Nioumachoua; by F. Glaw, M. Hiermeier, M. Kotrba. ZSM 312/2002; adult female; collected 20 April 2002; around Ouallah; by F. Glaw, M. Hiermeier, M. Kotrba. ZSM 43/2010; adult female; collected 2 March 2010; East of Ouallah; on tree in plantation area; by O. Hawlitschek, J. Berger, B. Brückmann. ZSM 45/2010; adult female; collected 30 March 2010; Lac Dziani Boundouni; on tree in degraded forest surrounding lake; by O. Hawlitschek, J. Berger, B. Brückmann. ZSM 1682/2008; adult female, gravid with 3 eggs; collected 5 March 2008; Chalêt St. Antoine; tree in clearing on summit near Chalêt; by O. Hawlitschek, B. Brenzinger. MNHN 8702; adult male; collected June 1957; by J. Millot. ZMB 19227; adult male; by A. Voeltzkow. SMF 19629; 3 specimens, all adult, 1 male, 2 females; collected 1905; by A. Voeltzkow.

Etymology. "Innocens", Latin adjective of identical ending in masculine, feminine and neutral gender, meaning "innocent". This name was given in reference to the fact that inhabitants of Mohéli are very afraid of this snake and kill many individuals although it is harmless to humans and generally non-aggressive. Although this is also true for other taxa of Comoran *Lycodryas*, local people seem most afraid of snakes on Mohéli.

Diagnosis. Large subspecies of Comoran *Lycodryas*, snout-vent length max. 835 mm; max. snout-vent length for *L. cococola cococola* sp. n. 650 mm, for *L. maculatus comorensis* 757 mm and for *L. maculatus maculatus* 867 mm. 19 middorsal scale rows, 17 in ZMB 19227 and 1 specimen of SMF 19629 (see Table 1 for comparison with other taxa of Comoran *Lycodryas*). 233 to 259 ventral scales, 85 to 115 subcaudal scales, posterior ones divided. Loreal in contact with supralabial 2, like in *L. cococola cococola* sp. n., but unlike the other 2 taxa of Comoran *Lycodryas*. Anal shield divided. Males dorsally grey, with pattern of light and darker brown spots. Lower part of posterior supralabials in males white, upper part brown or black; first 2 or 3 supralabials shareing the overall head coloration. In general, the coloration of the supralabials is less apparent that in *L. maculatus comorensis* and in *L. maculatus maculatus*. Dark ventral stripe always absent. Females show the typical pattern of reddish, brownish or yellowish dorsal and yellowish ventral side.

Description of the Holotype. Specimen in good condition. Tongue removed and separately stored as tissue sample for DNA extractions. Hemipenes not everted. Body slender, approximately as wide as high, snout-vent length 771 mm. Tail complete, length 200 mm. Head clearly distinct from neck, length 26.2 mm. Eye diameter 3.7 mm, pupil vertical, distance between eye and snout-tip 7.9 mm, distance between eyes 7.5 mm.

Scalation: Rostral concave, much wider than tall, hardly visible in dorsal view. Nostrils bordering prenasals, postnasals and supranasals. Loreals each 1 left and right, wider than tall, bordering postnasals, supralabial 2, preoculars and prefrontals. Supranasals 2, prefrontals 2, frontal 1, preoculars 1/1 (left/right), supraoculars 1/1, postoculars 3/3, parietals 2, supralabials 8/8, 4th and 5th in contact with eye. Mental triangular, broader than tall. Mental groove separating first infralabials and chin shields, extending to mental. Infralabials 10/10. Dorsal scales smooth, in 19 rows along body, ventrals 239, anal shield divided, subcaudals 87, posterior 66 divided.

Coloration in life. Dorsal and lateral base color grey. Diffuse pattern of lighter and darker grey and brown elements dorsally on head, extending onto body, but there with ill-defined small dots and spots of darker brown, also of lighter grey. Lower parts of rostral bright beige, of supralabials darker beige, darker than ventral color, upper part dark brown, almost black. Pattern more contrasted after conservation of specimen, if compared to life photograph. Iris silvery grey. Ventral side yellowish beige with blackish dots arranged in two irregular rows, darkening posteriorly. Mental showing dark median bar not connecting to mental groove.

Distribution, natural history and conservation. Endemic to Mohéli island. All living specimens for which exact locality data is available were found on trees in various kinds of forest-like habitats, including plantations, degraded forests and natural forests. A distribution map is given in Fig. 6. For additional geographic coordinates of localities and further comments on habitat and conservation, see Hawlitschek et al. [19].

Sexual dimorphism

Comoran *Lycodryas* express strong sexual dichromatism (Fig. 4), a very unusual phenomenon among snakes. Differences in morphometry, such as different total length, tail length or head proportions related to sex are observed in a number of snake species, such as Malagasy *Liopholidophis* [84] and many other species across all major families [113]). The expression of different color morphs within populations (e.g., *Vipera berus*, *Madagascarophis colubrinus*), sometimes related to life stage (e.g., *Agkistrodon contortrix*, *Morelia viridis*), is equally common [47,114]. However, differences in coloration between the sexes are rare and often restricted to slight variations in pattern or shading [84,113] (and note the possibility of cryptic dichromatism [115]). The color morphs of the Malagasy *Ithycyphus miniatus* have been considered to be related to sexes [116], but this is not supported by more recent observations (pers. obs.). One of the most striking examples of sexual dimorphism in snakes is presented by the Malagasy tree snake *Langaha madagascariensis* in which the sexes differ not only in coloration but also in the form of their rostral appendages [116]. Males are brown dorsally and females are greyish with darker spots, thus displaying a pattern opposite that of Comoran *Lycodryas* in which males are grey with spots and females are brown. Notably, among all species of *Lycodryas* and related genera in the family Lamprophiidae, the Comoran forms alone express this sexual dichromatism [116,117].

For *Langaha*, Krysko [118] hypothesized that the sexual dimorphism in the form of the rostral appendages may reflect microhabitat differences. This may also apply to color because selection pressures from different types of microhabitats could be assumed to favor different patterns of camouflage. For *Langaha madagascariensis*, this hypothesis seems plausible because this species is at least partly diurnal and has been shown to rely heavily on camouflage for foraging as well as predator avoidance [118]. Unfortunately, sufficient ecological data for testing the microhabitat hypothesis is available neither for *Langaha* nor for Comoran *Lycodryas*. Members of both sexes of the latter have been observed to be active both during the day and night, and the observations do not indicate different microhabitat preferences [19]. Greene [114] (p. 124) speculated that sexual dichromatism in snakes may reflect "divergent antipredator strategies and the increased vulnerability of male snakes as they search for females". This explanation does not appear to be convincing for Comoran *Lycodryas*. Their sexual dimorphism is expressed in an environment with relatively low predation pressure, given that native mammal and reptile predators are absent [13], and no observations of predation on Comoran *Lycodryas* by birds have been made. Additionally, on Madagascar the intensity of bird predation on snakes seems to be limited [119–121] but other *Lycodryas* species showing no sexual dichromatism are preyed upon by various mammals [122–123]. This agrees with the results of Macedonia et al. [124,125], who demonstrated that males of some subspecies of the collared lizard *Crotaphytus collaris* were conspicuously colored in areas with low predator density but cryptically colored in areas with high predator density; conversely, females always exhibited cryptic coloration.

Sexual selection remains a possible explanation for the sexual dichromatism of Comoran *Lycodryas*. In reptiles, this phenomenon has often been observed in lizards [126–128] but apparently not in snakes. In Comoran *Lycodryas*, the observation that color patterns of males but not females vary between island populations may be seen as the result of sexual selection by females. In the Malagasy species of *Lycodryas*, including the putative ancestral form of Comoran *Lycodryas*, predation (as discussed above) likely imposed a higher selection pressure on coloration towards camouflage. In contrast, it can be assumed that Comoran *Lycodryas* initially evolved in an insular habitat where predators were scarce. Thus, competition for mates possibly imposed a higher selection pressure on coloration than the need for predator avoidance [124,125]. Shine [113] argues against the function of color as a sexual signal in snakes because most snakes rely on chemical rather than visual cues to find mates [129,130], color vision in snakes was poorly developed, and dichromatism rarely takes a form that could be applied in courtship displays (reviewed in Shine [113]). However, later experiments demonstrated that at least some snake species are indeed capable of color perception [131]. The sexual dichromatism in Comoran *Lycodryas* may thus play a role in mate recognition. Even if color perception was poorly developed in this species, the pattern of dark spots on a lighter body expressed by males might function in social signaling based on contrast patterns rather than color. Notably, as mentioned above, the hemiclitores of female Comoran *Lycodryas* are very well developed. Their function is unknown. Females with well-developed hemiclitores are known from the insular pit viper species *Bothrops insularis*, where these specimens that are called "intersexes" by some authors are more numerous than males and (infertile) "normal" females [132]. Reproduction by a female in the absence of males, supposedly through facultative automictic parthenogenesis (FAP), is reported for this species [133]. Possible examples of FAP are recorded in an increasing number of reptiles and certainly provide an advantage for the colonization of new habitats, such as via overseas dispersal [134]. If the founder population of Comoran *Lycodryas* also possessed the ability to reproduce by FAP, then adaptations facilitating mate recognition, such as sexual dichromatism, may have been selected for in order to avoid FAP in favour of sexual reproduction. With the data currently available, however, FAP in Comoran *Lycodryas* remains pure speculation, and additional data should be collected to investigate possible explanations for the phenomenon of sexual dichromatism.

Effects of island evolution and the "island rule"

Aside from their unique sexual dimorphism, Comoran *Lycodryas* are distinguished from their Malagasy congeners by their larger size. The largest specimens recorded had total lengths of 1052 mm (ZSM 40/2010) and 1047 mm (ZSM 43/2010). The largest Malagasy *Lycodryas* specimen belongs to *L. granuliceps* (formerly *L. capuroni*) and has a total length of 1020 mm [102]. However, most specimens examined by Vences et al. [102] and Nagy et al. [82] were shorter, reaching maximum total lengths of 700 mm or less. In addition to larger body size, Comoran *Lycodryas* have an increased number of 19 dorsal scale rows at midbody, in contrast to 17 in the eight other *Lycodryas* species [82,102].

The same phenomena are expressed by *Liophidium mayottensis*, the only Comoran endemic in an otherwise Malagasy genus of terrestrial snakes. The maximum total length recorded for this species endemic to Mayotte was 978 mm (Table S1), whereas the largest Malagasy species *L. therezieni* reaches 726 mm according to Glaw & Vences [116]. *L. mayottensis* is also the only species of its genus with 19 dorsal scale rows; all 8 of its congeners have 17 dorsal scale rows [116,135].

The fact that both arboreal and terrestrial Comoran snakes are larger than their "mainland" congeners (even though Madagascar is an island itself, it can be considered mainland in relation to the much smaller Comoros) conforms to Van Valen's [28] "island rule". This rule states that on islands, small animals become larger and large animals become smaller in comparison to their mainland relatives. In Van Valen's original work, these phenomena were discussed for mammals alone. Lomolino [74] also discussed it for other vertebrates and described a more general pattern in which island species tend to approach the medium size for their clade (or 'optimal' size, see also Boback & Guyer [136]). This again leads to gigantism in small species and dwarfism in larger species (but see Meiri et al. [137]).

Among Malagasy snakes, the genera *Lycodryas* and *Liophidium* represent smaller body sizes in general. Several other genera of terrestrial and diurnal snakes (*Liopholidophis*, *Dromicodryas*, *Leioheterodon*) have larger body sizes than *Liophidium* species, with total lengths of over one meter. The same is true for the arboreal and nocturnal *Lycodryas* and related genera *Parastenophis* and *Phisalixella* [116]. This suggests that on Madagascar, relatively large specimens of *Lycodryas* and *Liophidium* are subject to competition by larger-bodied snake species [116,138]. Lomolino [74] hypothesized that on islands, where selection pressures due to interspecific competition and environmental heterogeneity are lower, species are less constrained to diverge from their modal size. On the Comoros, each species is the only species representing its guild (terrestrial/diurnal and arboreal/nocturnal snakes, respectively) and interspecific competition if effectively absent. This might have allowed Comoran *Lycodryas* and *Liophidium* to approach their 'optimal' body size and become 'giants'. Notably, the phenomenon of island gigantism is repeated on the Comoros by the iguanid lizard *Oplurus cuvieri comorensis*, a subspecies that attains larger sizes than its mainland congeners: Glaw & Vences [116] state a maximum of 373 mm total length for Malagasy *O. cuvieri* whereas Meirte [81,139] reports sizes of up to 500 mm for the Comoran subspecies.

The other feature distinguishing both Comoran *Lycodryas* and *Liophidium* from their mainland congeners is their increased number of dorsal scale rows. Previous studies indicated that variation in scale numbers is often correlated with climate; hotter and drier conditions may favor either fewer but larger scales [140,141] or a greater number of smaller scales [142,143]. Sanders et al. [144] studied variation in scalation in nocturnal and arboreal *Trimeresurus* snakes, which also show an increase in scale numbers in hotter and drier climates. They argue that larger and often highly sculpted scales are favored if animals are diurnal and exposed to high insulation and thus need to efficiently radiate excess heat. Smaller (and more numerous) scales, however, reduce the area of exposed interstitial skin due to their "tighter fit". Cutaneous evaporation via the exposed interstitial skin has been shown to be an important way of water loss in reptiles [145]. Since many Malagasy species of *Lycodryas* and *Liophidium* occupy large ranges with variable temperatures and precipitation, it is not easy to establish clear relationships between these factors and their scalation. However, *Lycodryas inornatus* and *L. guentheri* from the dry South and *Lycodryas gaimardii* from the wet east coast of Madagascar both have 17 dorsal scale rows. The same is true for *Liophidium torquatum*, which is from wetter regions all over Madagascar and for *Liophidium apperti*, *L. chabaudi* and *L. trilineatum*, which are from the dry South. The climate on the Comoros is intermediate between these mainland extremes (see climate layers on the worldclim data base [146]). It is therefore an unlikely driving force of variation in scalation.

Instead of climate, the increased number of dorsal scales may simply be correlated with the increase in size and body diameter. Comoran *Lycodryas* have body sizes similar to the larger members of the closely related genus *Phisalixella*, all of which have 19 to 25 dorsal scale rows [82]. These evolutionary trends might also be reflected in the insular species of *Lycodryas* and *Liophidium*.

The subspecies model

In this paper, we split the formerly recognized single species of Comoran *Lycodryas* into two species, each with two subspecies. However, many authors, particularly herpetologists, have argued against maintaining or newly describing subspecies at all. In the following section, we discuss the subspecies concept in the light of these developments.

As reviewed by de Queiroz [39,43], different species concepts, or operational criteria of the unified species concept, will identify the split from one into two species at different points in the process of speciation ("considering a separately evolving lineage to have become a species", according to de Queiroz [43]). Using Cracraft's [38] phylogenetic species concept as an operational criterion, all four island populations of Comoran *Lycodryas* clearly warrant species status: they are monophyletic and diagnosable by differences in the mtDNA markers studied. The monophyletic clusters are supported by the TCS analyses. Thus, they could be regarded as separately evolving metapopulation lineages [39] and may also be seen as independent lineages of the evolutionary species concept [32].

As acknowledged by de Queiroz [43], the question of whether the lineages studied are separate enough to qualify for species status remains. Clearly, all four lineages of Comoran *Lycodryas* share at least similar evolutionary tendencies and a common historical fate [32] due to their insular endemism, origin via overseas dispersal, lack of competition, and similar habitat and ecology. Whether their evolutionary tendencies and their historical fates are just similar or the same remains a decision that can hardly be bolstered by quantitative data and is thus arbitrary. Unfortunately, we feel that the data available for Comoran *Lycodryas* is at a level that allows neither unambiguous acceptance nor rejection of species level for the lineages. The morphological differences are scarce, but morphological diagnosability is not seen as a requirement for most species concepts (explicitly stated in Wiley [32]). A single diagnostic morphological character is found between, but not within, the two major clades of Comoran *Lycodryas* (Anjouan+Mayotte vs. Grand Comoro+Mohéli). The divergence in the nDNA markers studied is marginal, thus rather discouraging elevation to species rank, although it is known that many otherwise well-delimited species show no divergence in nuclear markers [147,148]. The most significant line of evidence is mtDNA. Divergences are higher between the two major clades confirmed by morphology, and lower within these clades. Notably, as stated above, the divergence between the major clades meets the threshold that was identified for lamprophiid snake species in Nagy et al. [104], while divergences within these clades are lower. Convergently, the divergences in cyt *b* between the major clades are higher than the lowest divergences between other congeneric species, whereas within-clade divergences are in the same range or below. Proposals for thresholds of mtDNA divergence for species delimitation have been made [97,149,150], but these are arbitrary and should be used for the preliminary designation of UCS or operational taxonomic units (OTU) rather than for species descriptions, as stated by the authors themselves. Nevertheless, we believe that the comparison with a threshold can be helpful in critical cases, such as that of Comoran *Lycodryas*. Thus, as discussed after the application of the "Lines of Evidence" approach, our data provide relatively good support for splitting Comoran *Lycodryas* into two species, but less convincing support for splitting it into four species. In our view, evidence suggests that speciation within the two major clades has reached a level that does not yet warrant species status. Unlike many authors who decline to recognize a taxonomic level below species [38,51,60–62], we adopt the view that there is a level of divergence on the way to speciation at which lineages are already diagnosably distinct, but should not yet be considered full species. In our opinion, this level

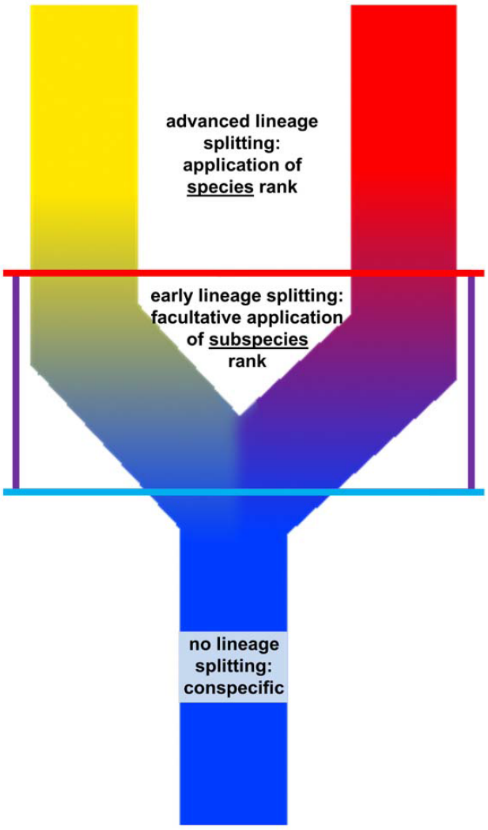

Figure 7. Simplified sketch showing a speciation event. Modified after de Queiroz [42]. A single lineage (blue) splits into two divergent lineages (red and yellow). Below the blue line, a single species is recognized unambiguously, while above the red line, two species are recognized unambiguously. In the zone framed by the lines, disagreement over the number of species (1 vs. 2) is possible, depending on which species concept is applied. Assigning subspecies rank to lineages in this stage appears as a possible solution.
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corresponds to the rank of subspecies (Fig. 7). Below, we will provide our reasoning for this view.

Subspecies have largely been neglected in species concepts other than the biological species concept because the main difference between species and subspecies is reproductive isolation [26,151]: subspecies differ from other subspecies of the same species but can interbreed with them. In the sense of Mayr [26] and Dobzhansky [27], reproductive isolation means isolation by intrinsic mechanisms and not by geographical barriers (as is the case in allopatric island populations). Finding evidence for intrinsic isolation mechanisms has always been a major issue in species delimitation. Therefore, many researchers adopted a view in which species were fully differentiated forms that could coexist in sympatry and allopatric differentiated forms were assigned subspecies status [67,152,153]. This corresponds with the definition of subspecies as a "stage in the process of allopatric speciation" [26,151]. Maintaining reciprocal isolation of parapatric or sympatric species despite the possibility to interbreed is seen as evidence for the existence, and also as reason for the evolution, of intrinsic isolation mechanisms [154].

For these reasons, insular forms with a certain degree of differentiation have often been described as subspecies. In part, this has contributed towards the tendency of herpetologists to reject subspecies. In the Mediterranean region, 23 subspecies of *Podarcis pityusensis* and 91 subspecies of *P. siculus* were recognized at some point. Many of these subspecies descriptions were based on poor evidence, representing local color morphs only and most were later synonymized. Other forms, for which good evidence existed, were elevated to species rank [155–159]. Such events have led many authors to reject the subspecies concept as a whole; we argue that this critizism should be directed against such misuse of the subspecies rank, but not to the concept of the subspecies per se. On the Comoros, two examples of island-specific subspecies groups exist. The first example is the four island-endemic subspecies of the skink *Cryptoblepharus boutonii* (Desjardin, 1831) [160], which are now mostly considered species according to Horner [161]. These are easily distinguished by their color patterns but show only shallow genetic divergence and are thus likely to be the result of very recent colonization events [162]. The second example is the radiation of *Phelsuma* day geckos endemic to the Comoros. One island (Mayotte) has two endemic species that most likely resulted from sympatric speciation and are clearly distinct in morphology, ecology and molecular characters. Their sister taxon is *Phelsuma v-nigra* Boettger, 1913 [104], which is present on the remaining three major islands with each one endemic subspecies [163–165]. The three subspecies are genetically distinct, show moderate morphological divergence, and have and no determinable differences in ecology [166]. Apparently, the divergence of these three lineages is less strong than between this clade and the Mayotte lineages; therefore, we can see the *P. v-nigra* subspecies as a "stage in the process of allopatric speciation" [26,151].

If seen as separately evolving metapopulation lineages according to the general lineage concept [39] or minimum diagnosable units according to Cracraft's [32] phylogenetic species concept, all of these geographically isolated insular subspecies would have to be elevated to specfic status. In the case of *Cryptoblepharus boutonii* alone, which inhabits not only the Comoros but a large number of islands in the Indian and Pacific oceans, this would lead to an increase in species numbers from one to 36 [162] (but see Horner [161]). Such an increase in species numbers was discussed broadly in papers on taxonomic inflation [167–170]. Taxonomic inflation in species is a problem because species are the taxonomic level that is most important inside and outside taxonomy: it is species, much less subspecies, genera or families, that are counted in species lists, evaluated for conservation purposes and are given most attention in evolutionary studies [64,65,171]. Describing minimum diagnosable units as species, or elevating such subspecies to species level, bears the risk that the lower limit of what is diagnosable will be reduced to the point at which diagnosable units no longer represent separately evolving entities. Subspecies, for which neither monophyly nor any type of unique evolutionary tendencies or historical fate are required, are not necessarily concerned by this problem: they describe a level below that of independently evolving lineages [63]. Fitzpatrick [66] considered this level a "zone of art" and subspecies a "heterogeneous mix of evolutionary phenomena", and before him, Mayr [172] saw subspecies as "convenient handles by which to describe, sort, store, retrieve, and discuss certain types of information about phenotypic geographic variation". Species, however, are thought to represent real evolutionary entities. We therefore believe that describing species purely based on a personal and subjective interpretation of "existence as a separately evolving metapopulation lineage", "minimum diagnosable unit" or lineage with "own evolutionary tendencies and historical fate" can lead to very divergent species counts, the same problem for which the subspecies was widely rejected.

While the search for a single overarching species concept continues [173], species delimitations have become more sophisticated in relation to earlier, purely descriptive approaches [174]. We argue that species delimitations should rely on more than one operational criterion and should incorporate modern criteria, such as the evolutionary and the general lineage concept, and guidelines for using the evidence provided by all types of data available. The lines of evidence approach by Miralles et al. [71] provides such a guideline. This concept is arbitrary in and of itself due to its definitions of species and subspecies according to the lines of evidence. Additionally, as shown in the present case study, the lines of evidence should not be followed blindly but instead be judged with a taxonomist's knowledge of related examples, more generalized species concepts and common sense. Nevertheless, the widespread use of integrative taxonomic approaches, with the application of a common guideline, may increase the objectivity of taxon descriptions in all groups of organisms. It may do so more than another new species concept.

Supporting Information

Table S1 Morphological data on specimens of *Lycodryas* from the Comoro islands and *Liophidium mayottensis*.
(XLS)

Table S2 Primers and PCR protocols. Forward (F) and reverse (R) primers are given, all in 5′-3′ order. Mitochondrial gene loci: 16S = 16S ribosomal RNA, cyt *b* = cytochrome B oxidase, ND4 = NADH dehydrogenase subunit 4, COI = cytochrome C oxidase 1. Nuclear gene loci: c-mos = nuclear genomic proto-oncogene *c-mos*, Rag2 = recombination activating gene 2, PRLR = prolactin receptor.
(PDF)

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Author Contributions

Conceived and designed the experiments: OH FG. Performed the experiments: OH ZTN. Analyzed the data: OH ZTN. Contributed reagents/materials/analysis tools: OH ZTN. Wrote the paper: OH ZTN FG. Specimen acquisition: OH FG. Morphological analyses and species description: OH.

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7 Spin-off products

7.1 What kind of spin-off products?

In the course of the work I did for this dissertation, I was able to make progress with some side projects. I decided to exclude two of these projects completely from my dissertation because I had only a small part in their making, but I nevertheless want to mention them here because they are comprised in the same complex of work as the previous chapters on Australian diving beetles:

HENDRICH L., HAWLITSCHEK O., BALKE M. (2009): The epigean Australasian species of *Neobidessodes* **gen.n.** diving beetles – a revision integrating morphology, cybertaxonomy, DNA taxonomy and phylogeny (Coleoptera: Dytiscidae, Bidessini). Zootaxa 2288: 1–41.

HÁJEK J., HENDRICH L., HAWLITSCHEK O., BALKE M. (2010): *Copelatus sibelaemontis* **sp. nov.** (Coleoptera: Dytiscidae) from the Moluccas with generic assignment based on morphology and DNA sequence data. Acta Entomologica Musei Naturalis Pragae 50: 437–443.

I call this chapter 'Spin-off products' because it includes two projects that do not really focus on the topic of 'Speciation and species delimitation'. These projects are nonetheless intimately connected with my other work on the Comoros and are – in my opinion – necessary to complete my dissertation, albeit in a less prominent place, because they lay the fundament for all the previous chapters on Comoran reptiles (Chapters 4 to 0) and sum the results for the public and for future researchers (Chapter 7.3).

7.2 PAPER: Integrating field surveys and remote sensing data to study distribution, habitat use and conservation status of the herpetofauna of the Comoro Islands.

During my field surveys on the Comoros, I collected numerous voucher specimens and tissue for phylogenetic and taxonomic analyses. Aside from these, however, I collected a considerable amount of habitat and distributional data on all reptiles and the two amphibian species of the archipelago. Together with the results of an examination of museum records, these data allowed the clarification of some questionable species records. More importantly, however, in combination with data from my diploma thesis they allowed me to estimate which species were abundant and which were rare, and which species were common also in degraded habitats, while others depended on natural areas. After the publication of this paper, but based on it, I officially proposed IUCN Red List stati of threat for all described endemic Comoran reptile species that had not been previously assessed by other authors. This happened under the management of PHIL BOWLES (IUCN) in the course of the Madagascar Reptile Assessment 2011.

Some species had been assessed by previous authors. The proposals given in the paper were so far published only for species that had not been previously assessed. Table 6 gives an overview over all species concerned. All species records can be reviewed at the IUCN website (http://www.iucnredlist.org/).

> "There is one last reason for caring, and I believe that no other is necessary. It is certainly the reason why so many people have devoted their lives to protecting the likes of rhinos, parakeets, kakapos and dolphins. And it is simply this: the world would be a poorer, darker, lonelier place without them."
>
> Mark Carwardine

*Table 6: IUCN Red List stati of Comoran endemic reptiles. *: Other Comoran taxa of* Cryptoblepharus *have not been assessed so far. **: Due to taxonomic changes (Chapters 5.1 and 6.1), the assessments of these taxa are in need of updating.*

Species	**Current official IUCN status assessed by**	**Proposed status (HAWLITSCHEK et al. 2012, Zookeys)**	**Official IUCN status**
Amphiglossus johannae	INEICH & MEIRTE 2011	Least Concern	Least Concern
Cryptoblepharus ater *	INEICH 2010	Least Concern	Data Deficient
Furcifer cephalolepis	CARPENTER 2011	Least Concern	Least Concern
Furcifer polleni	HAWLITSCHEK & GLAW 2011	Least Concern	Least Concern
Liophidium mayottensis	HAWLITSCHEK & GLAW 2011	Endangered B1ab(iii)+2ab(iii)	Endangered B1ab(iii)+2ab(iii)
*Lycodryas sanctijohannis***	HAWLITSCHEK & GLAW 2011	Near Threatened	Near Threatened
*Paroedura sanctijohannis***	HAWLITSCHEK & GLAW 2011	Endangered B1ab(iii)+2ab(iii)	Endangered B1ab(iii)+2ab(iii)
Phelsuma comorensis	INEICH 2010	Near Threatened	Least Concern
Phelsuma nigristriata	HAWLITSCHEK & GLAW 2011	Vulnerable D2	Vulnerable D2
Phelsuma pasteuri	HAWLITSCHEK & GLAW 2011	Near Threatened	Near Threatened
Phelsuma robertmertensi	HAWLITSCHEK & GLAW 2011	Endangered B1ab(iii)+2ab(iii)	Endangered B1ab(iii)+2ab(iii)
Phelsuma v-nigra	INEICH 2010	Near Threatened	Least Concern
Trachylepis comorensis	RAXWORTHY & BOWLES 2011	Least Concern	Least Concern
Typhlops comorensis	HAWLITSCHEK & GLAW 2011	Data Deficient	Data Deficient

HAWLITSCHEK O., BRÜCKMANN B., BERGER J., GREEN K., GLAW F. (2011): Integrating field surveys and remote sensing data to study distribution, habitat use and conservation status of the herpetofauna of the Comoro Islands. Zookeys 144: 21-79.

Post-publication comments and errata:

p. 43: Unconfirmed records and taxonomic comments – *Lycodon aulicus*: An inspection of CAS (California Academy of Sciences, San Francisco, USA) 135119 on 2012-08-23 showed that this is a specimen of *Lyocdryas sanctijohannis*. A number of other misidentified specimens of Comoran reptiles were detected at CAS. The only other recorded Comoran specimen of this species, FMNH (Field Museum of Natural History, Chicago, USA) 205907, could not yet be inspected, but it seems plausible that this is also a misidentification. In this case, the record of *Lyocdon aulicus* on the Comoros would be obsolete. I thank JENS VINDUM, San Francisco, for granting me access to the herpetological collection at CAS.

p. 69: *Furcifer cephalolepis* is erroneously stated as recorded at Mt. Benara, Mayotte. Correctly, this refers to *F. polleni*. *F. cephalolepis* is endemic to the island of Grand Comoro.

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RESEARCH ARTICLE

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Integrating field surveys and remote sensing data to study distribution, habitat use and conservation status of the herpetofauna of the Comoro Islands

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Abstract

We studied the non-marine reptile and amphibian species of the volcanic Comoro archipelago in the Western Indian Ocean, a poorly known island herpetofauna comprising numerous microendemic species of potentially high extinction risk and widespread, non-endemic and often invasive taxa. According to our data, the Comoro islands are inhabited by two amphibian species and at least 28 species of reptiles although ongoing genetic studies and unconfirmed historical records suggest an even higher species diversity. 14 of the 28 currently recognized species of terrestrial reptiles (50%) and the two amphibians are endemic to a single island or to the Comoro archipelago. The majority of species are most abundant at low elevation. However, a few endemic species, like the gekkonid lizards *Paroedura sanctijohannis* and *Phelsuma nigristriata*, are more common in or even confined to higher altitudes. We created habitat maps from remotely sensed data in combination with detailed species distribution maps produced using comprehensive data from field surveys between 2000 and 2010, literature, and historical locality records based on specimens in zoological collections. Using these data, we assessed the conservation status of the endemic terrestrial reptiles and amphibians according to the IUCN Red List criteria. Our results show that although little area of natural forest remains on the Comoros, many species are abundant in degraded forest or plantations. Competition and predation by invasive species appears to be the most important threat factor for the endemic herpetofauna, together with habitat degradation and destruction, which further favours invasive species. We propose the status Endangered for three species, Vulnerable for one species, Near Threatened for six species, Least Concern for four and Data Deficient for two species. The endemic subspecies *Oplurus cuvieri comorensis* is proposed for the status Critically Endangered. Based on the results of this study, seven areas of importance for reptile and amphibian conservation on the Comoros are identified. This study shows how remote sensing data can contribute to increasing accuracy and objectiveness of conservation assessments.

Résumé:

Nous avons étudié les reptiles et amphibiens non marins de l´archipel volcanique des Comores, Océan Indien occidental, une herpétofaune insulaire peu connue et comprenante à la fois de nombreuses espèces microendémiques à risque d´extinction potentiellement élevé, et des espèces non endémiques à grande aire de distribution, souvent invasives. D´après nos données, l´archipel des Comores est habité par 2 espèces d´amphibiens et au moins 28 espèces de reptiles, bien que des études génétiques en cours et des données historiques non confirmées suggèrent une diversité spécifique plus élevée. 14 des 28 espèces de reptiles terrestres actuellement référencés (50%) et les 2 espèces d´amphibiens sont endémiques d´une seule île ou de l´archipel. La majorité des espèces sont plus abondantes à basse altitude. Cependant quelques espèces endémiques comme les lezards Gekkonidae *Paroedura sanctijohannis* et *Phelsuma nigristriata* sont plus communs ou confinés à l'altitude élevée. Nous avons réalisé des cartes des habitats à partir de données de télédétection ainsi que des cartes détaillées de distribution des espèces en combinant des données obtenues au cours de mission de terrain entre 2000 et 2010, des données de la littérature et des données de distribution historiques basées sur des spécimens de collections zoologiques. A partir de ces données, nous avons évalué le statut de conservation des espèces endémiques de reptiles terrestres et d´amphibiens d´après les critères de la liste rouge de l´UICN. Nos resultats montrent que bien qu´il reste de petites zones de forêt naturelle aux Comores, beaucoup d´espèces sont abondantes dans les forêts dégradés et les plantations. La competition et la prédation par les espèces invasives apparaît comme la plus grosse menace pesant sur l´herpétofaune endémique, aux quelles il convient d´ajouter les dégradations et la destruction des habitats, qui en outré favorise les espèces invasives. Nous proposons le statut Endangered (en danger) pour 3 espèces, Vulnerable (vulnérable) pour une espèce, Near Threatened (quasi menacée) pour 6 espèces, Least Concern (preoccupation mineure) pour 4 et Data Deficient (données insuffisantes) pour 2 espèces. Le statut Critically Endangered (danger critique d'extinction) est proposé pour la sous-espèce *Oplurus cuvieri comorensis.* Sur la base de ces résultats, 7 régions d´importance pour la conservation des reptiles et amphibiens des Comores sont identifiées. Cette étude montre comment les données de télédétection peuvent contribuer à accroître la précision et l'objectivité des estimations de conservation.

Keywords

amphibians; area of occupancy; conservation planning; IUCN Red List; Landsat; protected areas; reptiles; satellite imagery; Western Indian Ocean

Introduction

Island faunas are considered especially vulnerable to threats and extinction because of the small areas occupied by species and the inability to compensate losses resulting from environmental influences (Diamond 1984a, 1984b, Purvis et al. 2000, Cole et al. 2005, Rödder et al. 2010, Lee and Jetz 2011). High extinction rates on islands have been documented for birds (Temple 1985) and reptiles (Case et al. 1992, Cheke and Hume 2008).

In order to predict and prevent future extinctions, it is necessary to detect threatened species and assess the degree of threat they are facing. The IUCN Red List has become the reference to the status of threatened species worldwide and an important tool in biodiversity monitoring and conservation planning (Stuart et al. 2004, Rodrigues et al. 2006, Vié et al. 2008, Hoffmann et al. 2010). Initially, Red List status assessments were based solely on the experience and opinion of the assessors; no formal criteria were available (Burton 2003). The first set of objective criteria was released in IUCN (1994), and revised in IUCN (2001a), to be henceforth applied in Red List assessments. These criteria are either based on known numbers of individuals or areas inhabited by the species in question. While numbers of individuals may be reliably estimated or counted in large, conspicuous and well-known species, this is often difficult if species are small, elusive or occur in remote areas. In such cases, the criteria based on area are more useful. Buchanan et al. (2008) used remote sensing data to estimate deforestation and its impact on the conservation status of birds in New Britain. To date we know of no other attempts to directly link remote sensing information with IUCN Red List assessments. We suggest that, in cases where sufficient distributional data are available, remote sensing may provide an important contribution towards the objectification of Red List assessments.

For a case study, we chose the Comoro archipelago in the Western Indian Ocean (WIO). The WIO is a region in which particularly many extinction events of reptiles have occurred. Half of the 20 reptile species listed as extinct in the IUCN Red List were endemic to this area (IUCN Red List of Threatened Species, Version 2010.4, http://www.iucnredlist.org; downloaded on 27 October 2010). Due to the relative scarcity of historical records and the absence of identifiable fossil and subfossil material (but see Gerlach and Canning 2004 for a note on possible fragments of giant tortoises), no reliable hypotheses on possible past extinctions on the Comoros can be made. Similarly, no information on possible threats to their endemic extant herpetofauna has been available so far.

The Comoro archipelago consists of four major islands: Grand Comoro (1010 sqkm), Anjouan (424 sqkm), Mohéli (217 sqkm) and Mayotte (377 sqkm). They are a result of hot-spot volcanism (Emerick and Duncan 1982) and were not connected to each other or any other landmasses at any point in their geological history (Colonna et al. 1996, Battistini and Verin 1984). Distances between the islands are from 50 to 90 kilometres and roughly 300 kilometres both to the East African coast and to Madagascar. According to various authors the archipelago's oldest island, Mayotte, has an estimated age of between 15 and 3.65 ma (million years) (Hajash and Armstrong 1972, Montaggioni and Nougier 1981, Emerick and Duncan 1982, Nougier et al. 1986, Bachélery and Coudray 1993). Climate on the Comoros is tropical and dominated by monsoon and trade winds.

Seven families of terrestrial reptiles and one family of amphibians are recorded on the Comoros. The highest species diversity is found in the geckos (14 species), followed by skinks (4 species), lamprophiid (3 species) and typhlopid snakes (3 species), chameleons (2 species), one species of iguana (*Oplurus cuvieri comorensis)*, and one species of agamid lizard (*Agama agama*). Two species of amphibians are endemic, but still await description (Vences et al. 2003). Sea turtles commonly use Comorian beaches for reproduction but are not included in this study as we focus on the terrestrial herpetofauna. The Nile Crocodile (*Crocodylus niloticus*) was reported to occur on the Comoros by Benson (1960), but has not been observed since. Benson's report probably refers to an erratic specimen (Meirte 2004).

The iguana *Oplurus cuvieri comorensis* is endemic to Grand Comoro at subspecies level, the nominate form inhabiting Madagascar (Münchenberg et al. 2008). Each of the four subspecies of *Cryptoblepharus boutonii* (Scincidae) is endemic to one of the four islands, while some other subspecies of the same species are distributed over a vast area in the Indian and Pacific oceans (Rocha et al. 2006). *Phelsuma v-nigra* (Gekkonidae) is endemic to the Comoros as a species, but Grand Comoro, Mohéli and Anjouan each have one endemic subspecies (Rocha et al. 2009a).

As in many regions worldwide, natural habitats on the Comoros are threatened by the impact of man. The archipelago was first colonized by man over a thousand years ago (Allibert and Verin 1996, Cheke 2010, Msaidie et al. 2011). Today, the Comoros are densely populated islands; calculated from numbers of various sources (Central Intelligence Agency 2010, INSEE 2007), densities are about 325 inhabitants per sqkm for Grand Comoro, 595 for Anjouan, 167 for Moheli and 533 for Mayotte. In comparison, the numbers are 117 inhabitats per sqkm for metropolitan France and 404 for the Netherlands, Europe's most densely populated country. An estimated growth rate of 2.7% makes the Comorian population one of the fastest growing on earth. Due to requirements for construction, ylang ylang distillation, and agriculture, most forests in easily accessible areas are lost, and the remaining forests are already modified by activities of logging and agroforestry (Anonymus 2000, Kothari 2003). Jolly and Fukuda-Parr (2000) calculated an average deforestation rate of 5.8% per year for the period from 1990 to 1995, which is the fourth highest rate worldwide.

Due to increasing sea and air traffic, foreign species are regularly introduced and sometimes become invasive. Most prominent among the invasive plants is the strawberry guava (*Psidium littorale*) on Grand Comoro, which has already had a devastating impact on native floras on Mauritius and Hawaii (Lowe et al. 2000, Tassin et al. 2006). Many animal species, among them rats, were already introduced in historical times (Allibert et al. 1983, Atkinson 1985). Four species of *Hemidactylus* house geckos inhabit the Comoros, all of which were probably introduced historically or recently (Vences et al. 2004, Rocha et al. 2005). Several *Hemidactylus* species including those occurring on the Comoro archipelago are considered invasive in many tropical areas and *Hemidactylus frenatus* has probably led to the extinction of the endemic species of *Nactus* geckos on the Mascarene Islands (Cole et al. 2005). A case of very recent introduction is the agamid lizard *Agama agama*. According to Meirte (2004) this species was introduced to Grand Comoro in 1998, and already in early 2000 it was found to be abundant in parts of the capital Moroni (Wagner et al. 2009).

In this paper, we present distribution maps for all terrestrial species of Comorian reptiles and amphibians. Furthermore, we present the first complete terrestrial habitat survey based on satellite imagery for the Comoros archipelago, which we utilised to evaluate which habitats are of importance for the herpetological species. We also present a key to the reptiles and amphibians of the Comoro Islands to facilitate future identification. Based on these results, we use IUCN Red List criteria to assess the conservation status of the Comorian reptiles and amphibians and propose areas of particular conservational importance.

Methods

Locality data and species identification

Locality data on reptiles and amphibians was obtained from zoological collections, field surveys, and literature. We surveyed all preserved Comorian specimens of terrestrial reptiles and amphibians from the following collections: Natural History Museum, London, United Kingdom (BMNH); Muséum National d'Histoire Naturélle, Paris, France (MNHN); Senckenberg Forschungsinstitut und Naturkundemuseum Frankfurt, Germany (SMF); Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK); Museum für Naturkunde Berlin, Germany (ZMB); Zoologische Staatssammlung München, Germany (ZSM). In addition we discuss further records of special interest fom the California Academy of Sciences, San Francisco (CAS) and the Field Museum, Chicago (FMNH) which were traced by searches in the HerpNET database (http://herpnet.org). Material from field surveys and museums were also used for creation of the identification key.

Only reliable localities of safely identified specimens have been included in this study. All recent locality data was collected in repeated field surveys in the Comoros in 2000, 2002, 2008 and 2010, with a total of 24 weeks of field work or obtained from Carretero et al. (2005). We also visited historical localities in an attempt to confirm the presence of the species historically recorded. To verify species identifications, voucher specimens (deposited in ZSM) and / or tissue samples were collected and photographs were taken. In the field, coordinates were stored with a Garmin (R) ETREX Venture GPS.

Habitat classes

During the herpetological surveys, we also collected ground truthing data for habitat mapping. Ten habitat classes were selected based on their different suitability as habitats for reptiles and amphibians, and ease of identification both in the field and in remote sensing applications. Where appropriate, classes were selected in accordance with the IUCN habitats authority file (IUCN 2001b), and classification numbers of this file are given in brackets. Basic information on Comorian vegetation was obtained from Anonymus (2000), Pascal (2002) and Louette et al. (2004).

Closed Forest: Forest with little or no traces of human impact and a closed canopy. The remote sensing classification does not allow the discrimination between areas of undisturbed pristine forest and forest which has a closed canopy but is modified by low intensity human activities such as limited agro-foresty, selective logging, and firewood collecting. However, the methods applied here have already been used to discriminate areas of forest under heavier human influence (Hayes and Sader 2001, Sader et al. 2001, Wilson and Sader 2002). Corresponsing classes in IUCN (2001b) are Tropical Moist Lowland (1.6) and Tropical Moist Montane Forests (1.9)

Mangrove: Tropical Mangrove Forests (1.7), dominated by *Rhizophora* and *Avicennia* trees, growing in estuaries and coastal areas.

Montane Dry Vegetation: Tropical High Altitude Shrubland (3.7), dominated by *Philippia (Erica) comorensis*. Restricted to Mt. Karthala on Grand Comoro, at altitudes of above 1400 meters.

Volcanic Rock: Areas covered by recent lava outflow. Vegetation is absent or very scarce. Also includes large cliff and shore areas of volcanic rock.

Inland Water: Water bodies within the terrestrial surface of the islands that have no connection to the sea. This includes freshwater (e.g. Lac Dziani Dzaha) as well as other types of water (e.g. Lac Salé).

Degraded Forest: Tropical Heavily Degraded Former Forest (11.6) in forest areas where logging, intensive agroforestry, and clearing for small plantations are present. It also includes regenerating secondary forests. This habitat is distinguished from natural forest types by a more open canopy and thus less reflectance in the near-IR bands in relation to reflectance in the visible spectrum. Patches of crop plants are present, either under the original canopy or in clearings. Neophyte stands are common.

Plantation: Areas widely dominated by plantations of arboreal and arbusteal crops (11.3). Only isolated stands, single trees or nothing is left of the primary forest. In relation to forest and degraded forest, near-IR reflectance is lower. This class does not include pure open crop plantations (manioc, potato etc.). On the Comoros, however, such crops are rarely planted in sufficiently large pure plantations to justify a separate class. Patches of open crop plantations may be included in the classes “Plantation” or “Dry / Low Vegetation”.

Dry / Low Vegetation: Characterized by complete or near complete vegetation cover in dry areas. It is dominated by shrubby or herbaceous plants (Tropical Dry Shrubland: 3.5), but may also contain stands of short trees or single larger trees, often forming savanna-like structures (Dry Savanna: 2.1). Baobabs (*Adansonia digitata*) often occur in this class. This class also includes plantations in dry areas, which are often to a high degree mixed with natural vegetation (11.3). Near-IR reflectance is lower than in other vegetation classes because of relatively low photosynthetic activity due to drought.

Openly Populated Area: Urban areas (11.5) with gardens (Rural Gardens: 11.4), small plantations, and trees which often shade buildings. More than 50% vegetation cover.

Densely Populated Area: Denser urban areas (11.5), dominated by buildings and infrastructure, with less than 50% vegetation cover.

Habitat and distribution maps

We created habitat maps of all four Comoro islands based on Landsat ETM+ images of 30 m resolution, purchased from Landsat.org, Global Observatory for Ecosystem Services, Michigan State University (http://landsat.org, 2007-09-20; images et1610690228032, et1620680814032, et1620690520012, and et1630681114052, years 2001 to 2007). Elevation data was obtained from SRTM-DEM images of 90 m resolution from the information technology center at the University of Tokyo (http://itc.u-tokyo.ac.jp/GIS, 2007-10-10; images S13E044, S12E043, S13E043, and S13E045).

Habitat mapping was conducted using the maximum likelihood supervised classification in ENVI 4.3 to 4.7, ITT Industries, Inc., followed by manual processing in ESRI ArcGIS 9.2 and 9.3. We based our supervised classification on ground verification points collected in the field, using 10% to 25% of the available field points as test points instead of training points. Further accuracy assessment of the classification was conducted by visual comparison of habitat classes between the classified map, topographic maps (1:25,000 for Mayotte and 1:50,000 for all other islands, © Institut Géographique National, France, 1993 and 1995) and GoogleEarth scenes (http://earth.google.com/). We then used ArcGIS 9.2 to 10.0 to measure the areas covered by the different habitat classes and to create distribution maps for all species.

Conservation assessments

We evaluated all endemic species of reptiles and amphibians in the Comoros using the IUCN Red List criteria (IUCN 2001a). These assessments are considered proposals for a national conservation status as well as IUCN Red List status and are currently under review at the CI-IUCN Biodiversity Assessment Unit. To allow an objective application of this guideline, we calculated extent of occurrence (area potentially inhabitable by a species) and area of occupancy (part of this area in which a species actually lives; see IUCN 2001a for further explanation) based on our habitat classification. Historical records which could not be confirmed by surveys after 1999 were not included in these calculations, because the habitat class of these localities may have changed between the time of recording and today. Unless a species was known to be restricted to a certain part of an island, we considered the total area of all islands inhabited by a species as this species' extent of occurrence. To calculate the area of occupancy, we subtracted all area that was outside the species' altitudinal range or that was covered by habitat classes in which the species was not recorded from the extent of occurrence. For certain species, habitat classes were excluded from the area of occupancy even if specimens had been observed there because they were considered unsuitable for maintaining a viable population, as explained for each of these species in the discussion. Conservation assessments and proposals of IUCN Red List status are presented under Discussion: Conservation assessments.

Results

Distribution maps and species composition

We examined 680 historical specimens in herpetological collections which are known to harbour significant material from the Comoro islands (BMNH, MNHN, SMF, ZFMK and ZMB), representing 306 localities recorded between 1845 and 1991. Of all historical localities, 56 had very imprecise locality data (e.g., "Comoros"). 205 were referable to one particular island of the archipelago. Only 45 had locality data precise enough to be used for our distribution maps, and all but two of these could be confirmed in our field surveys. Thus, these rather precise historical records provided very valuable support in our surveys. In our field surveys from 2000 to 2010, we collected 311 specimens and recorded 952 localities. We obtained another 133 locality records collected in 2003 from Carretero et al. (2005). After exclusion of overlapping records, a total of 1038 localities were used in our analyses and for the creation of the distribution maps (Appendix 1, 2).

Our data (Table 1) show that seven families of reptiles are present on the Comoros. 14 of the 28 recognized species of terrestrial reptiles (50%) and both amphibian species (100%) are endemic to a single island or to the Comoros. Of the 36 taxa of both groups recognized at species or subspecies level, 22 (66.7%) are endemic. Within the Comoro archipelago amphibians have only been recorded on Mayotte, where the two species are endemic, but to date undescribed. One species of blindsnake (*Typhlops* sp.) is probably undescribed. Photographs of the species and subspecies are shown in Figs 5–9.

Habitat classification

Our habitat classification is supported by a total of 738 ground verification points. Habitat maps are given in Figure 1. The results show that plantation (28%), degraded forest and dry / low vegetation (each 27%) equally contribute to a total of 82% of the land cover of the Comoros (Table 2 and Figure 2). Mayotte is clearly dominated by plantation (38%), Grand Comoro by dry / low vegetation (36%), Anjouan (38%) and Mohéli (35%) by degraded forest. Closed forest makes up only 9% of the total land area of the Comoros (Figure 3). The largest areas remain on Grand Comoro (68.5 sqkm) and cover only 7% of the island's area. In proportion to island size, the largest area of forest is detected on Mohéli with 16% (34.5 sqkm), followed by Anj-

Table 1. An overview of species and subspecies of Comorian reptiles and amphibians. EI = Endemic to this island, EC = Endemic to the Comoros, P = Present on this island, but not endemic.

	Gd. Comoro	*Anjouan*	*Mohéli*	*Mayotte*
Typhlopidae:				
Ramphotyphlops braminus	P	P	P	P
Typhlops comorensis	EC	EC	-	-
Typhlops sp.	EI	-	-	-
Lamprophiidae:				
Leioheterodon madagascariensis	P	-	-	-
Liophidium mayottensis	-	-	-	EI
Lycodryas sanctijohannis	EC	EC	EC	EC
Chamaeleonidae:				
Furcifer cephalolepis	EI	-	-	-
Furcifer polleni	-	EC	-	EC
Gekkonidae:				
Ebenavia inunguis	P	P	P	P
Geckolepis maculata	P	P	P	P
Hemidactylus frenatus	P	P	P	P
Hemidactylus mercatorius	P	P	P	P
Hemidactylus parvimaculatus	-	P	P	-
Hemidactylus platycephalus	P	P	P	P
Paroedura sanctijohannis	EC	EC	EC	EC
Phelsuma comorensis	EI	-	-	-
Phelsuma dubia	P	P	P	P
Phelsuma laticauda	-	P	-	P
Phelsuma nigristriata	-	-	-	EI
Phelsuma pasteuri	-	-	-	EI
Phelsuma robertmertensi	-	-	-	EI
Phelsuma v-nigra anjouanensis	-	EI	-	-
Phelsuma v-nigra comoraegrandensis	EI	-	-	-
Phelsuma v-nigra v-nigra	-	-	EI	-
Iguanidae:				
Oplurus cuvieri comorensis	EI	-	-	-
Scincidae:				
Amphiglossus johannae	EC	EC	EC	EC
Cryptoblepharus boutonii ater	EI	-	-	-
Cryptoblepharus boutonii degrijsii	-	EI	-	-
Cryptoblepharus boutonii mayottensis	-	-	-	EI
Cryptoblepharus boutonii mohelicus	-	-	EI	-
Trachylepis comorensis	P	P	P	P
Trachylepis striata	-	P	-	-
Agamidae:				
Agama agama	P	-	-	-
Mantellidae:				
Blommersia sp.	-	-	-	EI
Boophis sp.	-	-	-	EI

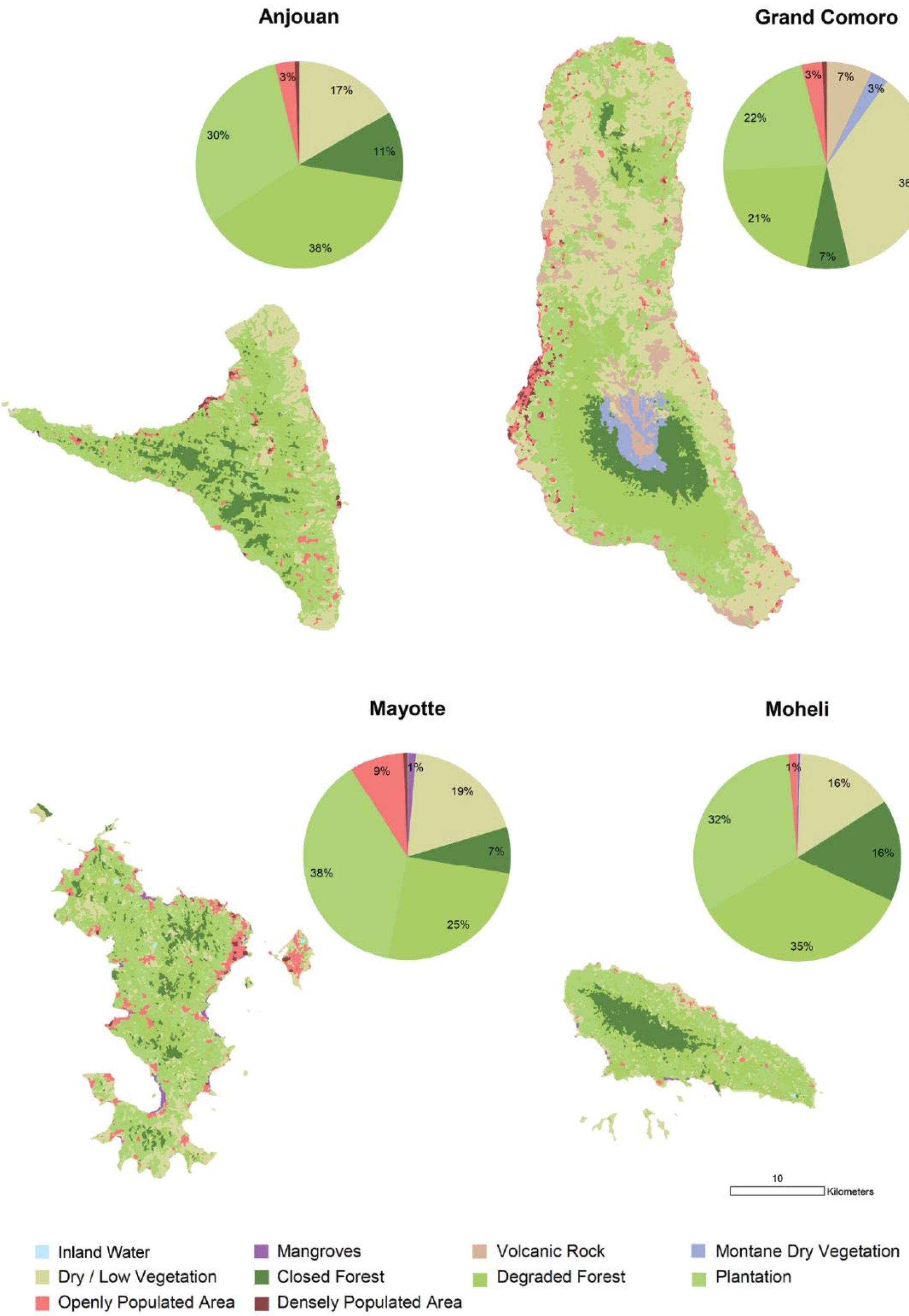


Figure 1. Habitats on the Comoro islands. Maps of habitat classes and relative land cover are given for each island.

Table 2. Land cover by habitat classes. The areas of each habitat class per island and as total are given in square kilometres.

	Anjouan	Grand Comoro	Mayotte	Mohéli	Total
Inland Water	0.00	0.04	0.06	0.02	0.12
Mangroves	0.01	0.07	4.44	0.08	4.60
Volcanic Rock	0.00	73.23	0.00	0.00	73.23
Montane Dry Vegetation	0.00	27.00	0.00	0.00	27.00
Dry / Low Vegetation	70.24	367.95	71.64	33.53	543.37
Closed Forest	46.60	68.48	27.47	34.47	177.02
Degraded Forest	161.27	212.64	95.06	74.84	543.81
Plantation	129.41	220.69	143.50	69.68	563.28
Openly Populated Area	13.22	32.91	31.52	3.03	80.68
Densely Populated Area	2.79	6.85	2.27	0.00	11.91
	423.54	**1009.85**	**375.96**	**216.65**	**2025.01**

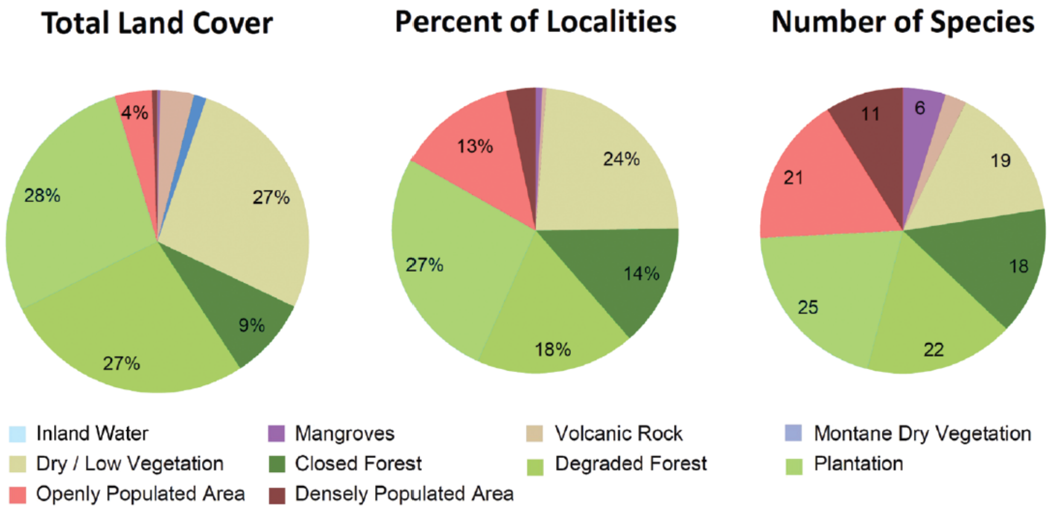


Figure 2. Land cover, localities and number of recorded species

ouan with 11% (46.6 sqkm). Mayotte displays the smallest remaining area of forest (27.5 sqkm, 7%).

Habitat use

Most specimens (27%) and species (25 species) out of the total number of 30 species of reptiles and amphibians were recorded in the habitat class plantation (Figure 2). A similarly high percentage of specimens was recorded in dry / low vegetation (24%), followed by degraded forest (18%). Of specimen records, 14% were made in forest and

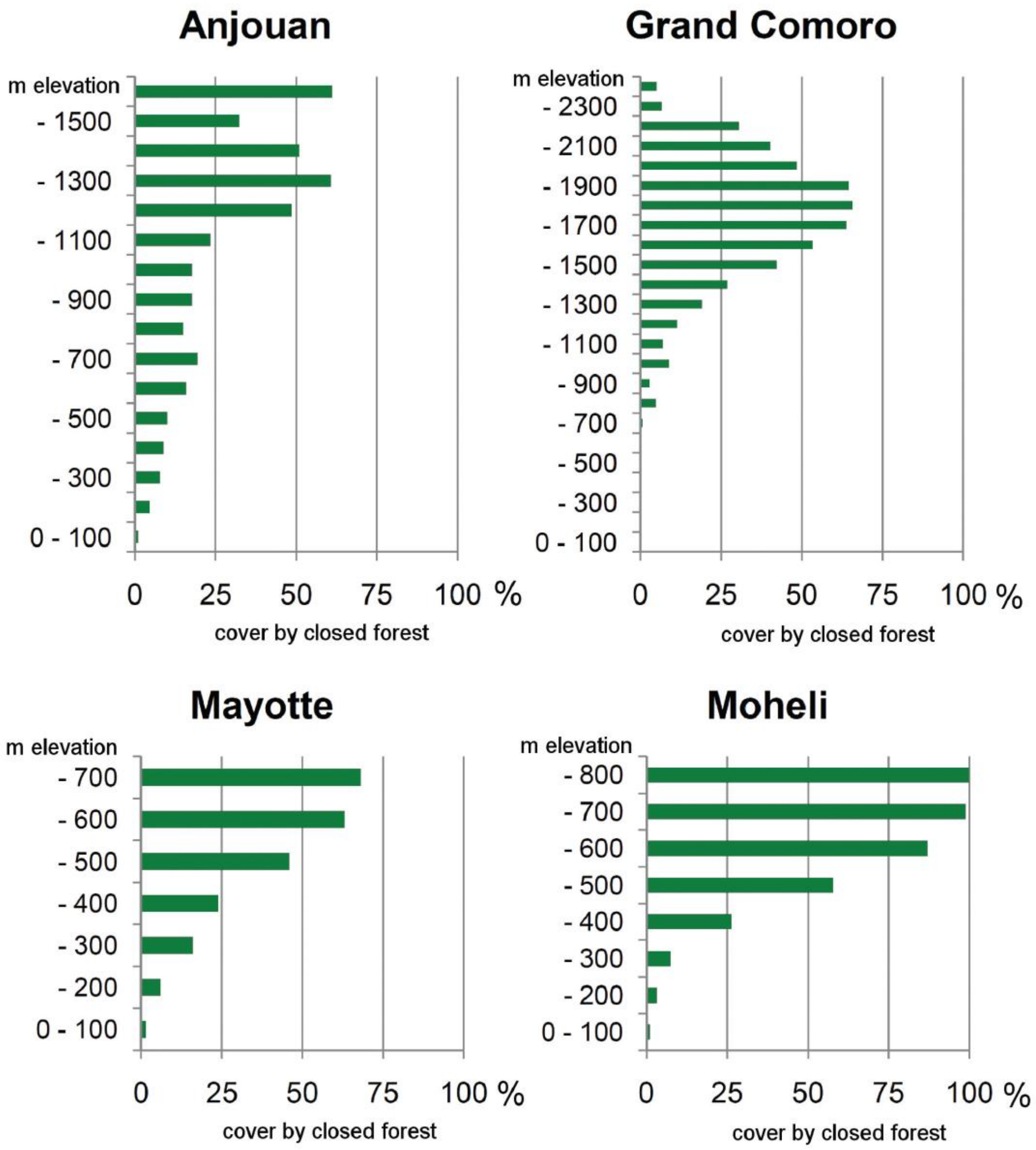
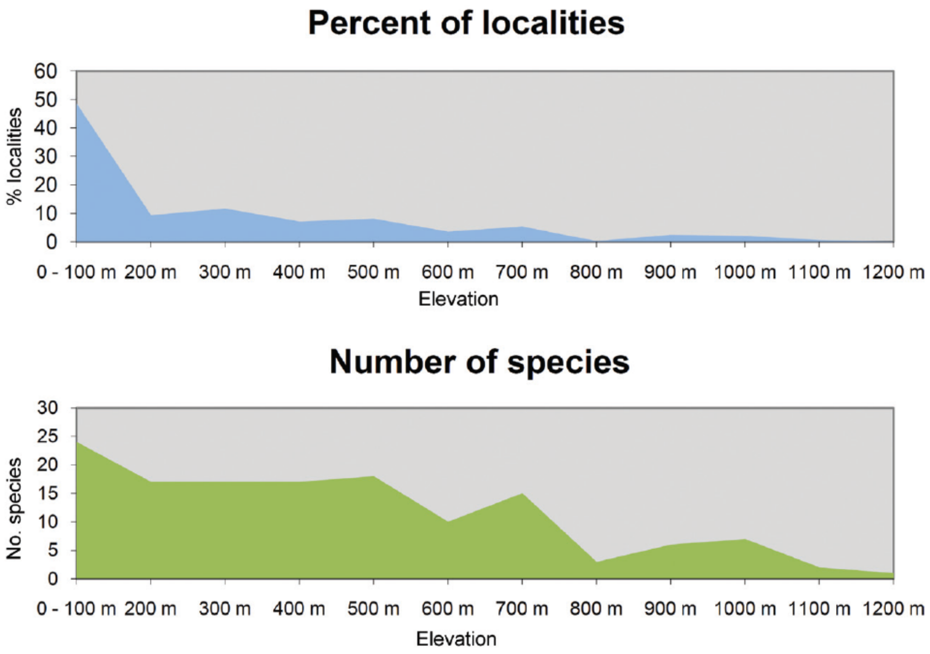


Figure 3. Forest areas on the Comoros. For each level of altitude (in intervals of 100 m), the area occupied by forest is given as percentage of the total area occupied by all habitat classes in this level.

13% in openly populated areas. The second highest numbers of species were recorded in degraded forest (22) and in openly populated areas (21), followed by dry and low vegetation (19), forest (18), densely populated areas (11), mangroves (6) and volcanic rock (3).

Regarding altitude, almost half of all localities (48.9%) and 25 species (out of 30) were recorded in altitudes below 100 m asl (Figure 4). The number of species recorded then drops to 18 up to 200 m altitude and still 16 at elevations of up to 700 m. The relative number of records decreases to around 10% up to 200 m and further to a single specimen of *Furcifer cephalolepis* on the slope of Mt. Karthala, Grand Comoro, at 1135 m asl.

Distribution maps and distribution over habitats and altitude levels for all species are given in Figs 10–19.


Percent of localities

Number of species

Figure 4. Localities and number of species in relation to altitude.

Table 3. Proposed Red List status, extent of occurrence (EOO) and area of occupancy (AOO) of reptile and amphibian species endemic to the Comoros. EOO and AOO are given in sqkm. Endemic subspecies that are not endemic at species level are marked with an asterisk (*).

Species	*Status*	*EOO*	*AOO*
Typhlops comorensis	DD	1433.5	?
Typhlops sp.	DD	1009.9	?
Liophidium mayottensis	EN B1abiii + B2abiii	376.0	65.2
Lycodryas sanctijohannis	NT	2025.0	1737.4
Furcifer cephalolepis	LC	1009.9	718.1
Furcifer polleni	LC	376.0	371.5
Paroedura sanctijohannis	EN B1abiii + B2abiii	2025.0	60.8
Phelsuma comorensis	NT	250.1	183.5
Phelsuma nigristriata	VU D2	376.0	24.8
Phelsuma pasteuri	NT	376.0	127.0
Phelsuma robertmertensi	EN B1abiii + B2abiii	376.0	98.9
Phelsuma v-nigra	NT	1650.1	1368.2
Oplurus cuvieri comorensis *	CR B1abv + B2abv	7.6	<7.6
Amphiglossus johannae	LC	2025.0	1737.4
Cryptoblepharus boutonii ater *	LC	?	?
Cryptoblepharus boutonii degrijsii *	LC	?	?
Cryptoblepharus boutonii mayottensis *	NT	?	?
Cryptoblepharus boutonii mohelicus *	LC	?	?
Trachylepis comorensis	LC	>2025.0	>1811.0
Blommersia sp.	NT	376.0	297.6
Boophis sp.	NT	376.0	297.6

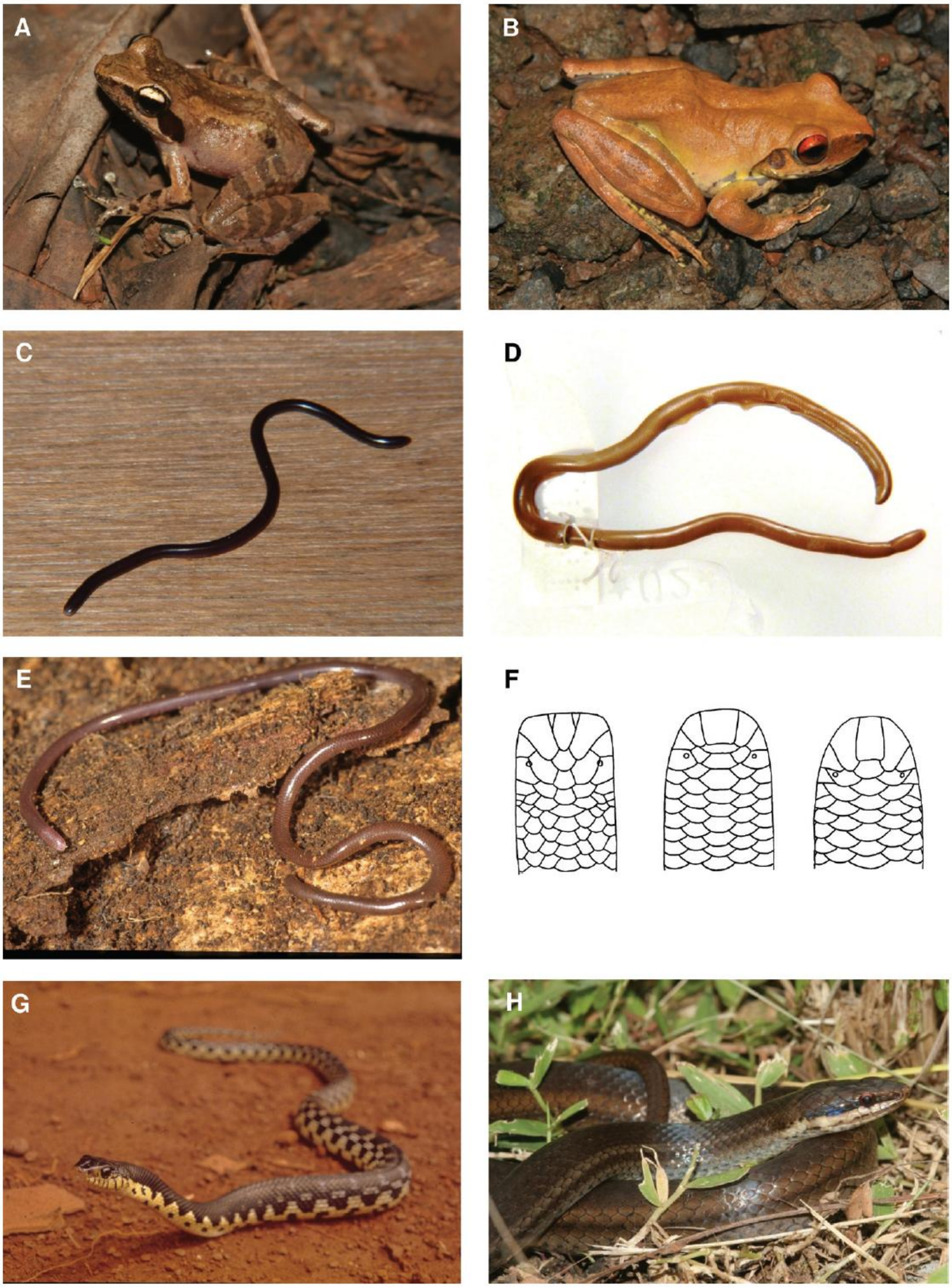


Figure 5. Species photographs. **A** *Blommersia* sp., ZSM 1706/2008, Mayotte **B** *Boophis* sp., ZSM 1711/2008, Mayotte **C** *Ramphotyphlops braminus*, ZSM 163/2010, Anjouan **D** *Typhlops comorensis*, MNHN 1895/126, Grand Comoro **E** *Typhlops* sp., ZSM 361/2002, Grand Comoro **F** drawings of heads of *R. braminus*, *T. comorensis* and *Typhlops* sp. (left to right) **G** *Leioheterodon madagascariensis*, Nosy Boraha, Madagascar **H** *Liophidium mayottensis*, ZSM 1693/2008, Mayotte.

Identification key

We created an identification key including all non-marine species and subspecies of amphibians and reptiles on the Comoros. We also included species whose occurrence on the Comoros could not be confirmed. For every species, the islands of its occurrence are indicated in round brackets (AN = Anjouan, GC = Grand Comoro, MA = Mayotte, MO = Mohéli). The key is based on data published in Meirte (2004), Vences et al. (2004), Glaw and Vences (2007) and unpublished data in Hawlitschek (2008) and by F. Glaw and V. Wallach.

1 Skin moist, no scales present; tail absent... **Amphibians / Anurans (frogs), 2**

– Skin dry, covered with scales; tail present .... **Reptiles, 3**

2 Small species (adult snout-vent length 25-35 mm); tympanal region blackish; hind limbs with distinct dark bands; relatively small eyes; iris silvery; adult males with distinct femoral glands on ventral surface of thighs; only on Mayotte .... ***Blommersia* sp. (MA)**

– Medium-sized species (adult snout-vent length 35-56 mm); tympanal region beige to brown; hind limbs without or with indistinct dark bands; relatively large eyes; iris reddish; adult males without femoral glands on ventral surface of thighs; only on Mayotte .... ***Boophis* sp. (MA)**

3 Limbs and external ear opening absent .... **Serpentes (snakes), 4**

– Limbs and external ear opening present .... **Lacertilia (lizards), 11**

4 Worm–like snakes with burrowing habits; very small, total length ca. 100-250 mm; scalation and coloration uniform all over body; eyes absent or only visible as small black spots covered by scales .... **Typhlopidae, 5**

– Typical snakes, ground- or tree-dwelling; medium-sized to large, adult total length > 50 cm; one distinctly enlarged row of ventral shields and 17–23 dorsal scale rows at midbody; pupil of eyes clearly visible... **other families, 7**

5 Maximum total length 181 mm; 20 midbody scale rows; 261-364 total middorsal scales; superior nasal suture visible dorsally .... ***Ramphotyphlops braminus* (AN, GC, MA, MO)**

– Not as above .... ***Typhlops*, 6**

6 Maximum total length 245 mm; 22 midbody scale rows; 414-485 total middorsal scales .... ***Typhlops comorensis (AN, GC)***

– Extremely small, known total length 68-70 mm; 18 midbody scale rows; 384-408 total middorsal scales; species poorly known...... ***Typhlops* sp. (GC)**

7 Dorsal coloration with dark and bright crossbands, dark bands at least twice as broad as bright bands; less than 100 ventral scales; only on Grande Comoro, occurrence in need of confirmation .... ***Lycodon aulicus* (Natricidae, GC – UNCONFIRMED)**

– Dorsal coloration other than 7 A); more than 150 ventral scales .... **Lamprophiidae, 8**

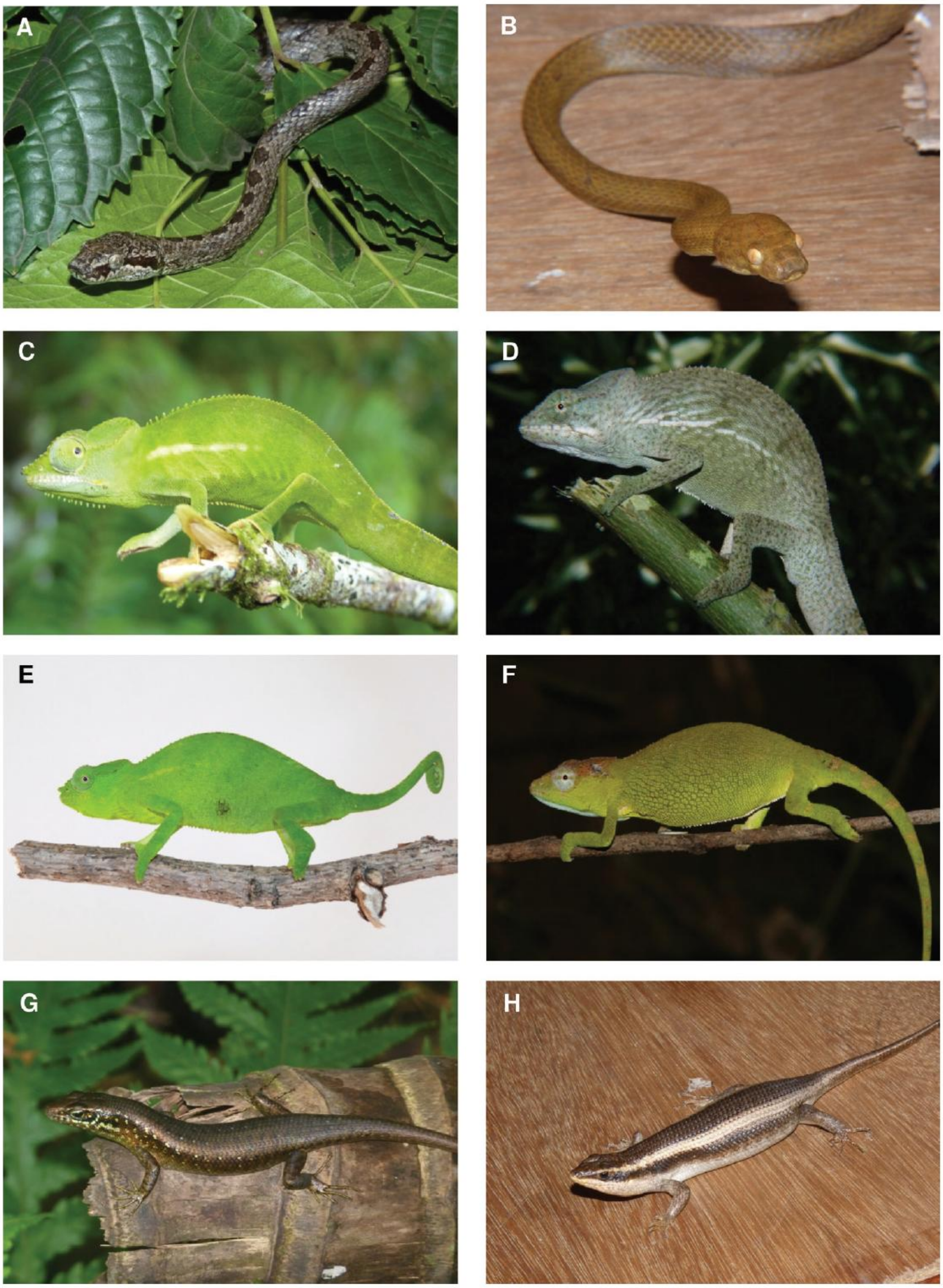


Figure 6. Species photographs. **A** *Lycodryas sanctijohannis*, male, ZSM 38/2010, Anjouan **B** *Lycodryas sanctijohannis*, female, ZSM 40/2010, Anjouan **C** *Furcifer cephalolepis*, male, Grand Comoro **D** *Furcifer polleni*, male, Anjouan **E** *Furcifer cephalolepis*, female, Grand Comoro **F** *Furcifer polleni*, female, Mayotte **G** *Trachylepis comorensis*, Mohéli **H** *Trachylepis striata*, ZSM 70/2010, Anjouan.

8 Robust, ground-dwelling and digging snake; rostral scale directed upwards; total length of adults up to 150 cm; 23 dorsal scale rows .... ***Leioheterodon madagascariensis*** **(GC)**

– Slender, terrestrial or arboreal, non-digging snakes; rostral scale not directed upwards; total length of adults up to 100 cm; 17-19 dorsal scale rows **9**

9 Pupil round; head not broader than neck; coloration dorsally grayish, ventrally bright with small dark spots on each ventral scale; ground-dwelling.......... ***Liophidium mayottensis*** **(MA)**

– Pupil vertical; head distinctly broader than neck; ground- or tree-dwelling.......... ***Lycodryas*****, 10**

10 Mostly 19 dorsal scale rows (sometimes 17) at midbody; coloration sexually dimorphic, males grayish, sometimes with dark spots or blotches, iris gray, females uniformly orange or brown, iris brownish.......... ***Lycodryas sanctijohannis*** **(AN, GC, MA, MO)**

– 17 dorsal scale rows at midbody, coloration brown with dark crossband.......... ***Lycodryas gaimardii*** **(MA – UNCONFIRMED)**

11 Eyes largely covered with skin and moving independently; prehensile tail; tong-like hands and feet with two or three lateral fingers opposing inner fingers..........**Chamaeleonidae, 12**

– Eyes not covered with skin; no prehensile tail; fingers and toes not fused.......... **other families, 13**

12 Snout-vent length maximum 77 mm in males, 54 mm in females; rostral crests with very distinctly enlarged scales, fusing anteriorly and reaching or exceeding snout tip; distinctly elevated parietal crest; gular crest with distinctly enlarged cones.......... ***Furcifer cephalolepis*** **(GC)**

– Snout-vent length maximum 104 mm in males, 63 mm in females; rostral crests with weakly enlarged scales, poorly elevated, diminishing posterior to snout tip; parietal crest visible, but not elevated; gular crest poorly developed .... ***Furcifer polleni*** **(AN, MA)**

13 Head dorsally with enlarged, symmetrical scales distinct from scales following posteriorly..........**Scincidae / Lacertidae, 14**

– Head dorsally with scales similar to those following posteriorly.......... **other families, 21**

14 Dorsal scales granular, ventral scales cycloid and overlapping, forming a distinct collar of enlarged scales in the throat region.......... ***Meroles knoxii*** **(Lacertidae, AN – UNCONFIRMED)**

– Scales uniformly cycloid and overlapping.......... **Scincidae, 15**

15 Body cylindrical, head and tail not clearly distinct from body; limbs small; coloration light brown with dark brown spots.......... ***Amphiglossus johannae*** **(AN, GC, MA, MO)**

– Body fusiform, distinct neck, tail distincly less broad than body, lizard-like appearance; ground coloration dark brown to black..........**16**

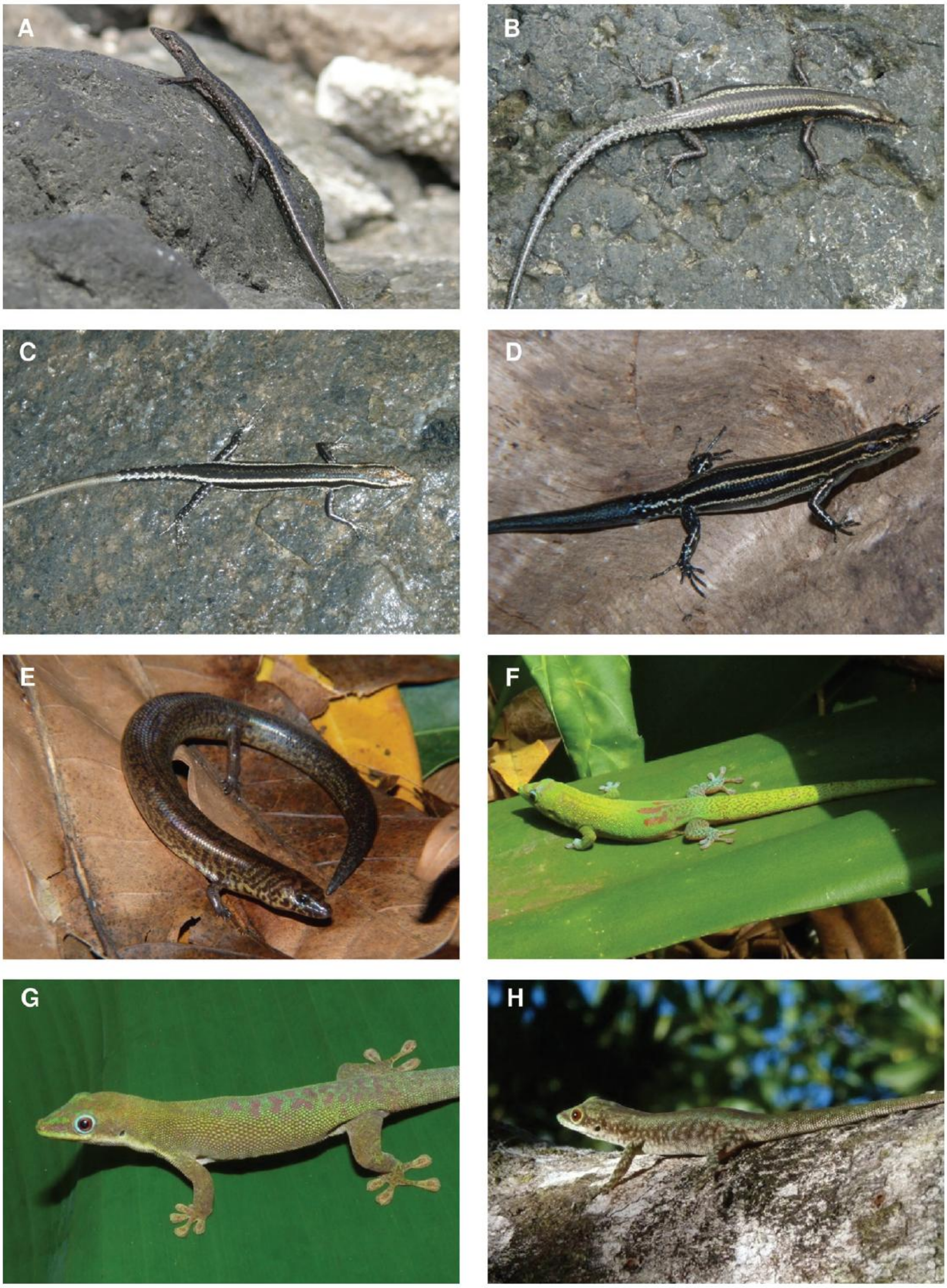


Figure 7. Species photographs. **A** *Cryptoblepharus boutonii ater*, Grand Comoro **B** *Cryptoblepharus boutonii mohelicus*, ZSM 1680/2008, Mohéli **C** *Cryptoblepharus boutonii mayottensis*, ZSM 1703/2008, Mayotte **D** *Cryptoblepharus boutonii degrijsii*, ZSM 63/2010, Anjouan **E** *Amphiglossus johannae*, ZSM 54/2010, Anjouan **F** *Phelsuma laticauda*, Anjouan **G** *Phelsuma dubia*, Mayotte **H** *Phelsuma robertmertensi*, Mayotte.

16 Medium-sized, often robust lizards, total length up to more than 200 mm; scale rows around midbody 28 to 39.................................***Trachylepis*, 17**
– Small lizards, less than 100 mm total length; dorsoventrally depressed; coloration blackish, often with white stripes; scale rows around midbody 21 to 29; only in rocky intertidal regions ....***Cryptoblepharus boutonii*, 18**
17 Common lizard; coloration dark with patterns of brighter spots; never with white stripes.......................... ***Trachylepis comorensis* (AN, GC, MA, MO)**
– Coloration blackish with two white lateral stripes .. ***Trachylepis striata* (AN)**
18 Coloration uniformly black .... ***Cryptoblepharus boutonii ater* (GC)**
– White stripes present **19**
19 Black with two white lines along the body ....***Cryptoblepharus boutonii mohelicus* (MO)**
– More than two white lines present **20**
20 Black with four white lines along the body, tail sometimes bluish .... ***Cryptoblepharus boutonii mayottensis* (MA)**
– Black with five white or yellowish lines along the body, tail often blue.......... .... ***Cryptoblepharus boutonii degrijsiii* (MO)**
21 Ventral surface of fingers and toes with adhesive lamellae, allowing movement on vertical surfaces; total length never exceeding 160 mm; usually not ground-dwelling ....**Gekkonidae, 22**
– Adhesive lamellae absent; total length up to 500 mm; ground-dwelling, climbing only on rough surfaces; robust lizards............... **other families, 38**
22 Dorsal ground color green, sometimes in shades of olive or brownish, often with pattern in red; pupil round; diurnal ....***Phelsuma*, 23**
– Dorsal ground color brown or grey, green and red colors absent; pupil vertical; nocturnal...**31**
23 Dark lateral stripe along body present..**24**
– No dark lateral stripe present **25**
24 Neck dorsally without dark stripes, limbs dorsally marbled with bright spots, tail dorsally with dark spots............................. ***Phelsuma comorensis* (GC)**
– Neck dorsally with three dark stripes, limbs dorsally not marbled, tail with narrow dark crossbands..................................***Phelsuma nigristriata* (MA)**
25 Dorsal ground color rather green with olive, brown or turquoise shades than deep green **26**
– Dorsal ground color deep green **27**
26 Flanks with brighter spots, dorsum dark green or brown, with orange median line (sometimes interrupted), sometimes with blue blotch on neck.............. .... ***Phelsuma robertmertensi* (MA)**
– No bright spots on flanks, often red dots on dorsum, no median line, tail often bluish.....................................***Phelsuma dubia* (AN, GC, MA, MO)**
27 Numerous small yellowish or golden spots dorsally in neck region and on tail; three large red blotches posteriorly on dorsum ....***Phelsuma laticauda laticauda* (AN, MA)**

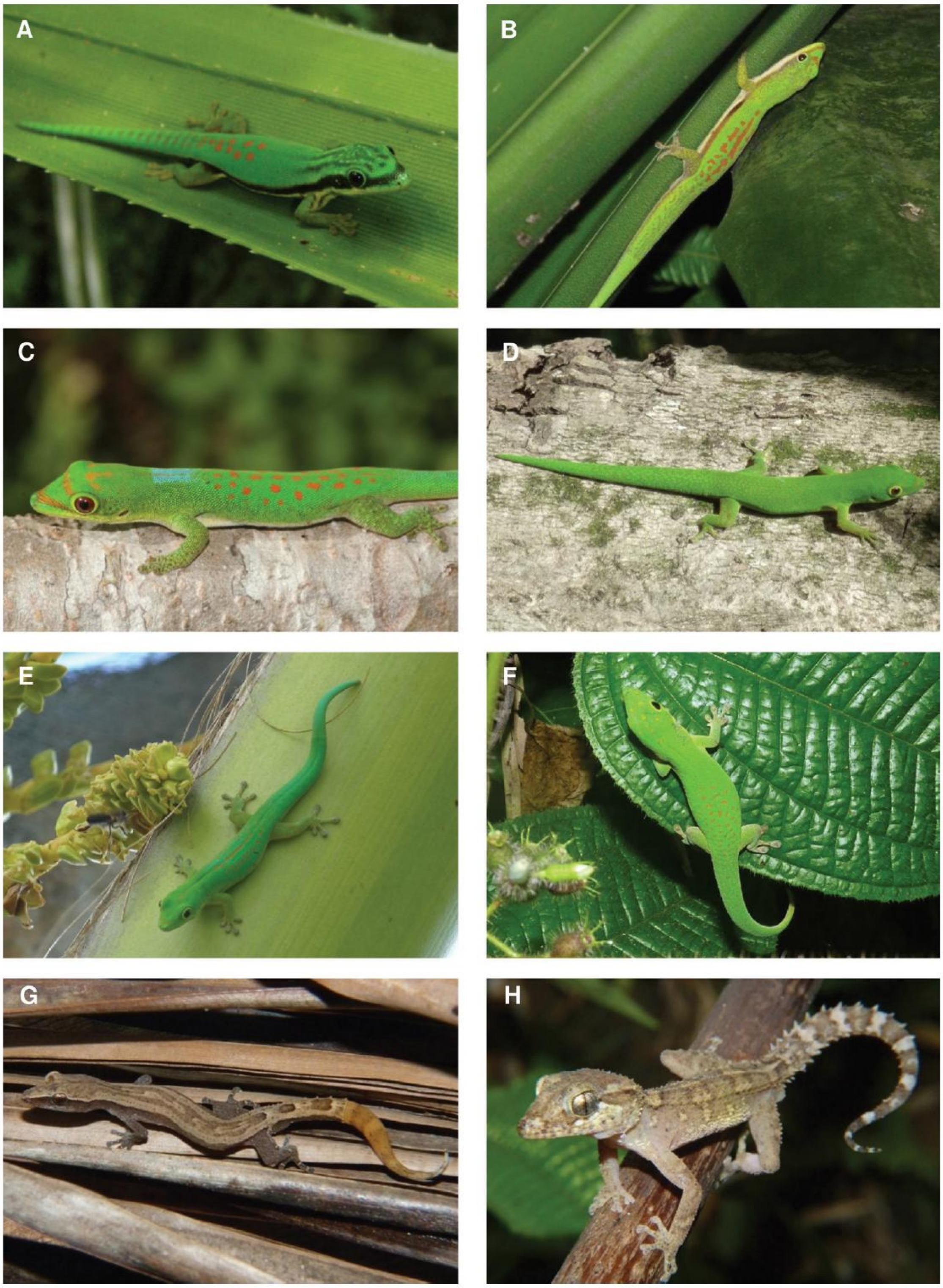


Figure 8. Species photographs. **A**. *Phelsuma nigristriata*, Mayotte **B** *Phelsuma comorensis*, Grand Comoro **C** *Phelsuma pasteuri*, Mayotte **D** *Phelsuma v-nigra anjouanensis*, Anjouan **E** *Phelsuma v-nigra comoraegrandensis*, Grand Comoro **F** *Phelsuma v-nigra v-nigra*, Mohéli **G** *Ebenavia inunguis*, ZSM 68/2010, Anjouan **H** *Paroedura sanctijohannis*, ZSM 98/2010, Mayotte.

– Neck and tail not yellowish or golden, dorsum with numerous smaller red dots and spots **28**

28 Blue spot on neck; gular region uniformly bright.. ***Phelsuma pasteuri*** **(MA)**

– No blue color elements dorsally; gular region with more or less visible pattern in the form of a "V" .... ***Phelsuma v-nigra*****, 29**

29 Ventral side yellow, dorsal side green with relatively large red dots .... ***Phelsuma v-nigra v-nigra*** **(MO)**

– Ventral side whitish, dorsal red dots smaller **30**

30 Dorsal side green to bluish, small red dots, sometimes with red median line. .... ***Phelsuma v-nigra comoraegrandensis*** **(GC)**

– Dorsal side green, red dots very small, sometimes forming a reticulated pattern or absent .... ***Phelsuma v-nigra anjouanensis*** **(AN)**

31 Scales rather large, cycloid, overlapping, can be shed when touched; tubercles and striped color patterns absent... ***Geckolepis maculata*** **(AN, GC, MA, MO)**

– Scales small, granular, in part tubercular; sometimes with striped color pattern **32**

32 Adhesive lamellae covering entire ventral side of digits, in double rows of several pads each; when resting, ventral side of body usually in contact with substrate; colors brown during day, grayish, whitish or pinkish at night .... ***Hemidactylus / Gehyra*****, 33**

– Adhesive lamellae only in two pads near tips of the toes; when resting, ventral side of body usually lifted from substrate; colors always brownish other genera **37**

33 Body and tail smooth, no tubercles present; coloration grey, sometimes pinkish .... ***Gehyra mutilata*** **(UNCONFIRMED)**

– Body and tail covered with tubercles; coloration grey to brownish; very common in urban areas and plantations, rare in natural habitats .... ***Hemidactylus*****, 34**

34 Maximum snout-vent length of adults 55 mm; 2 to 8 rows of tubercles along the back, with 7 to 24 tubercles per row; dorsal side of body mostly uniformly white, beige or yellowish at night, pattern of dark stripes and spots visible during the day .... ***Hemidactylus frenatus*** **(AN, GC, MA, MO)**

– Pattern of dark stripes and spots mostly also visible at night; 8 or more rows of tubercles along the back, with 21 or more tubercles per row **35**

35 Maximum snout-vent length of adults 51 mm; dorsal side covered with relatively prominent brown and whitish tubercles; 3 to 5 dorsal rows of brown spots often visible; 14 to 17 rows of tubercles along the back, with 23 to 30 tubercles per row .... ***Hemidactylus parvimaculatus*** **(AN, MO)**

– Snout-vent length of adults up to more than 51 mm; dorsal side usually with pattern of brighter and darker colors, but no whitish tubercles present **36**

36 Maximum snout-vent length of adults 59 mm; original tail with whorls of spiny, enlarged scales extending far into the distal half of the tail; males with 25 to 32 femoral pores; 8 to 16 rows of tubercles along the back, with 25 to 37 tubercles per row; cranial depression extending anteriorly from between

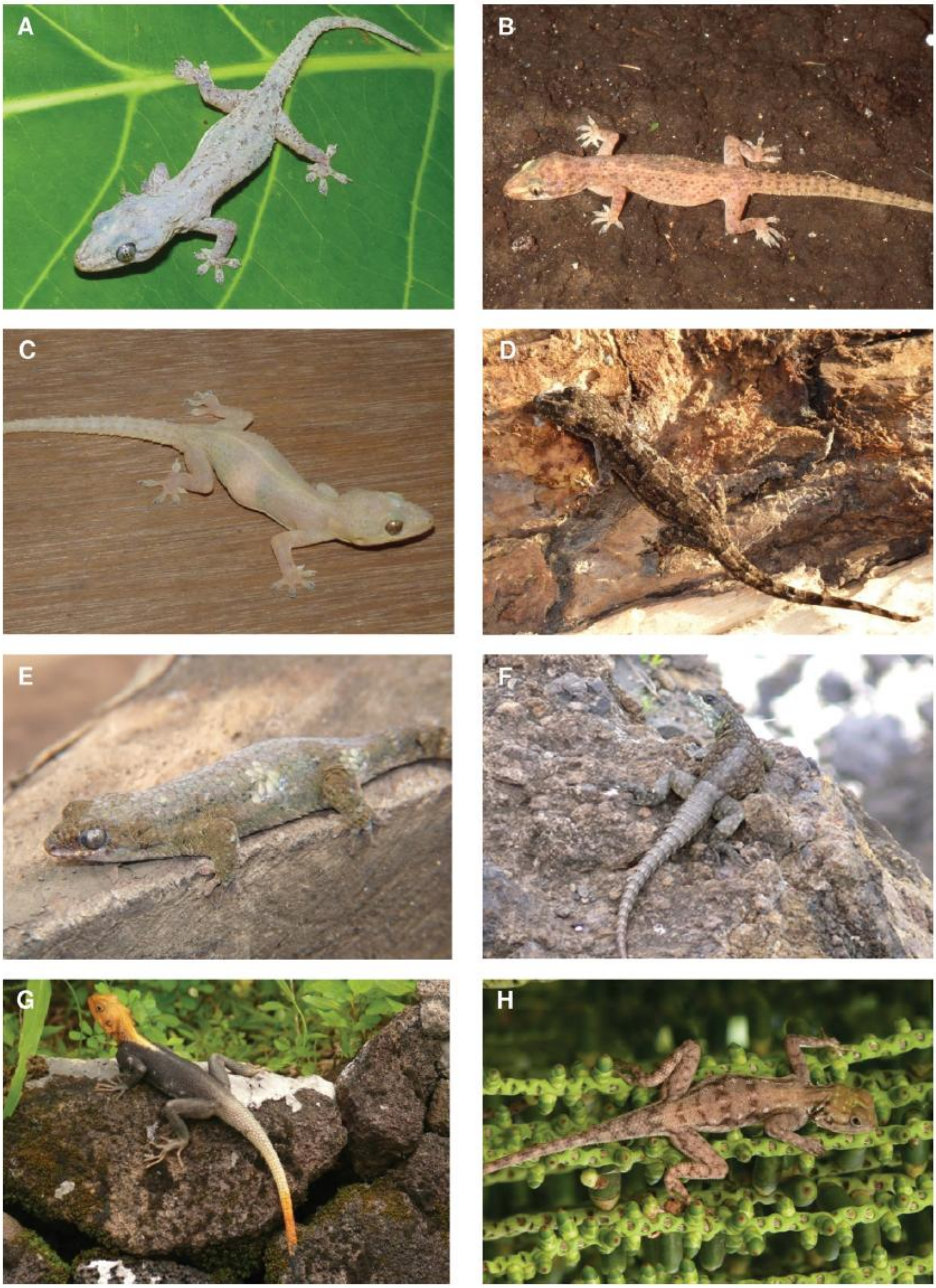


Figure 9. Species photographs. **A** *Hemidactylus frenatus*, ZSM 116/2010, Mohéli **B** *Hemidactylus parvimaculatus*, ZSM 370/2002, Mohéli **C** *Hemidactylus mercatorius*, ZSM 121/2010, Anjouan **D** *Hemidactylus platycephalus*, Anjouan **E** *Geckolepis maculata*, ZSM 83/2010, Anjouan **F** *Oplurus cuvieri comorensis*, Grand Comoro **G** *Agama agama*, male, Grand Comoro **H** *Agama agama*, juvenile, Grand Comoro.

the eyes less distinct than in 36 B) .... ***Hemidactylus mercatorius*** **(AN, GC, MA, MO)**

– Maximum snout-vent length 85 mm; original tail with whorls of spiny, enlarged scales largely restricted to the proximal part of the tail; males with 41 to 59 femoral pores; 8 to 14 rows of tubercles along the back, with 21 to 31 tubercles per row; cranial depression extending anteriorly from between the eyes more distinct than in 36 .... ***Hemidactylus platycephalus*** **(AN, GC, MA, MO)**

37 Small, slender geckos, maximum snout-vent length 40.8 mm; flanks darker than dorsum, with sharpest contrast in head region; never with crossbands on body .... ***Ebenavia inunguis*** **(AN, GC, MA, MO)**

– Medium-sized geckos with robust heads, maximum snout-vent length 66.9 mm; four bright crossbands on dorsum, best visible at night; coloration of flanks not clearly distinguished from that of dorsum.................................... .... ***Paroedura sanctijohannis*** **(AN, GC, MA, MO)**

38 Neck with crest of spiny scales; scales on tail similar to those on body; males with contrasting black and orange color pattern............ ***Agama agama*** **(GC)**

– Crest of spiny scales on neck absent; tail with enlarged scales, spiny............. .... ***Oplurus cuvieri comorensis*** **(GC)**

Discussion

Unconfirmed records and taxonomic comments

For all the species records given in Table 1 at least one voucher specimen for each island was either collected during our surveys or traced in natural history collections. The only exception is *Hemidactylus mercatorius* on Mohéli, of which we do not know of any voucher specimen. The following species of terrestrial reptiles were previously recorded in the Comoros, but were not confirmed during our study.

Lycodon aulicus

Specimens of the Wolf snake *Lycodon aulicus* were neither available for examination nor observed. The locality of one specimen, CAS 135119, collected by E.J. Morris and traced by a search in the HerpNET database, is given as “Moroni, hospital ground North of Moroni”. During our field surveys, we searched at the hospital ground and interviewed employees, but could not find any evidence for an extant population of this species. Another specimen, also traced by HerpNET (FMNH 205907), was collected by J. Visser in 1973 on Grand Comoro, but no further information about this specimen is available. The presence of this species possibly resulted from a single introduction event and no evidence of an extant population

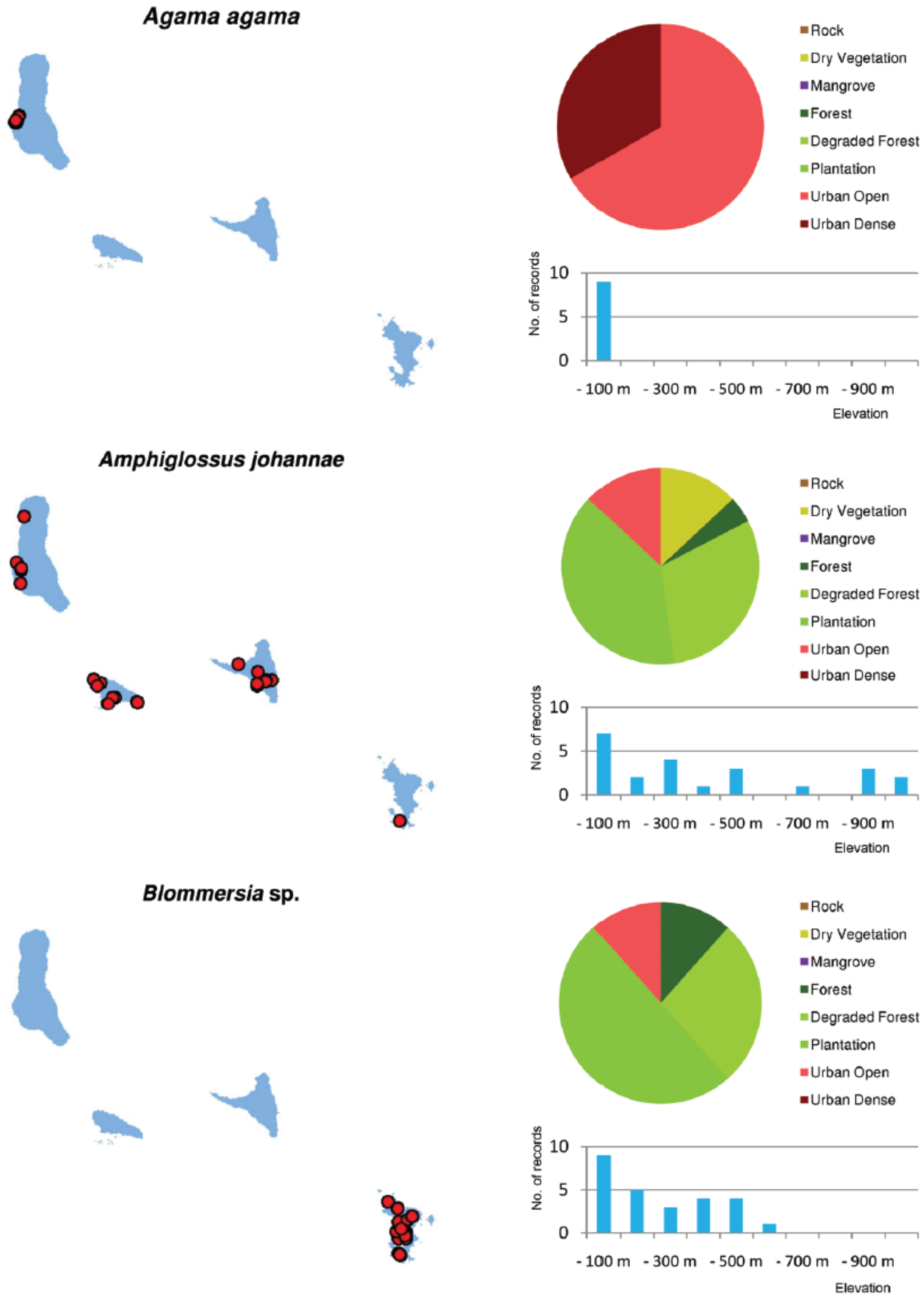


Figure 10. Distribution maps, and distribution over habitat and altitude classes, for *Agama agama*, *Amphiglossus johannae* and *Blommersia* sp.

of *L. aulicus* is known to exist. *Lycodon aulicus capucinus*, now often considered as distinct species *L. capucinus*, is an invasive species on the Mascarene islands (Cheke and Hume 2008).

Lycodryas gaimardii and *Lycodryas maculatus*

No unambiguous records of these species on the Comoros exist. Historical records and their possible taxonomic implications will be discussed in an upcoming paper on *Lycodryas sanctijohannis* (Hawlitschek et al., unpublished data, see also Hawlitschek 2008).

Gehyra mutilata

This nocturnal gecko, similar in appearance to the genus *Hemidactylus*, is widespread in the Indian Ocean area and considered an invasive species in many regions (Denny et al. 2005, Witmer et al. 2007, Rocha et al. 2009b). Its existence on the Comoros was apparently first suspected by Blanc (1971), without indicating any specific island, and subsequently by Cole (1992) and Paris (1999), but was never confirmed. During our field surveys in the Comoros, we did not detect any signs of the presence of this species, and did not find any voucher specimens in the studied collections.

Meroles knoxii

As described in Günther (1879), the single Anjouan specimen of the lacertid lizard *Meroles knoxii* was collected by C.E. Bewsher together with other material from Anjouan, including the type specimens of *Paroedura sanctijohannis* and *Lycodryas sanctijohannis*. While many localities from this period are doubtful, this latter fact supports the assumption that this specimen was indeed collected on Anjouan. However, as no other report of *M. knoxii* from the Comoros has come to be known since, the status of this species on Anjouan remains uncertain and its possible presence on the Comoros is in need of confirmation. For biogeographical reasons it is very unlikely that this species colonized the Comoros by natural dispersal but it may be that at some point, *M. knoxii* was introduced to this island but later became extinct. In another case, the scincid lizard *Trachylepis striata* remained hidden for several decades (e.g. Brygoo 1981, Meirte 2004), and it was rediscovered only in 2003 at Anjouan (Carretero et al. 2005). This demonstrates that the future rediscovery of species, whose occurrence on the Comoros is considered dubious is possible, and even the presence of yet unknown reptile species cannot be excluded.

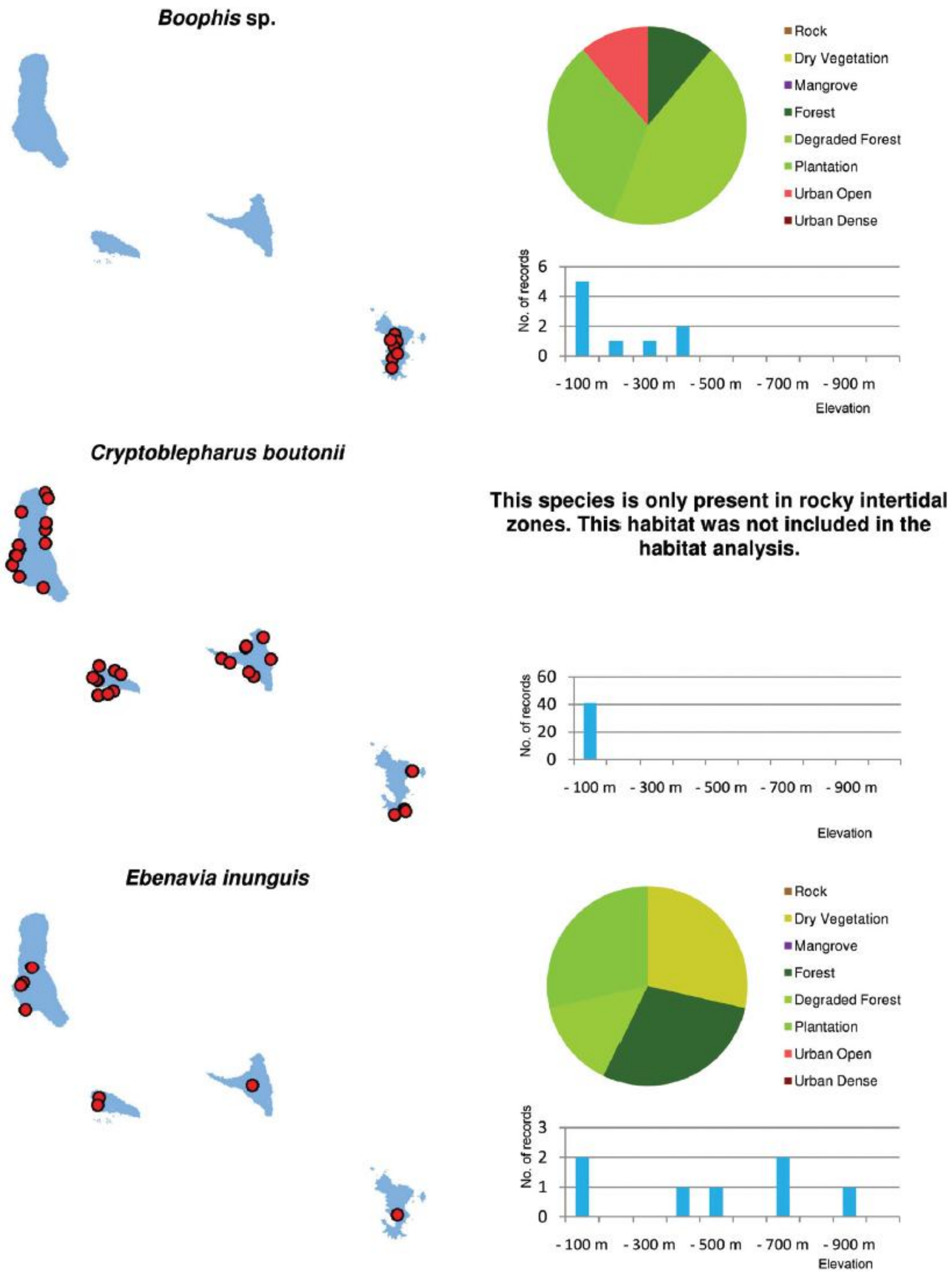


Figure 11. Distribution maps, and distribution over habitat and altitude classes, for *Boophis* sp., *Cryptoblepharus boutonii* and *Ebenavia inunguis*.

Hemidactylus mabouia and *H. brooki*

The Comorian populations of *Hemidactylus* which were formerly assigned to *H. mabouia* (Blanc 1971, 1972, Meirte 2004) are now assigned to *H. mercatorius* and *H. platycephalus* (Vences et al. 2004, Carretero et al. 2005, Rocha et al. 2010b). The Comorian populations formerly assigned to *H. brooki* (Carretero et al. 2005) are now assigned to *H. parvimaculatus* (Bauer et al. 2010, Rösler and Glaw 2010).

Land cover changes and state of the natural vegetation

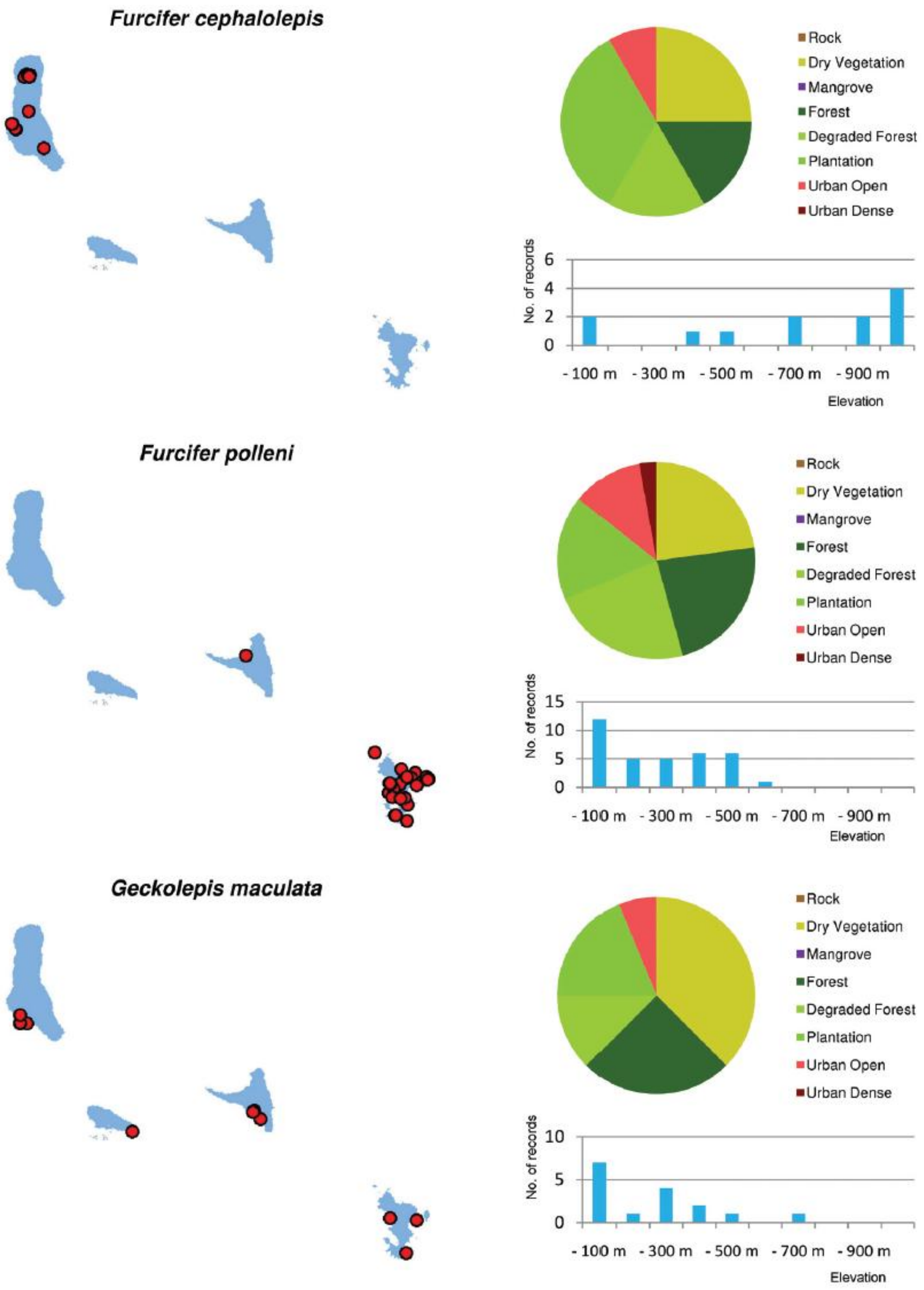
Similar to other tropical oceanic islands, the Comoros were likely covered by large areas of forest before the arrival of man (Pascal 2002, Louette et al. 2004). Today, as shown by the present results, land cover of natural or near natural forest on the Comoros is classified at less than 7%. As the remote sensing classification relies mainly on photosynthetic activity and canopy closure, minor modifications from selective logging or agro-forestry may be undetected. This means that the less than 7% coverage of this class of forest contains pristine undisturbed forest but may also comprise areas where significant human impact is present.

Opening of the forest canopy is one of the prime factors inducing changes in forest microclimate and understory vegetation and thus affecting the species composition of the fauna (Chen et al. 1999, Jennings et al. 1999). Large areas still covered with forest vegetation are heavily degraded and their canopy has been opened. Areas where intensive logging has taken place are often cultivated with arborescent crops, small-scale plantations of other crops and neophyte stands. In lowland areas, forests have been replaced by extensive crop plantations. On Anjouan, Mayotte and Mohéli, the largest forest areas remain in the highest altitude ranges. Only on Grand Comoro, they are replaced by montane dry vegetation in altitudes too high for tropical forest (Figure 3).

Nearly a third of the area of the Comoros is covered by dry and low vegetation. This definition includes natural types of non-forest vegetation as well as crop stands and areas in which erosion and devegetation has led to soil fatigue to such an extent that crops cannot be grown (called “Padzas”). It can be assumed that even before the arrival of man, dry and low vegetation already made up a certain proportion of the Comorian vegetation. However, large areas which are now covered by dry / low vegetation were originally covered by dry forests, of which today only fragments are left (Pascal 2002, Louette et al. 2004).

Habitat use and the impact of altitude

As mentioned above, it is assumed that the Comoros were originally covered largely by rainforest. This might lead to the assumption that forest and other original vegetation classes host a larger diversity than secondary vegetation, since plantation and degraded


Furcifer cephalolepis

Furcifer polleni

Geckolepis maculata

Figure 12. Distribution maps, and distribution over habitat and altitude classes, for *Furcifer cephalolepis*, *Furcifer polleni* and *Geckolepis maculata*.

forest are the results of human impact of the last millennium. However, relatively low numbers of species were recorded in closed forest. This may be attributed to the relatively small area occupied by this habitat, but this alone cannot easily explain why fewer species were recorded in natural forests than in plantations, degraded forests, dry and low vegetation and even in urban areas.

Several reasons for this pattern can be discussed. (1) Introduced species, mainly among the genera *Hemidactylus* and *Phelsuma*, are adapted to anthropogenic habitats, abundant, and easy to observe, and therefore make up a large part of the total records. (2) As several studies have shown (Fredericksen and Fredericksen 2002, Bell and Donnelly 2006), degraded habitats generally seem to show a higher diversity especially of reptiles than undisturbed forest in similar areas. This is explained with the higher diversity in microhabitats, as patches of original forest remain very close to a system of clearings and secondary vegetation, and the higher proportion of insolated sites, which is important for ectothermic animals. (3) For similar reasons, many reptile species are less abundant in closed forest areas and prefer perch sites higher above the ground, where insolation is higher. This makes observation more difficult. (4) The possibility of extinction should be considered. Lowland forest areas have probably been deforested over a long period of time, and species restricted to these habitats may have already gone extinct. (5) At least on Grand Comoro, regular volcanic eruptions of the Mt. Karthala might have disturbed the evolution of reptile communities specialized on undisturbed forest.

Of the endemic species recorded, most are more abundant in secondary habitats than in pristine forest (Figure 5). The endemic reptiles *Amphiglossus johannae*, *Furcifer cephalolepis*, *Lycodryas sanctijohannis*, and *Phelsuma v-nigra* were all most commonly recorded in plantations or degraded forests. This is also true for both endemic amphibian species in Mayotte. There are only two endemic reptile species whose preference for pristine forest habitats can be supported by a significantly large number of records. These are the geckos *Paroedura sanctijohannis* and *Phelsuma nigristriata*. Nearly all *P. sanctijohannis* on Anjouan, Grand Comoro and Mohéli were observed in undisturbed forests, while on Mayotte this species was also commonly found in degraded habitats. *P. nigristriata* is known almost exclusively from pristine forests on Mayotte and largely depends on *Pandanus* plants as microhabitat which are restricted to relatively undisturbed forest. Only few, mainly juvenile, specimens were observed in neighbouring degraded habitats.

As shown in Figure 3, the majority of all species are most abundant in the low elevation ranges. However, a few endemic species are more common in or even confined to higher altitudes. Namely, these are *Phelsuma comorensis* and *Furcifer cephalolepis* on Grand Comoro and *Phelsuma nigristriata* and *P. pasteuri* on Mayotte. *Paroedura sanctijohannis* is almost exclusively found at high altitudes on all islands but Mayotte, where it covers the entire altitudinal range. In *Paroedura sanctijohannis* and *Phelsuma nigristriata*, this preference for higher altitudes can probably be explained by the availability of suitable habitat, since as stated above these are species more or less dependent on forest. Climate may also play a role especially for *P. nigristriata*, *P. comorensis* and *P. pasteuri* (B. Brückmann et al., unpublished data).

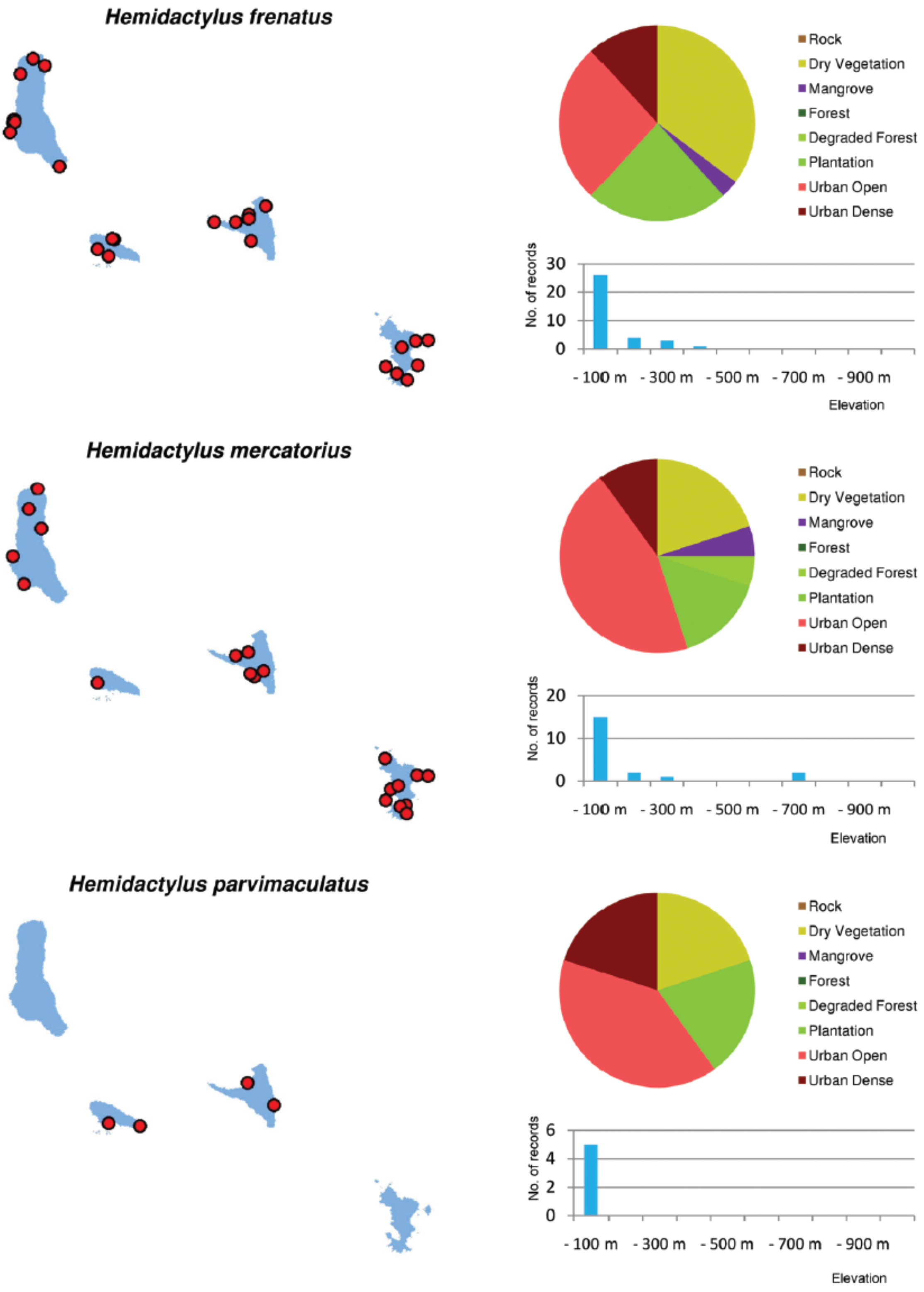


Figure 13. Distribution maps, and distribution over habitat and altitude classes, for *Hemidactylus frenatus*, *Hemidactylus mercatorius* and *Hemidactylus parvimaculatus*.

Potential factors of threat for endemic Comorian reptiles and amphibians

The majority of endemics among the Comorian herpetofauna appear to have adapted to the habitat changes imposed by man. *Paroedura sanctijohannis* and *Phelsuma nigristriata* are the only species for which a true dependency on pristine forests can be postulated. Even these two species can also be found in degraded habitats, albeit in lower densities.

A much more potent threat for Comorian endemics may be competition by invasive species. In 1998, according to Meirte (2004), the agamid lizard *Agama agama* was introduced to Grand Comoro. It has since established a dense population in the capital Moroni, but has not yet been observed outside urban areas. While it may be a competitor to or even predator on other local species, the species most affected may be the Comoro iguana *Oplurus cuvieri comorensis*, which resembles the agama in habitus and ecology. The Comoro iguana inhabits only a narrow strip of rocky cliffs along the northeast coast of Grand Comoro. If the agama expands its range and reaches that of the Comoro iguana, there would be no areas of refuge for the latter species. If both species prove to be direct competitors, this might lead to the extinction of *O. cuvieri comorensis*.

An even more evident situation of direct competition is that of the diurnal *Phelsuma* geckos, which are present on the Comoros with five endemic and two introduced species. While *P. comorensis* on Grand Comoro, as well as the Mayottean species, seem to prefer habitats where invasive *Phelsuma* species are rare or absent, *P. v-nigra* resembles the invasive species in terms of distribution and preferences. Focused studies are necessary to address the situation of competition between the various Comorian *Phelsuma* species.

The overwhelming majority of nocturnal geckos that can be observed on the Comoros belong to introduced species. These are the four species of *Hemidactylus*, all of which cover more or less extensive distribution ranges in the tropics. Three of these species are rarely seen outside urban and plantation areas of low altitudes, but *H. platycephalus* has colonized all available types of habitats including undisturbed forests and has been observed in altitudes of over 600 m. On Anjouan, Grand Comoro and Mohéli, the endemic and nocturnal *Paroedura sanctijohannis* inhabits almost exclusively areas above this altitude. This could be explained by possible exclusion from lower altitudes through competition from *H. platycephalus*. Only on Mayotte, *P. sanctijohannis* regularly occurs in syntopy with *H. platycephalus*.

While *P. sanctijohannis* currently inhabits areas in elevations too high for competing invasive species, this may change if global warming would allow these competitors to colonize areas at higher altitudes. Raxworthy et al. (2008) describe the phenomenon of "upslope displacement" of habitat areas, which particularly can affect endemic highland species often restricted to mountaintops. With an increase in temperature, the lower distribution limit of these species rises; if it rises up to a level higher than the point of highest elevation in the species' habitat area, the entire habitat of the concerned species is lost, leading to extinction.

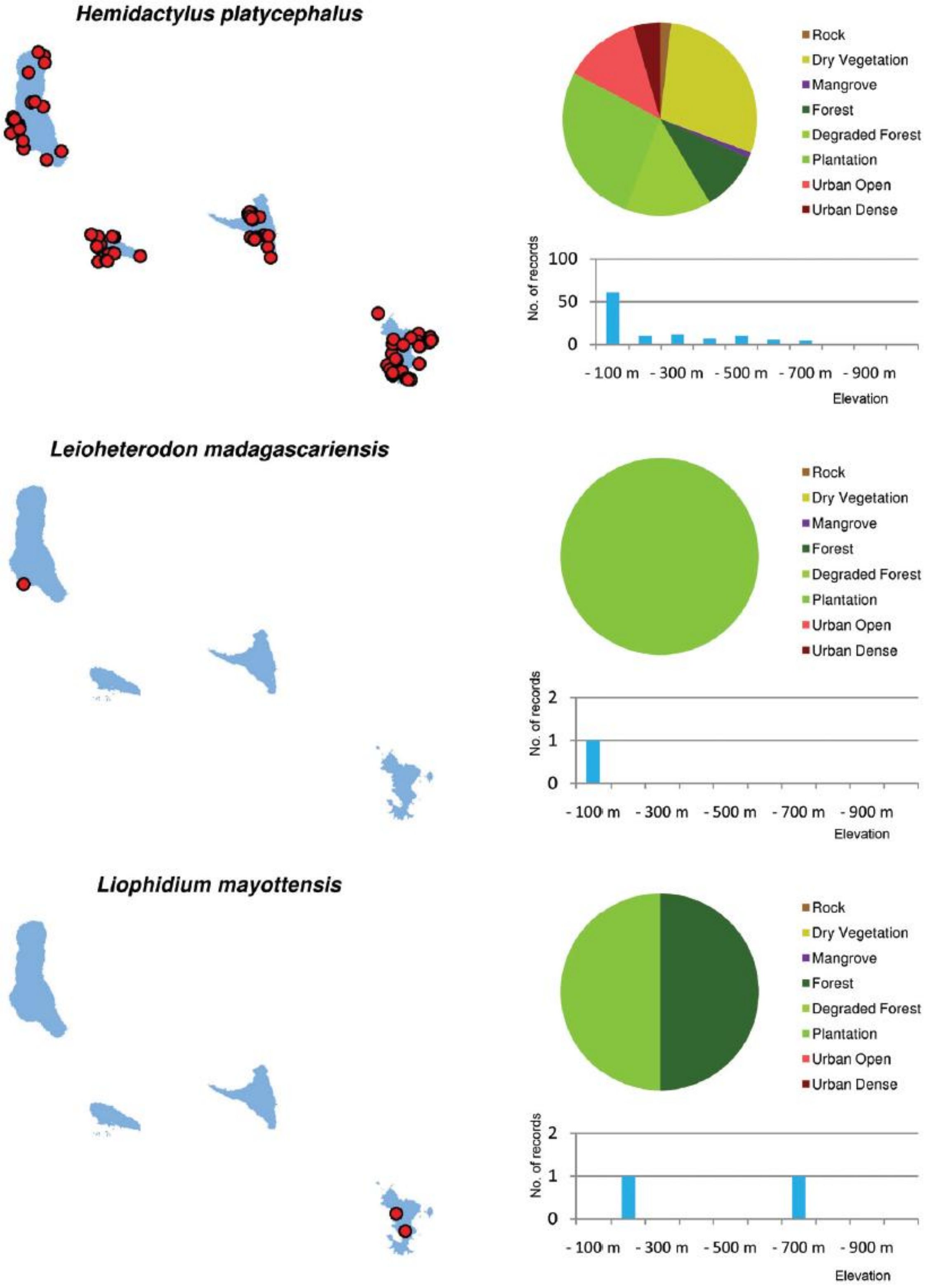


Figure 14. Distribution maps, and distribution over habitat and altitude classes, for *Hemidactylus platycephalus*, *Leioheterodon madagascariensis* and *Liophidium mayottensis*.

No studies have yet been undertaken concerning the impact of predatory mammals on the Comorian herpetofauna. Tenrecs (*Tenrec ecaudatus*) inhabit all islands of the Comoro archipelago and were probably introduced in historical times (Louette et al. 2004), although this is unclear. This species mainly feeds on invertebrates but to some extent also on small, ground-dwelling vertebrates. On the Comoros, typhlopid snakes, skinks and frogs might form part of its nurriture. Two introduced species of viverroid carnivores may be stronger predators on reptiles: the Small Indian Civet (*Viverricula indica*) on Mayotte and the abundant Small Asian Mongoose (*Herpestes javanicus auropunctatus*) on Grand Comoro. They prey on a variety of small animals; in both species, insects and other invertebrates make up the largest part of the diet, but they also feed on amphibians and reptiles, including snakes (see Chuang and Lee 1997 for *Viverricula* and summary in Hays and Conant 2007 for *Herpestes*). Thus, both species, although this has not yet been proven, may also prey on reptiles, especially snakes, on the Comoros and therefore reduce the populations of these reptile species. The species most strongly affected might be the terrestrial Mayotte endemic snake *Liophidium mayottensis*.

These observations suggest that competition and predation by invasive species pose a more serious threat to Comorian endemic reptiles than destruction of the remaining natural habitats. However, habitat degradation and destruction, extension of settlements and intensification of land use also create more suitable conditions for invasive species and should therefore also be considered an important threat factor.

Several endemic Comorian reptile species have been subject to international trade as pets. Data are available only for species listed on the CITES appendices (UNEP-WCMC 2011). According to this database, a considerable number of animals was exported from the three islands of the Union of the Comoros. 22004 specimens of the day gecko *Phelsuma comorensis* and 23850 specimens of *P. v-nigra* are listed for the period of 2000 to 2010. The numbers for Mayotte endemics are much lower at 2000 specimens for *P. nigristriata*, 100 for *P. pasteuri* and 280 for *P. robertmertensi*. The situation of the chameleons of the genus *Furcifer* is similar: 14420 specimens of *F. cephalolepis* from Grand Comoro were listed as exported since 2000, but only 1560 specimens of *F. polleni* from Mayotte.

The relatively low export numbers of species from Mayotte are unlikely to have a significant impact on populations. For species endemic to the islands of the Union of the Comoros, these numbers are much higher. In the Union of the Comoros, little effort is required to obtain export permits for CITES-listed species in relation to Madagascar or Mayotte, where recent quotas for commercial export numbers are relatively low or even zero for most species. This condition may have directed pet traders to exploit populations in the Union of the Comoros more intensively. For comparison, 16252 *Phelsuma madagascariensis* and 14963 *P. laticauda*, among the most popular vivarium species of the genus *Phelsuma*, are listed in the same period (2000 to 2010). These numbers are lower than those from the Comorian species. Notably, in the period of 1990 to 1999, export numbers of the Malagasy species were five-fold higher than in the following decade (91083 and 95391, respectively). The decrease of export in Malagasy species may have been a further incentive to exploit the populations of other countries.

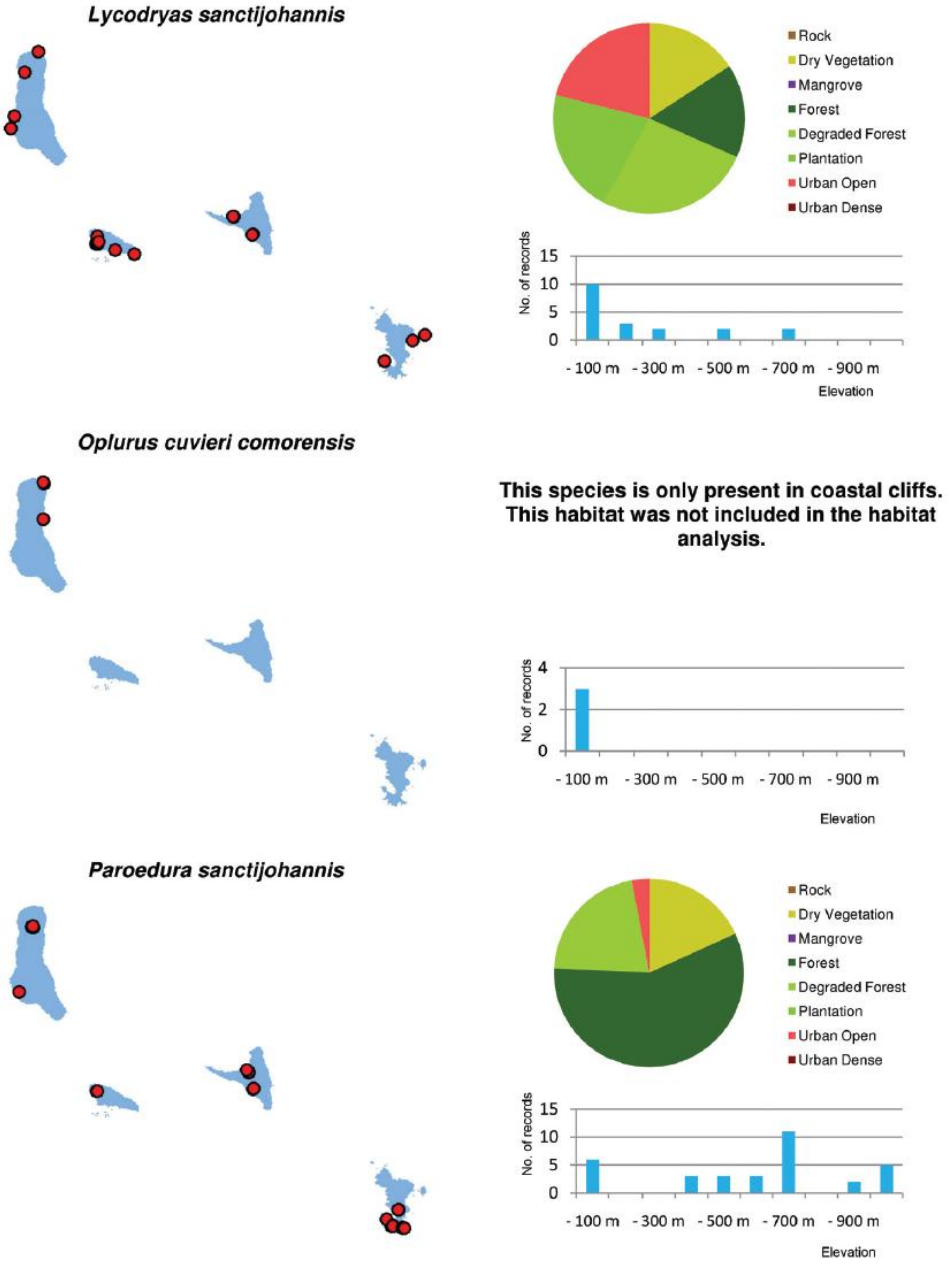


Figure 15. Distribution maps, and distribution over habitat and altitude classes, for *Lycodryas sanctijohannis*, *Oplurus cuvieri comorensis* and *Paroedura sanctijohannis*.

It is difficult to assess the impact of the export of specimens for pet trade on the wild populations of the species concerned. Our results show that the relatively heavily exploited species *Phelsuma comorensis* and *P. v-nigra* are more abundant in their natural habitats than the less heavily exploited species from Mayotte. This suggests that the collection of specimens so far did not have a significantly negative effect on their populations. The chameleon *Furcifer cephalolepis* from Grand Comoro, which has been much more intensely exploited than its relative from Mayotte, *F. polleni*, is also less often observed in the wild than its congener. This could indicate that collection of specimens for pet trade in high numbers may negatively influence the populations of *F. cephalolepis*. Since no reliable data on the abundance of this species before its exploitation is available, we have no means of investigating this speculation.

Amphibians are globally threatened by the chytrid fungus *Batrachochytrium dendrobatidis* (Daszak et al. 2003, Weldon et al. 2004, Mendelson et al. 2006, Skerratt et al. 2007, Wake and Vredenburg 2008, Rödder et al. 2009), which has led to massive reductions and even extinctions of amphibian populations. So far, no amphibian population declines have been noticed on Mayotte and surveys for this fungus have not been conducted. It is unknown how strongly it might affect the endemic anuran species, but reviewing its impact on other amphibian species worldwide, it must be assumed to cause severe losses among Mayotte's amphibians if introduced.

Conservation assessments

We evaluate all species of reptiles and amphibians endemic to the Comoros against the criteria for Red List categories in IUCN (2001a). These assessments are proposals for national conservation status and are also currently under review at the CI-IUCN Biodiversity Assessment Unit for inclusion in the IUCN Red List (P. Bowles, pers. comm. 2011-05-17 to 2011-09-06). Some endemic reptile species from the Comoros were previously evaluated by other authors and were all given the status "Least Concern". Our results do not always confirm these assessments, and we propose changing the Red List entries to better reflect the threat situations of these species.

We also present an assessment of *Trachylepis comorensis* although a population of this species is known on the islet of Nosy Tanikely, Madagascar (Köhler et al 1997). Additionally, genetic studies by Mausfeld-Lafdhiya et al. (2004) and Rocha et al. (2010a) show that the subspecies *T. maculilabris casuarinae* from Casuarina Island off the coast of North Mozambique is nested within *T. comorensis*. According to these studies, however, the two non-Comorian areas were probably colonized with human aid, and the Comoros are the centre as well as the original distribution range of *T. comorensis*.

Taxonomic units below species level currently do not receive any status in the IUCN Red List. Nevertheless, we present assessments for the endemic subspecies of *Oplurus cuvieri* and *Cryptoblepharus boutonii*. The Comoro Iguana *Oplurus cuvieri comorensis* was already considered a separate species by Meirte (1992, 2004). Münchenberg et al. (2008) later found no genetic divergence between the Comorian and the

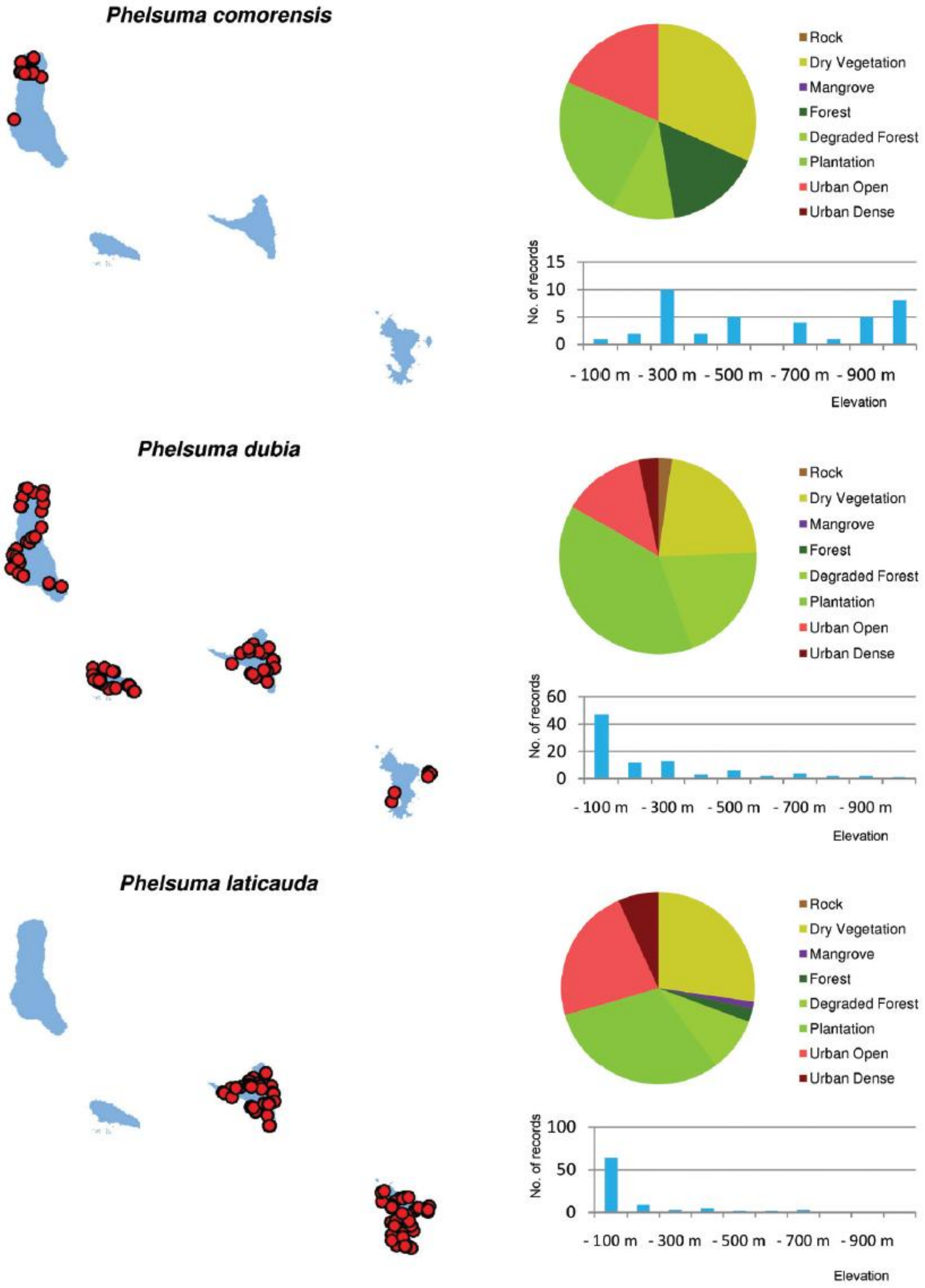


Figure 16. Distribution maps, and distribution over habitat and altitude classes, for *Phelsuma comorensis*, *Phelsuma dubia* and *Phelsuma laticauda*.

Malagasy *O. cuvieri* and it is now considered a subspecies. We agree with this view but still evaluate the Red List status of *O. cuvieri comorensis* because we recognize the differences in morphology and behaviour to the nominate form (Meirte 1992) and because, according to our evaluation, it is the most threatened reptile taxon on the Comoros. Several subspecies of *Cryptoblepharus boutonii*, including those from the Comoros, have been treated as species (Horner 2007). We follow Rocha et al. (2006) in considering all WIO forms as subspecies.

Although IUCN does not support Red List assessments for undescribed species, we here provide an evaluation of their national conservation status applying the Red List Criteria, in order to help inform assessment of these species once they are formally described.

A summary of the assessments is given in Table 3. Future taxonomic studies may reveal further endemic species on the Comoros, possibly in the genera *Ebenavia*, *Geckolepis*, *Lycodryas* and *Paroedura*.

Oplurus cuvieri comorensis

This iguana inhabits only rocky shores in a very small area on Grand Comoro. We measured the length of coastline potentially inhabitable for *O. cuvieri comorensis* as 38 km. Assuming that the iguana inhabits areas as far away as 200 m from the coast, which is probably not true for most of its range, this would mean an extent of occurrence of roughly 7.6 sqkm. The actually inhabited area (area of occupancy) is even smaller, as not all coastal areas are colonized. The two foremost threatening factors are competition with invasive species and collection of individuals by locals for food and entertainment (Meirte 2004 and pers. obs.), which cause a continuing decline in number of mature individuals (CR B1bv + B2bv). If the invasive *Agama agama* extends its range to the North of Grand Comoro, the entire range of *O. cuvieri comorensis* will be affected.

According to IUCN (2001a), “locations” are areas where “a single threatening event can rapidly affect all individuals of the taxon present”. Thus, all known localities of *O. cuvieri comorensis* can be considered a single location (CR B1a+B2a) because of the uniformity of the habitat and the short distances in between. According to the facts presented, the status of Critically Endangered (CR B1abv + B2abv) is proposed for *O. cuvieri comorensis*.

Typhlops comorensis and *Typhlops* sp.

Due to their cryptic and fossorial habits virtually nothing is known on the biology, distribution and ecology of both putatively endemic *Typhlops* species. *Typhlops comorensis* is known from only a few specimens, and only two were recorded in our surveys. *Typhlops* sp. is a very tiny species known from only very few individuals (including ZSM 361/2002 and 362/2002) from the area around Moroni, the capital of Grand

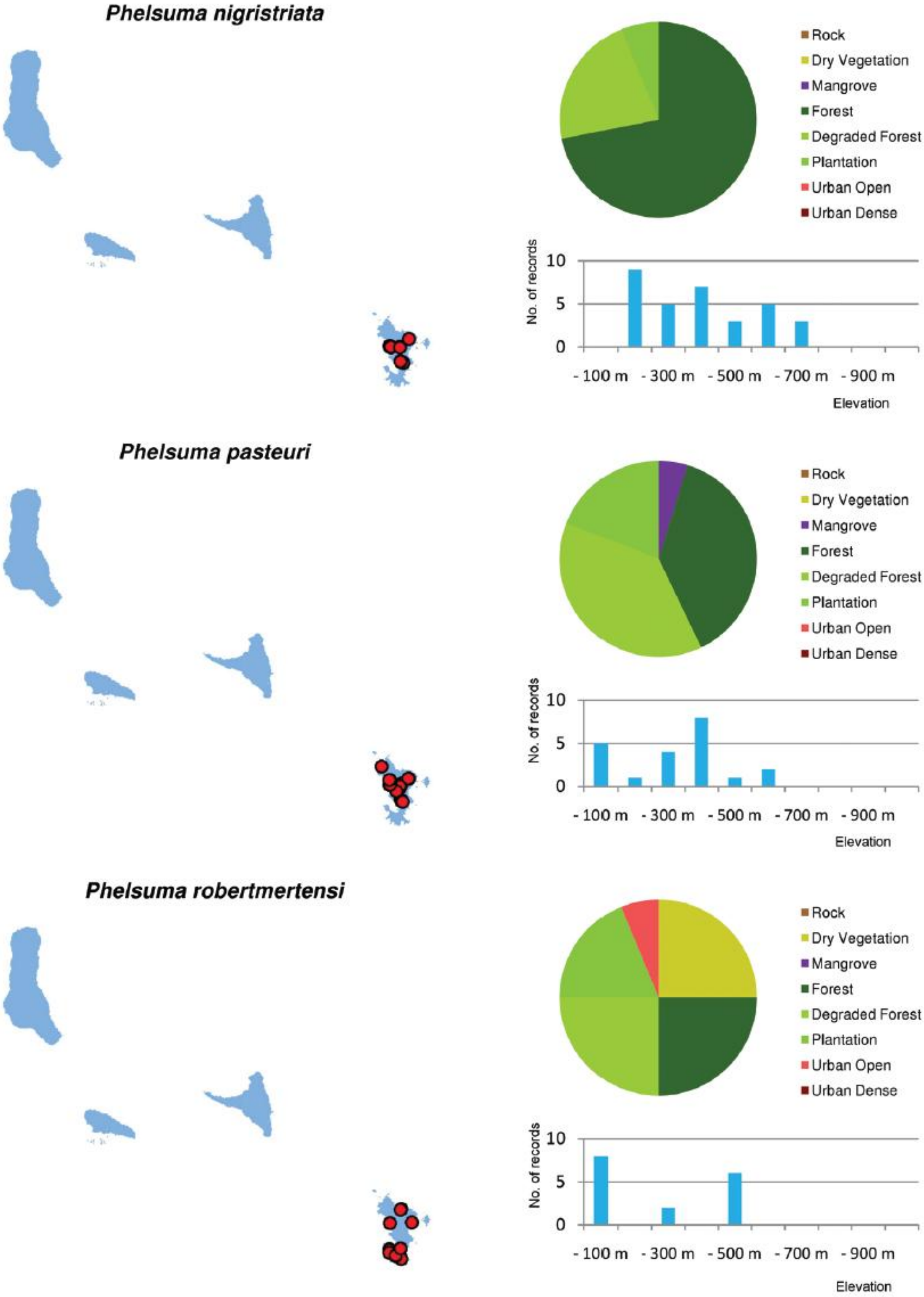


Figure 17. Distribution maps, and distribution over habitat and altitude classes, for *Phelsuma nigristriata*, *Phelsuma pasteuri* and *Phelsuma robertmertensi*.

Comoro. It is likely to represent a new, undescribed species (Wallach pers. comm.) but current knowledge does not exclude the possibility that this species has been accidentally introduced from somewhere else. There are no indications that these two species are restricted to the remaining undisturbed forest areas or that *R. braminus* or any other possibly introduced species impose high pressure on them. With regards to the poor knowledge available the status Data Deficient appears appropriate for both species.

Liophidium mayottensis

Only two localities of this terrestrial and diurnal snake endemic to Mayotte were available for this analysis and therefore no specific statements about its biology, distribution and population size can be made. As no specific data on the distribution of this snake is available and one of the two specimens was caught in forest, it should be assumed that *L. mayottensis* will be affected by a further degradation and fragmentation of its habitat and probably also by the introduced carnivorous Small Indian Civet (*Viverricula indica*). While the total area of Mayotte (376.0 sqkm) is recognized as extent of occurrence, we conservatively assume that *L. mayottensis* is restricted to an area in central Mayotte forming a single location, where both observations were made. Assuming that plantation and closed forest, where specimens have been observed, and degraded forest as "intermediate" are suitable habitat classes, we calculate an area of occupancy of 65.2 sqkm. Therefore, the status Endangered (EN B1abiii + B2abiii) is applied for this very rarely encountered and poorly known species that should become a focal species for future studies and monitoring programs.

Lycodryas sanctijohannis

This largely arboreal and nocturnal snake was observed in a variety of habitat classes on all islands (extent of occurrence 2025.0 sqkm, area of occupancy 1737.4 sqkm) and seems to be well adapted to living in natural forests as well as in plantation and urban areas. Its French vernacular name "serpent des cocotiers" (coconut palm snake) was probably given by locals who frequently encounter this snake while collecting coconuts. Thus, further destruction of the remaining natural vegetation is unlikely to affect the species' area of occurrence. Considering the crepuscular and mostly tree dwelling habits of this snake, the low number of observations does not necessarily point to a population decline or general rareness. The fact that locals state to observe, on average, about one snake per day per village (pers. observation), may even mean that *L. sanctijohannis* is still relatively abundant. Potential threats for this species may come in further extension of the infrastructure in settlements and intensification of horticulture, which may lead to the result that these areas are less suitable as habitats for *L. sanctijohannis*, and subpopulations will be isolated from each other by habitat fragmentation (EN B1biii). Snakes are regularly killed by farmers and planters, and extension of settlements and intensification of horticulture will also increase this threat factor. Since none of these threats is imminent and the species is unlikely

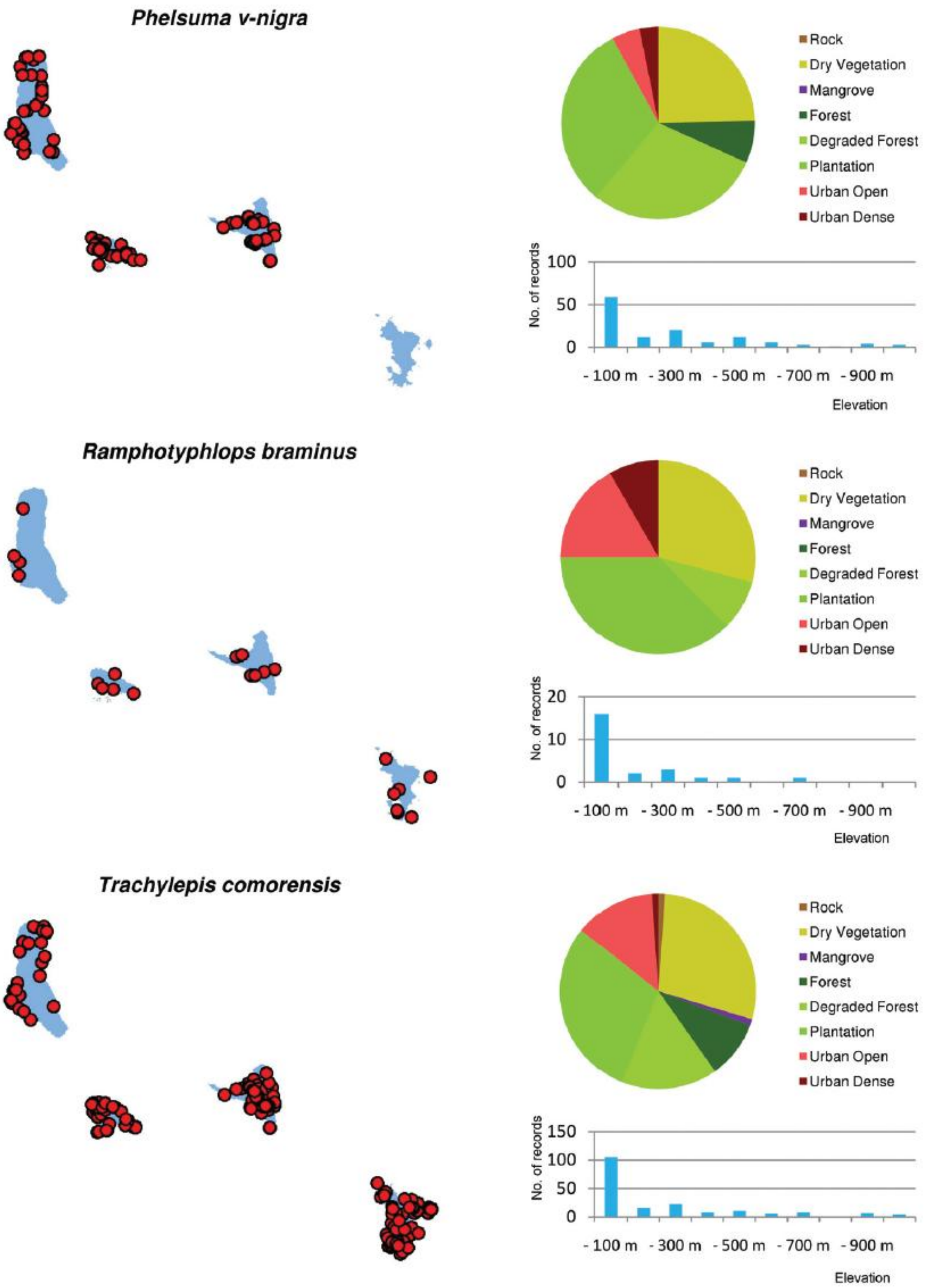


Figure 18. Distribution maps, and distribution over habitat and altitude classes, for *Phelsuma v-nigra*, *Ramphotyphlops braminus* and *Trachylepis comorensis*.

to be strongly affected by the introduced species (carnivores, rats, tenrecs, other reptiles or birds) at present, the status Near Threatened is proposed.

Furcifer cephalolepis

Current listing: Least Concern (Carpenter 2009)

This chameleon inhabits various habitats on Grand Comoro, in an area of occupancy of 718.1 sqkm. It is most common at the mountain of La Grille. Since *F. cephalolepis* is also abundant in plantation and degraded forest, deforestation cannot be considered an imminent threat. However, relatively high export numbers from 2000 to 2010 could have had a negative impact on this species, although this cannot be proven at present. We therefore propose to maintain the status Least Concern, but note that future monitoring programs, if implemented, should also regard this species.

Furcifer polleni

Although limited to a much smaller extent of occurrence and thus also area of occupancy (371.5 sqkm) than *F. cephalolepis*, *F. polleni* from Mayotte island is more commonly observed and inhabits a variety of habitats from pristine forest to gardens in densely populated areas. Blanc (1971, 1972, and cited in Raxworthy et al. 2002) reports *F. polleni* for Mohéli, but not for Mayotte. We have found no indications of a presence of this species on Mohéli and consider this record erroneous, in accordance with Meirte (2004). An allochthonous population on Anjouan, which is not considered for the calculation of area of occurrency, is so far only known from gardens in the town of Hombo (Meirte 2004 and pers. obs.). Export numbers from 2000 to 2010 were relatively low (1562 specimens) and significant threats were not observed. We propose to assign the status Least Concern to *F. polleni*.

Paroedura sanctijohannis

This nocturnal gecko is exposed to a greater risk than many other Comorian reptile species. While the Mayotte population shows no clear preference for any habitat or altitudinal range, the populations of the other three islands are almost exclusively restricted to high altitude forest which is subject to ongoing deforestation. Another potential threat to which *P. sanctijohannis* may be subjected in the future, due to its apparent restriction to mountain summits, is upslope displacement of its suitable habitats induced by global warming (Raxworthy et al. 2008). Increasing temperatures may also lead to an upslope displacement of its potential invasive competitor *Hemidactylus platycephalus* which is currently restricted to elevations below the distribution range of *P. sanctijohannis* on Grand Comoro, Mohéli, and Anjouan. As the area recorded for

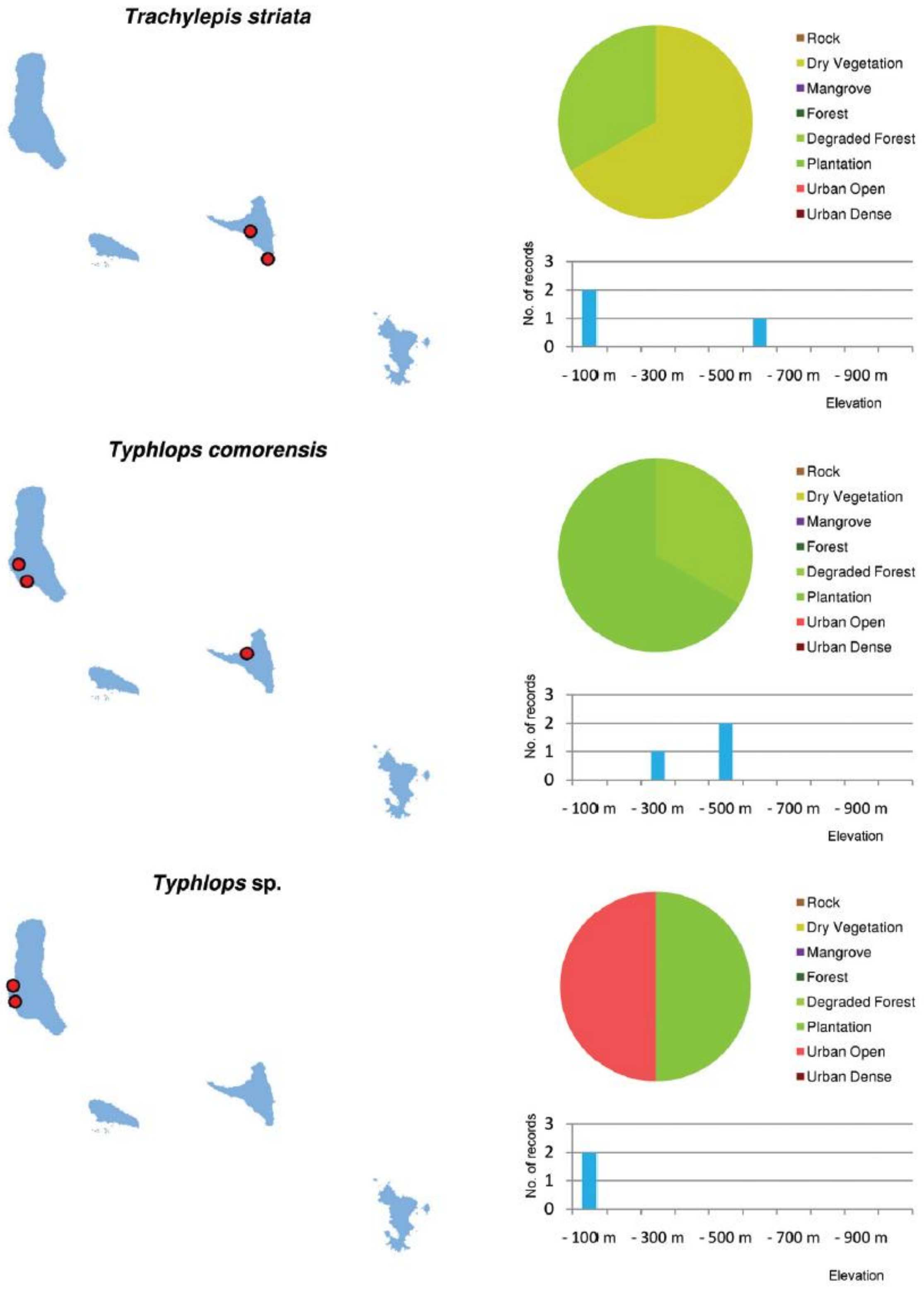


Figure 19. Distribution maps, and distribution over habitat and altitude classes, for *Trachylepis striata*, *Typhlops comorensis* and *Typhlops* sp.

P. sanctijohannis on these three islands is already reduced to a narrow range just below the summits of the mountains concerned, there is little buffer zone for displacement left. While the extent of occurrence is calculated to be 2025.0 sqkm, the area of occupancy is much smaller. Since mostly erratic juvenile specimens were observed outside closed forest, we assume that this habitat is not suitable for maintaining viable and reproducing populations. We calculate the area of occupancy as the sum of all areas of closed forest in which *P. sanctijohannis* was observed, plus the dry vegetation on Saziley peninsula of Mayotte, resulting in a severly fragmented area of 60.8 sqkm. This qualifies for the category Endangered, although it is much closer to Critically Endangered (below 10 sqkm) than to Vulnerable (500 sqkm). The status Endangered (EN B1abiii + B2abiii) is proposed for *P. sanctijohannis*.

Phelsuma comorensis

Current listing: Least Concern (Ineich 2009b)

This day gecko is restricted to a relatively small area on Grand Comoro, the mountain of La Grille, where it can be found in considerable abundance in degraded forests as well as in areas which are subject to heavy human influence, including banana plantations and open urban areas. An erratic individual was also observed near the capital Moroni, but no populations outside the La Grille area are known. Therefore, an extent of occurrence of 250.1 sqkm and an area of occupancy of 183.5 sqkm are calculated (EN B1biii+B2biii), and La Grille can be considered a single location (EN B1a+B2a). *Phelsuma comorensis* lives syntopically with the invasive species *P. dubia* throughout most of its range, although strong competition has not been observed so far. Further human development and population growth may lead to intensification of agriculture and horticulture, and to unpredictable general changes in land use including intensified use of pesticides which could lead to strong degradation of its area of occupancy. This would qualify the species for the status Endangered (EN B1abiii + B2abiii). We therefore propose the status Near Threatened.

Phelsuma nigristriata

As discussed above, among the Comorian day geckos *P. nigristriata* is the species most dependant on the remaining natural forest vegetation. Excluding the altitude level below 100 meters, where *P. nigristriata* was not observed, the remaining area covered by the habitat class forest on Mayotte is calculated as 24.83 sqkm. This qualifies for the IUCN criteria Endangered with less than 500 sqkm (EN B2), but is relatively close to the limit of 10 sqkm for the status Critically Endangered (CR B2). A small number of presumably erratic juvenile specimens was also observed in degraded forest, however, we assume that natural forests are necessary to maintain viable and reproducing populations. The forest areas are severly fragmented, and due to the high rates of human

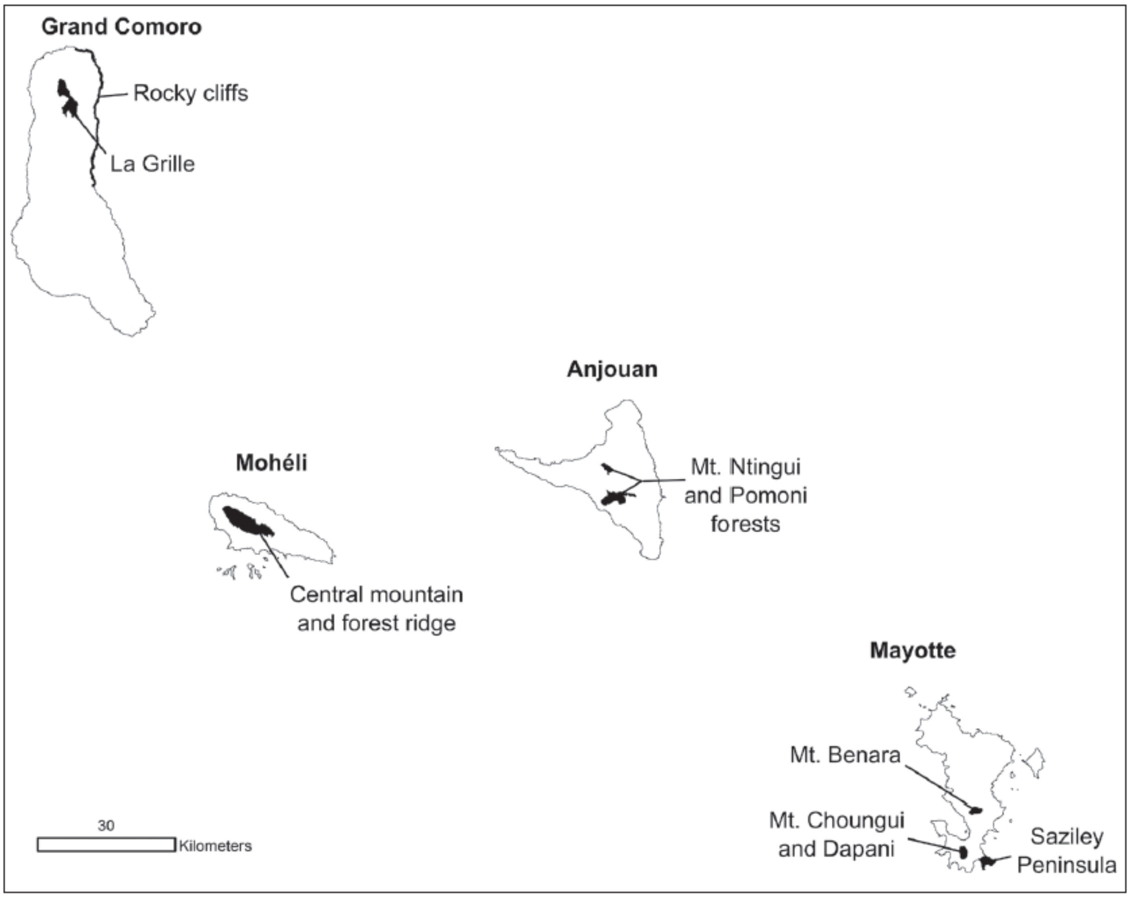


Figure 20. Proposed areas of conservational importance for the herpetofauna on the Comoro islands.

population growth on Mayotte, there is a high risk that increased degradation of pristine forests will lead to a continuing decline in the extent and quality of this habitat. This might drive the population of *P. nigristriata* to Critically Endangered in a very short time (criterion VU D2). We therefore propose the status Vulnerable (VU D2) for this species.

Phelsuma pasteuri

Among the species of *Phelsuma* endemic to Mayotte, *P. pasteuri* appears to be best adapted to degraded habitats, as discussed above. In areas of human influence, especially plantations, there is potential high competitive pressure imposed by *P. laticauda*, thus natural forest should be seen as the more adequate habitat for *P. pasteuri*. We calculate an area of occupancy of 127.0 sqkm, which brings this species close to meeting the criteria for the status Endangered (EN B1abiii + B2abiii). Only few specimens were exported for the pet trade (100 specimens). Currently, the status Near Threatened should be applied.

Phelsuma robertmertensi

As discussed above, *P. robertmertensi* is found mainly in mangroves and coastal tree stands, but also in the forest at Choungui on Mayotte. Despite the formal protection of mangroves and forests on Mayotte, these preferred habitats should still be considered threatened by human activities and fragmentation. An area of occupancy of 98.9 sqkm is calculated for *P. robertmertensi*. Almost the entire population of this species inhabits areas where individuals are exposed to competition by the introduced *P. laticauda*. As defined by this threat, the species is considered to occur at a single location. The status Endangered (EN B1abiii + B2abiii) should be assigned.

Phelsuma v-nigra

Current listing: Least Concern (Ineich 2009c)

On all the islands where *P. v-nigra* subspecies occur, they inhabit a variety of habitats in a total area of occupancy of 1368.2 sqkm and live in sympatry with at least one introduced species of *Phelsuma*. Though partially more abundant in higher altitude zones than the competitor *P. dubia* (and probably also *P. laticauda* on Anjouan), as shown by the results discussed above, no evidence clearly points to a decline of this species (EN B1bv). Elucidating this situation by long-term observations would be desirable. Moreover, many localities of *P. v-nigra* are situated in plantations and urban areas, so currently the area suitable for this species does not appear to be subjected to fragmentation and degradation (EN B1abiii). Therefore, the status Near Threatened (EN B1abiii + B1bv) appears appropriate for this species and should also be applied to all subspecies.

Amphiglossus johannae

Current listing: Least Concern (Ineich and Meirte 2009)

Amphiglossus johannae was observed on all islands in many different habitat classes, including habitats under human influence, and altitudes. Given the cryptic and fossorial habits of this skink a relatively high abundance cannot be excluded. No threat factors are currently visible. The status Least Concern should be maintained.

Cryptoblepharus boutonii subspecies

Current listing: *Cryptoblepharus ater*: Least Concern (Ineich 2009a)

Cryptoblepharus boutonii subspecies occupy an ecological niche unusual for reptiles. They live in rocky tidal zones, a habitat comprising little area and subjected to continuous change, as suitable areas may appear and disappear with erosion and tides. It is difficult to measure extent of occurrence and area of occupancy using our methodology, since rocky tidal zones are patchy and hard to detect using Landsat imagery. The subspecies *C. b. ater* (Grand Comoro), *C. b. degrijsii* (Anjouan) and *C. b. mohelicus* (Mohéli) are regularly observed in suitable habitats, also inhabit harbours and shores near settlements and are common in syntopy with potentially competitive *Trachylepis comorensis* and – on Grand Comoro – *Agama agama*. We propose the status Least Concern for these subspecies. In contrast, *C. b. mayottensis* in Mayotte could often not be detected in seemingly suitable habitats and was never recorded in harbours, which raises the suspicion of a past decline in area, extent or quality of its habitat (EN B1biii). We propose the status Near Threatened for this subspecies.

Trachylepis comorensis

On all islands, *T. comorensis* is widespread and occurs in primary forest as well as in areas under heavy human influence. In addition to the Comoros, it inhabits only small islets. No signs of reduction or fragmentation of the area of occupancy or population decline can be detected. The status Least Concern is applied to this species.

Blommersia sp. and *Boophis* sp.

Both anuran species treated here were recorded from various habitat classes, mainly classes under heavy human influence. Areas of occupancy of 297.6 sqkm were calculated for both species. Threats by habitat degradation or population declines can neither be observed nor inferred. However, the populations of both species might rapidly break down in the case of the introduction of the amphibian chytrid fungus, which is considered one of the prime factors of the global amphibian decline as has already been introduced to many previously unaffected areas (Daszak et al. 2003, Weldon et al. 2004, Mendelson et al. 2006, Skerratt et al. 2007, Wake and Vredenburg 2008, Rödder et al. 2009). This might lead to a classification of Endangered (EN B1bv + B2bv). At present, the status Near Threatened should be applied for both species.

Areas of conservational importance and future measures

In this section, we propose seven areas of highest importance for the conservation of the Comorian herpetofauna. Our proposals are based on the distributions of native, threatened species, on the occurrence of natural vegetation types and on the degree of potential threats. Figure 20 shows these proposed areas.

Studies concerning α- and β-diversity of birds and butterflies on Anjouan have been undertaken by the ECDD (Engagement Communautaire pour le Developpement Durable, BP 279, Mutsamudu, Anjouan, Comoros; Marsh et al. 2010) that highlight areas of conservational importance for these groups. As stated in this paper, "using just one taxon when prioritising areas for conservation may lead to a poor representation of wider diversity". Therefore, we underline that in order to benefit the Comorian fauna as a whole, further taxa and factors must be taken into consideration for the planning of protected areas.

Rocky cliffs along the north-eastern coast of Grand Comoro

This area encompasses an almost linear habitat of coastal cliffs of volcanic rocks. It stretches over a length of about 38 km from the area of Ivouani southwards to Itsikoudi. This is the only habitat of the Comoro iguana *Oplurus cuvieri comorensis*. This taxon inhabits the mostly vertical cliff structures, but also neighbouring areas of volcanic rock which extend several hundreds of meters inland. The only other reptile species detected in this habitat were *Cryptoblepharus boutonii* and *Trachylepis comorensis*. Since the cliff structures do not border the coastline continuously, *O. cuvieri comorensis* cannot be observed along the entire area of the coast. Instead, the cliff areas are home to subpopulations divided by unsuitable habitats.

Further studies should be undertaken to estimate the population size of *O. cuvieri comorensis*, to detect all of its subpopulations and to infer the degree of separation of these subpopulations. Additionally, the development of the agama population on Grand Comoro should be closely monitored. *O. cuvieri comorensis* is currently protected by Comorian law. Nevertheless, efforts should be made to ensure the effectiveness of this protection by preventing the regular capture of individuals by locals for food, entertainment or for the international pet trade. This could be achieved by communication with authorities in the local village communities and particularly by the participation of locals in the works mentioned above.

La Grille (Grand Comoro)

Grand Comoro is an island of two volcanoes, the northern, older, and lower of which is called La Grille. Aside from several reptile species widespread on Grand Comoro, La Grille is home to the microendemic *Phelsuma comorensis* and probably to the largest population of *Paroedura sanctijohannis* on Grand Comoro. Further endemic species present at La Grille are *Furcifer cephalolepis*, which is very common in this area, and *Lycodryas sanctijohannis*. Forest, of a total area of 747 hectares, remains only in the highest elevations. The mountain is populated up to areas of around 600 m, but plantations and degraded forests with understory crop planting are common up to near the peaks. While *P. comorensis* mainly inhabits plantations and urban areas and seems not immediately threatened, *P. sanctijohannis* is found only in or near the forest. Increasing deforestation pressure by a growing population might thus lead to the loss of what to our current knowledge forms the core habitat of the Grand Comoro population of this species.

Efforts should be undertaken to halt the deforestation on La Grille. The populations of *P. comorensis* and *P. sanctijohannis* should be monitored, especially as *P. comorensis* might be exposed to threats by international pet trade and the introduction of further invasive species.

Central mountain and forest ridge on Mohéli

The largest continuous area of natural and near-natural forest on the Comoros is the forest covering the slopes of Mt. Karthala, Grand Comoro. However, these forests mainly correspond to altitudes of over 1000 m asl and are thus hardly suitable for most reptile species. The second largest forest area is the forest growing on the central mountain ridge of Mohéli. This forest extends from altitudes of around 500 m asl to the peak at 790 m, encompassing an area of about 3190 hectares. It is home to the native reptile species *Paroedura sanctijohannis*, *Phelsuma v-nigra* and *Lycodryas sanctijohannis*.

The forest on Mohéli's mountain ridge should be considered one of the most important forest areas for conservation on the Comoros not only due to its biodiversity, but also due to its still relatively untouched state. Since Mohéli is more sparsely populated than the other islands of the archipelago, the future rate of deforestation is also expected to be lower. Measures to protect this forest and to halt logging and underplanting would benefit not only reptiles, but also many other groups of organisms (Louette et al. 2004).

Forests on Anjouan: Mt. Ntingui, Pomoni

Anjouan is the island of the Comoro archipelago most densely populated. Forest remains in higher altitudes and in inaccessible areas. Large parts of the forested areas are in elevations too high for reptiles. In lower altitudes, remarkable forests can be found at the north-western slope of Mt. Ntingui, on the way from Mutsamudu, and above Pomoni. There, pristine forests already grow around 600 m asl. *Paroedura sanctijohannis* is relatively common there. Though not considered endemic, *Geckolepis maculata*, a species rather rare on the Comoros, is regularly observed above Pomoni. Further endemics are *Lycodryas sanctijohannis* and *Phelsuma v-nigra*. The forest at Ntingui, up to about 1000 m asl, covers an area of about 198 hectares, at Pomoni about 940 hectares.

It is currently unknown if the populations of *P. sanctijohannis* at Mt. Ntingui and Pomoni are separated or if there is gene flow between them. This gene flow might be facilitated either by a more or less closed corridor of suitable habitat between these areas or by a sufficiently large number of wandering individuals (which have also been observed in degraded forest areas). To protect *P. sanctijohannis* and *G. maculata* on Anjouan, measures should be taken to protect these two forest areas.

Mt. Benara (Mayotte)

As also stated in Pascal (2002), the forest at Mt. Benara is one of the best conserved on Mayotte. Two of the three endemic species of day geckos, *Phelsuma nigristriata* and *P. pasteuri*, are very common there. Mt. Benara is also the only high-altitude forest on Mayotte in which *Paroedura sanctijohannis* has been observed and the top of this mountain is one of only two known locality records for the endemic species *Liophidium mayottensis*. The chameleon *Furcifer cephalolepis*, the two endemic amphibians *Boophis* sp. and *Blommersia* sp. and the non-endemic but rare *Ebenavia inunguis* are also present at Mt. Benara. The forest area is about 185 hectares.

Mt. Benara is already a forest protection area, and on Mayotte, all endemic reptile species are protected. Nevertheless, the importance of Mt. Benara for reptile protection should be emphasized.

Mt. Choungui and Dapani (Mayotte)

Due to its isolation in the drier southern part of Mayotte, the forest at Mt. Choungui and Dapani is distinguished from other forests of similar altitudes on Mayotte by its vegetation and fauna (Pascal 2002). Most conspicuous among the reptiles is the endemic *Phelsuma robertmertensi*, otherwise a species of coastal habitats. There are also populations of *Paroedura sanctijohannis* and *Furcifer cephalolepis*. This forest encompasses around 233 hectares. Similarly to Mt. Benara, Mt. Choungui is a protected area.

Dry forest and bush on the Saziley peninsula (Mayotte)

In our classification, the vegetation at Saziley was assigned the habitat class “dry / low vegetation”. This is because, in contrast to forest of the habitat class “closed forest”, this vegetation consists of low trees and bushes which even in their natural state form a canopy only partly continuous and less dense than humid forests do. Saziley is the most remarkable habitat of *Paroedura sanctijohannis*, being the only place where this species has been recorded at sea level and in a dry area. It is furthermore remarkable because a population of *P. sanctijohannis* exists in syntopy with the invasive *Hemidactylus platycephalus*. The non-endemic but rare *Geckolepis maculata* also occurs.

Saziley is not a forest protection area, but is protected and closely monitored because of its beaches, which are breeding sites of marine turtles. As in the cases of Mt. Benara and Mt. Choungui, the importance of Saziley peninsula for reptile protection should be emphasized. Saziley is a peninsula of about 593 hectares.

Conclusions

On the Comoro islands, only fragments of the original forest vegetation are left, mostly at high altitudes. However, reptiles and amphibians are more common and species-rich in non-natural habitats, especially plantations, and urban areas are home to a remarkable diversity. This can in part be attributed to the high number of invasive, anthropophilous species. However, many native species are recorded commonly outside the natural forests. *Paroedura sanctijohannis* and *Phelsuma nigristriata* appear to be the only reptiles for which pristine forest is of high importance as habitat. As most species show little dependency on natural habitats, loss of these habitats alone could be identified as a strong threat factor only for the two forest species. The most important threat factor at present is invasive species, primarily among the genera *Hemidactylus*, *Phelsuma* and *Agama*, and mammals. Therefore, habitat degradation and destruction remain an important threat factor, as it creates more suitable conditions for invasive species.

The use of satellite imagery, ground truthing, and species distribution maps allowed us to reliably estimate habitat availability and thus Area of Occupancy according to the criteria of IUCN (2001a). According to our evaluation, three endemic species are proposed for the status Endangered, one for Vulnerable, six for Near Threatened, and two for Data Deficient. Four species are proposed for the status Least Concern. The single most threatened taxon, though endemic only as a subspecies, is the Comoro iguana *Oplurus cuvieri comorensis*.

In comparison to Madagascar, where conservation is in the focus of international attention (Kremen et al. 2008), the Comoros are in urgent need of plans and measures for conservation of their native biodiversity. Legislation already provides nominal protection for the Comorian species on the international (CITES regulations for genera *Furcifer* and *Phelsuma*) and national level (see Anonymus 2000, Louette et al. 2004). However, any measures can be effective only if the local communities are conscious of the value of biodiversity and its fragility, and they participate in translating these measures into action.

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Appendix I

List of locality data used in the analyses. (doi: 10.3897/zookeys.144.1648.app1). File format: Microsoft Excel (XLS).

Explanation note: List of locality data used in the analyses. Altitude level is given in hundred meters asl (e.g., 4 = 300 to 400 m asl). Abbreviations: APO = Authors' personal observation (including reports by locals), MCPO = M. Carretero personal observation, see Carretero et al. (2005). SOH (tissue samples, Oliver Hawlitschek personal collection) numbers, like ZSM numbers, are stored at ZSM.

Citation: Hawlitschek O, Brückmann B, Berger J, Green K, Glaw F (2011) Integrating field surveys and remote sensing data to study distribution, habitat use and conservation status of the herpetofauna of the Comoro Islands. ZooKeys 144: 21–79. doi: 10.3897/zookeys.144.1648.app1

Appendix II

List of specimens examined. (doi: 10.3897/zookeys.144.1648.app2). File format: PDF.

Explanation note: List of specimens examined. Museum abbreviations are explained under "Locality data and species identification" in the "Methods" section.

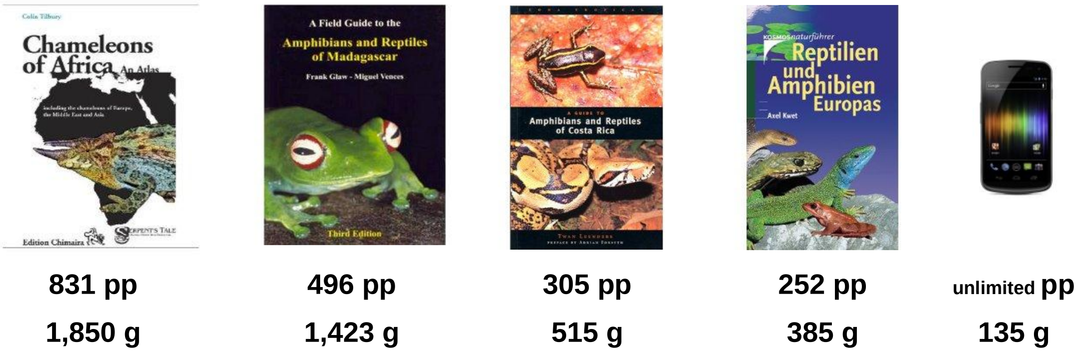
Citation: Hawlitschek O, Brückmann B, Berger J, Green K, Glaw F (2011) Integrating field surveys and remote sensing data to study distribution, habitat use and conservation status of the herpetofauna of the Comoro Islands. ZooKeys 144: 21–79. doi: 10.3897/zookeys.144.1648.app2

7.3 SmartHerper Comoros

Cybertaxonomy makes access to taxonomic data easier by making use of online media. Currently, this mostly refers to online databases, online identification numbers, and the publication of taxonomic articles in online media (see Chapter 3.2.3). Printed books are still widely used as tools for the identification of species in the field by professional and non-professional naturalists. Books are complemented by further accessory tools, such as GPS receivers, digital cameras, pens, and notebooks.

The increasing availability of electric power supply, and mobile internet and telephone connection, even in remote regions of the planet also increase the utility of small portable computers, tablets, and cell phones for naturalist field work. If the prerequisites of power supply and connectivity are given, smartphones can be excellent tools in field surveys. Modern models have high data storage capacity, include a GPS receiver and a camera of sufficient quality, and allow the up- and download of data to and from the internet. Additionally, many models can be used for the short-range communication with other team members even in the absence of mobile phone network (Fig. 15).

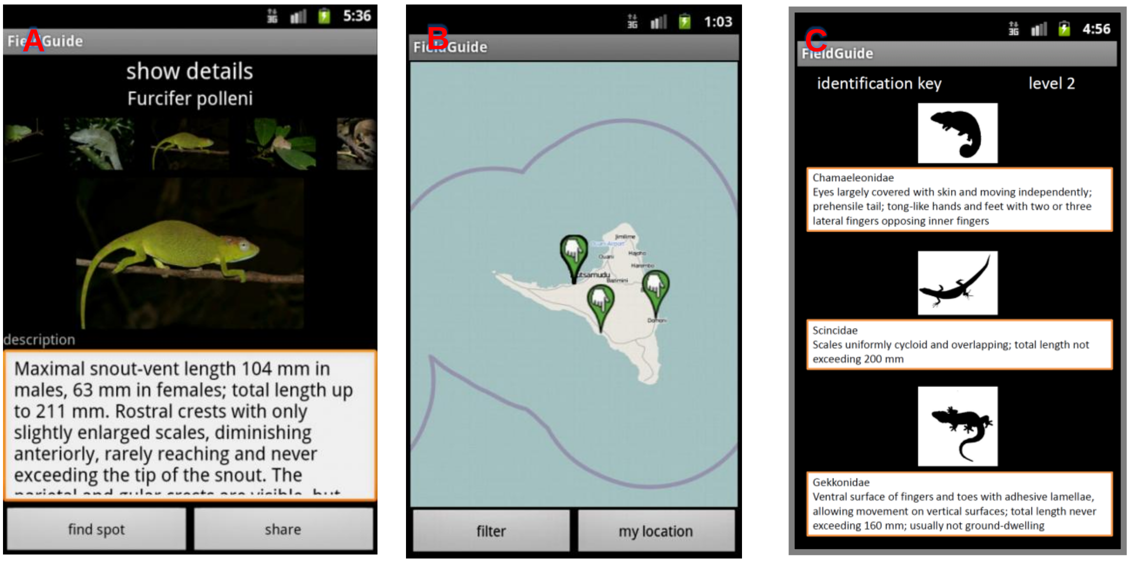
Figure 15: Weight and volume of some printed herpetological field guides and a smartphone. The ration of volume (of information) versus weight is an important factor in naturalist field work because the total weight of the equipment that can be carried to the field is limited. Smartphones with digital field guides, e.g., as mobile applications, may provide a useful alternative to printed guides. The pictured field guides are: Tilbury (2010), Glaw & Vences (2007), Leenders (2001), and Kwet (2005).



A number of commercial mobile applications ('apps') are available on the market that contain picture, text, and map information on a certain set of species sorted by region or taxonomic group, similar to a traditional field guide (e.g., Green Mountain Digital 2012, Search Life Forms 2012, Valois 2012). A few non-commercial apps allow and even encourage the contribution of locality and picture data by non-professional naturalists, using the GPS and camera functions of the users' smartphones (e.g., iNaturalist 2013). This 'citizen science' approach has already been used in a number of projects and has the advantage that much higher amounts of data on the distribution of the target organisms become available than could be collected by professionals alone. The disadvantages are that little data become available from regions that are hard to reach for non-professionals, and that the error rate in species identification is much higher (Bonney et al. 2009, Silvertown 2009). With smartphone apps, citizen scientists are not required to carry much equipment (camera, GPS, notebook) for surveying, but can collect data if they carry nothing but their smartphone (iNaturalist 2013).

SmartHerper Comoros is such a smartphone app that functions similar to a traditional field guide by providing text, picture, and map information on the reptiles and amphibians of the Comoros Archipelago. An interactive identification key based on text and image information, users can identify all included species. Users can also see their own position in relation to known localities of reptiles and amphibians in the area of interest using the GPS function of their smartphone (Fig. 16).

Figure 16: Screenshots of SmartHerper Comoros (developmental version). A: Species Accounts for every species of amphibians and reptiles present on the Comoros include text information and a photo gallery. B: A map view displays known localities of the queried species in relation to the user's position according to the smartphone GPS. C: An interactive identification key helps with identifying species in the field.



Maximal snout-vent length 104 mm in males, 63 mm in females; total length up to 211 mm. Rostral crests with only slightly enlarged scales, diminishing anteriorly, rarely reaching and never exceeding the tip of the snout. The

Chamaeleonidae
Eyes largely covered with skin and moving independently; prehensile tail; tong-like hands and feet with two or three lateral fingers opposing inner fingers

Scincidae
Scales uniformly cycloid and overlapping; total length not exceeding 200 mm

Gekkonidae
Ventral surface of fingers and toes with adhesive lamellae, allowing movement on vertical surfaces; total length never exceeding 160 mm; usually not ground-dwelling

The app is currently under development by collaboration of the Zoologische Staatssammlung, Munich (OLIVER HAWLITSCHEK, FRANK GLAW), and the University of Applied Sciences Rosenheim (DANIEL GUGGENBICHLER, RENÉ RÖSNER). A translation for a future French language version is provided by EMMANUEL F.A. TOUSSAINT (ZSM). SmartHerper Comoros is developed for the Android 2.3.3 (or higher) operating system for smartphones. Future versions for other operating systems (IOS, Windows Phone) are planned.

The final version of SmartHerper Comoros will include a function by which the user can contribute locality and picture data via the camera and the GPS of his or her smartphone. This citizen scientist data will be uploaded to a database, checked by the administrator, and made available to the user community via regular updates. The app will be made available for free. I hope that SmartHerper Comoros will contribute to the dissemination and generation of knowledge on the biodiversity of its focus regions, the Comoros Archipelago, and thus stimulate interest in the observation and conservation of the regional biodiversity in visitors, e.g., ecotourists, and residents alike.

8 Discussion

8.1 What different biogeographic models tell us about the origin of species

8.1.1 The case studies presented in this dissertation

In my work for this dissertation, I followed the path that many researchers in evolutionary biology have taken before me: I studied an island system in order to gain insights into to mechanisms of speciation and evolution. At the same time, I studied similar phenomena in a continental system, attempting to make use of my experience with the supposedly less complex island system (WALLACE 1865). Indeed, the 28 reptile species of the Comoros are a much simpler system than 300+ species of Australian diving beetles. However, in both study systems a variety of biogeographic scenarios with different degrees of complexity was found (Table 7).

Table 7: Summary of the speciation events studied in this dissertation. The proposed scenario of speciation is stated here, summarized below, and described exhaustively in the respective chapters.

Study group	Type	Proposed scenario	Chapter
Comoran *Paroedura*	reptiles / Comoros	two independent colonization events of true oceanic islands	5.1
Comoran *Lycodryas*	reptiles / Comoros	archipelago speciation on true oceanic islands	6.2
Hygrobiidae	diving beetles / Australia	'relic' distribution after breakup of Pangea	5.3
Antiporus femoralis	diving beetles / Australia	splitting of ancestral range of mesic habitats by Miocene forming of Australian arid zone	6.1
Sternopriscus tarsalis radiation	diving beetles / Australia	isolation and re-expansion during Pleistocene climate oscillations, with ecological diversification and reproductive isolation	5.2

8.1.2 Summarizing discussion: speciation events in the Comoros

I found that the biogeographic patterns of the Comoran system, too, were relatively complex despite its apparent relative simplicity. This was indicated by preliminary DNA-Barcoding studies (Chapter 4) and evaluated in more details for two endemic groups of reptiles in phylogenetic analyses (Chapters 5 and 0). In these studies, none of the colonization events of the Comoros by reptiles could be shown to clearly follow the Progression Rule (FUNK & WAGNER 1995) (but possibly the endemic *Phelsuma* radiation shows this pattern, see ROCHA et al. 2009 and Chapter 4). Instead, a variety of biogeographic patterns is indicated, including intra-island radiation, back-dispersal, and repeated colonization from the 'mainland' Madagascar (Table 8). This diversity in biogeographic patterns is in line with what was found for many groups of organisms in other oceanic archipelagoes, although the Progression Rule seems to be followed more often in other archipelagoes than in the Comoran organisms studied so far (see review in WHITTAKER & FERNÁNDEZ-PALACIOS 2007 and WHITTAKER et al. 2008). This may be because other archipelagoes are larger, geologically older, have more diverse biota, and have been studied more comprehensively (WHITTAKER & FERNÁNDEZ-PALACIOS 2007).

Table 8: Biogeographic patterns in Comoran reptiles, based on the biogeographic-evolutionary patterns proposed in FUNK & WAGNER (1995) and WHITTAKER & FERNÁNDEZ-PALACIOS (2007). See also Table 4.

Group	**Pattern**	**Comment**	**Reference**
Comoran *Phelsuma* endemic radiation	Progression Rule (?)	relationship between populations of youngest islands not fully resolved	ROCHA et al. (2009)
Phelsuma pasteuri and *P. robertmertensi*	intra-island radiation	sympatric *Phelsuma* sister-species of Mayotte	ROCHA et al. (2007, 2009)
Paroedura of Anjouan	back-dispersal	possible re-colonization of Anjouan from geologically younger Grand Comoro after extinction event (volcano?)	Chapter 5.1
Comoran *Lycodryas*	stochastic	apparent stochasticity in distribution in relation to the developmental history of the archipelago	Chapter 6.2
Comoran Geckolepis	stochastic	close to 'Progression Rule' pattern	Chapter 4.1
Trachylepis comorensis	back-dispersal	likely colonization in opposite direction of the 'Progression Rule' because of African instead of Malagasy origin	ROCHA et al. (2010)
Liophidium mayottensis	recent colonization	colonization of only a single island, genetically nested within mainland clade	NAGY et al. (2012) and Chapter 6.2
Phelsuma comorensis	recent colonization	colonization of only a single island, genetically nested within mainland clade	ROCHA et al. (2007, 2009)
Oplurus cuvieri comorensis	recent colonization	colonization of only a single island, genetically nested within mainland clade	MÜNCHENBERG et al. (2008)
Comoran *Cryptoblepharus*	recent colonization	above-average dispersal abilities	ROCHA et al. (2006)
Comoran *Ebenavia*	repeated colonization / extinction	paraphyletic; Mayotte population is not sister group to other Comoran populations; possible extinction of older populations	Chapter 4.1
Comoran *Paroedura*	repeated colonization / extinction	paraphyletic; Mayotte population is not sister group to other Comoran populations; possible extinction of older populations	Chapters 4,1 and 5,1
Comoran *Paroedura*	fusion of paleoislands	possible for the Grand Comoro population	Chapters 4,1 and 5,1
Amphiglossus johannae	unresolved	likely recent colonization, mainland clade probably nested within Comoran populations; insufficient data	Chapter 4,1
Typhlops comorensis	unresolved	insufficient data (rare species)	Chapter 4,1
Comoran *Furcifer*	unresolved	insufficient data; only two islands colonized, lack of comprehensive phylogeny of the genus	Chapter 4,1, ROCHA et al. (2005b)

Among the more remarkable patterns in Comoran biogeography are the various events of repeated colonization of the Comoros. According to ROCHA et al. (2005b), the two endemic chameleon species of the genus *Furcifer* are the result of two separate colonization events, but this deserves further review in a comprehensive phylogeny of the entire genus. Most likely, *Cryptoblepharus* skinks also colonized the Comoros in two independent events (Chapter 4 and ROCHA et al. 2006). The opposite case is found in the skink *Amphiglossus johannae*, where the presumed mainland sister taxon *A. ardouini* is nested within the Comoran populations (Chapter 4). The cases of *Cryptoblepharus* and *Amphiglossus* should be treated with caution because of the shallow divergences between the taxa in question. The Comoran clade of *Lycodryas* snakes appears paraphyletic in the DNA-Barcoding study (Chapter 4), but monophyletic in a multigene phylogeny (Chapter 6.2), and similar results might be obtained for the skink genus *Amphiglossus*. Deeper divergences were found in the gecko genera *Ebenavia* and *Paroedura*. In both cases, the Comoran populations are paraphyletic with respect to 'mainland' populations from Madagascar; the populations from the oldest island Mayotte are the sister groups to

Malagasy clades, whereas the other Comoran populations form a separate clade (Chapters 4 and 5.1). For *Paroedura*, this relationship is supported by a multigene phylogeny and morphological studies. The question of why the populations inhabiting the oldest Comoran island Mayotte (Table 5) are more closely related to populations from Madagascar than from the Comoros is not easy to answer. Options are that: (1) Initially, Mayotte was not colonized at all, but only after the colonization of the other islands of the archipelago. However, in most other phylogenies of Comoran reptiles Mayotte appears to be the island colonized first, and there is no evidence why Mayotte should not have been initially colonized by *Paroedura* and *Ebenavia*. (2) The second colonization event took place while the original populations of *Paroedura* and *Ebenavia*, respectively, were still extant, but the original populations were later outcompeted by the new colonizers and went extinct. However, it is likely that in this case some kind of genetic introgression from the hypothetical original *Paroedura* populations could be found in the molecular markers studied. (3) The original population of Mayotte was extinct, e.g., due to volcanic activity, and the island was later re-colonized from Madagascar (see also discussion in Chapter 5.1). Both options (2) and (3) highlight that extinction events probably played a major role in the biogeography of the herpetofauna of the Comoros archipelago and should not be underestimated in future studies on island biogeography.

One of the goals of my study of the phylogenies and the biogeography of the Comoran herpetofauna was to investigate the utility of this system for molecular clock studies. The use of oceanic island endemics as calibration points for divergence time estimates has been a matter of recent debate (HEADS et al. 2011, MELLO & SCHRAGO 2012); fossils are generally considered preferable to geological calibration points (DONOGHUE & BENTON 2007, FOREST 2009), but as explained in Chapter 5.3, the fossil record is not equally abundant for all groups of organisms, and molecular clock studies focusing on reptiles at low taxonomic levels (but also on beetles, as discussed below) often present the problem of a lack of fossil calibration points (HEADS 2005, PEPPER et al. 2011a). Species that are endemic to oceanic islands with available geological age estimates have been used widely, but this requires the assumption that the species in question diverged from its ancestral clade around the time of the emergence of the oceanic island, i.e., the ancestors of the endemic species colonized the island shortly after its origin. Consequently, a colonization and radiation pattern following the Progression Rule must be presumed if an island endemic species is to be used for the calibration of a molecular clock.

As shown above and discussed in Chapter 5.1, there are several alternatives to this basal scenario: (1) The colonization event took place at a later date. (2) The clade of the species in question is older than the island it is endemic to, either because its closest mainland relative was extinct between the colonization event and present, or because the colonization took place via stepping stones that today no longer exist (see possible examples in RENNER 2004, RENNER et al. 2010). The latter scenario appears highly likely in the Comoros, where the Geyser and Leven Banks formed stepping stones for the colonization from Madagascar to the Comoros during the Pleistocene regression (Chapter 3.4.2). (3) The geological age estimates for the islands are not correct. Most estimates of the geological ages of oceanic islands (and all of the Comoros Islands) are based on rocks exposed on the surface of the islands. However, these surface rocks might cover older volcanic rocks that provide older and more correct age estimates, but are mostly inaccessible to scientists. This might be the case if older volcanoes become active after a period of inactivity. Alternatively, a volcano may be active over a longer period and 'wander', growing at one end and eroding at the other; in the result, the island would be old as a habitat for terrestrial organisms, but its stratigraphy would be composed entirely of younger rock (HEADS 2011). Such events are not expected in the hot spot theory, but most geologists are aware that the mechanisms of the formation of volcanic islands are still poorly understood (SHERROD 2009), and some molecular clock studies have yielded results that question the estimates for the geological ages of some islands (RASSMANN 1997, THORPE et al. 2005).

The Comoros include an example of an island whose biota appear to be much older than the geological age estimates. This was first speculated by ROCHA et al. (2005b), who found surprisingly high divergence between haplotypes within the population of the chameleon *Furcifer cephalolepis*. This species is endemic to Grand Comoro, the presumably geologically youngest island with an estimated age of 0.01 to 0.5 my (Table 5). ROCHA et al. (2005b) estimated, based on mutation rates of mitochondrial markers, that the haplotype divergence pointed towards an age of the population of *F. cephalolepis* of 0.63 to 1.76 my. In Chapter 5.1, I presented estimates for the age of the Grand Comoro population of the gecko *Paroedura sanctijohannis* of 0.7 to 2.7 my. As discussed in Chapter 4, the haplotype diversities of the Grand Comoro populations of many reptiles (e.g., *Lycodryas cococola*, *Paroedura sanctijohannis*) are relatively high. This supports the view that the populations of reptiles of Grand Comoro are older than the current age estimates for the island.

8.1.3 Summarizing discussion: speciation events in Australian diving beetles

The application of molecular clocks is even more difficult in Australian diving beetles: there are no reliable calibration points applicable to endemic Australian radiations. Fossils of aquatic beetles are globally scarce and almost completely missing from the region, and the existing fossils often cannot be reliably placed in the phylogenies (BALKE et al. 2004, 2007). Past geological events, such as the spreading of the arid zones and the genesis of the mesic biota, were gradual events over longer periods of geological history and are not useful for calibrating divergence time estimates (Chapter 6.1). However, these events were intimately linked to the diversification of the groups of diving beetles I studied (Table 9). The diversification events of diving beetles span a timescale an order of magnitude larger than those of Comoran reptiles, from the Mesozoic to the very recent past, and the mechanisms driving speciation can be assumed to be very different to those of the island reptiles.

Table 9: Biogeographic hypotheses for Australian diving beetles studied in this dissertation.

Group	Distribution	Origin	Biogeography	Reference
Hygrobiidae	Europe, China, Australia	Mesozoic	Vicariance after Pangea breakup	Chapter 5.3
Antiporus femoralis	disjunct SW and SE Australia	Lower Pleistocene	disjunction of formerly connected habitat by aridification	Chapter 6.1
Sternopriscus tarsalis radiation	mainly SE Australia and Tasmania	Upper Pleistocene	repeated isolation and re-connection of habitats during Pleistocene climate oscillations	Chapter 5.2

Attempts of molecular dating of the diversification events of Australian waterbeetles could be made only using either fossils of outgroups (Chapter 5.3) or standard mutation rates (Chapters 5.2 and 6.1). The results from both methods have to be treated with particular caution, but remain without alternatives. The analyses show the stark contrasts between ancient and recent diversification events: Only six extant species of *Hygrobia* are known worldwide, whose origin is dated to 221 to 163 mya, whereas an explosive radiation in the *Sternopriscus tarsalis* group estimated as recent as 130,000 to 60,000 years ago yielded ten species. Speciation in other groups of waterbeetles seems to have occurred at a pace between these extremes, as shown by the relatively lower rates of other *Sternopriscus* species (Chapter 5.2) and by the split of *Antiporus femoralis* and *A. occidentalis* estimated at 1.0 to 1.9 mya.

In chapter 5.2, I discuss whether the term 'species flock' is appropriate for the *Sternopriscus tarsalis* radiation. In this group of beetles, species are clearly distinguishable by male genital morphology and show a certain degree of ecological differentiation, but share haplotypes of mitochondrial and nuclear genetic markers. Species flocks have previously been described from radiations in lakes and on islands (GREENWOOD 1994, SCHÖN & MARTENS 2004), but not from continental regions. SCHÖN & MARTENS (2004) summarize the criteria to term a radiation of species a species flock as "speciosity [species-richness], monophyly and endemicity". While I argued that the former two criteria are met by the *Sternopriscus tarsalis* radiation, the species do not share a clearly circumscribed common area of endemism today. However, they likely did so during a stage in the speciation process: As argued in Chapter 5.2, the origin of this radiation is in time with the Pleistocene climate oscillations, and these can be suggested as a driving force of speciation in this group. In this scenario, populations of the ancestral species were repeatedly forced to retreat into refugia from the unsuitable climate from which they would expand during periods of favorable climate. These cycles might have led to divergent ecological adaptations in isolated populations, depending on the environment of the respective refuges, and to the fixation of morphological characters supporting reproductive isolation, thus promoting speciation. In the current period of stable climate, the species had time to disperse and were no longer confined to their ancestral areas. Therefore, the term 'species flock' may rather be attributed to a past than to the present stage of speciation in the *Sternopriscus tarsalis* radiation.

The sister species *Antiporus femoralis* and *A. occidentalis* present a less extreme example of speciation at a presumably slower pace (Chapter 6.1). Just like in *Sternopriscus*, climate change was likely the driving force of speciation. However, this climate change was a more gradual one – over millions of years, the formation of the southern Australian arid zone presumably separated the population of the ancestral species of *A. femoralis* and *A. occidentalis*. Today, the two sister species live in allopatric habitats of mesic climate that are more similar to each other than to the separating arid zone regarding temperature and precipitation. Yet the habitat of *A. occidentalis* shows a stronger seasonality in precipitation than that of *A. femoralis*. According to the identity and background tests of the Ecological Niche Models of the two species, the divergence of their climate envelopes is higher than what would be expected from the climatic difference in the allopatric ranges alone. This suggests that some degree of niche diversification has evolved between these two taxa as an adaptive response to the respective habitats.

In contrast to both previously discussed groups of Australian beetles, Hygrobiidae has a very ancient origin. As discussed in Chapter 5.3, the present-day distribution of this morphologically conservative family is thought to be formed by vicariance rather than by dispersal. In global terms, this distribution with one species in Europe, one in China, and four in Australia, can be considered relictual, although this term implies that the species inhabit very small 'relictual' areas. Instead, the European and Australian species of this family are very widespread. The deep divergences between the Eurasian and the Australian clades favor vicariance over dispersal in explaining the family's present-day distribution. According to my estimate, the clades represented by the extant species formed around the time of the breakup of Pangea. Therefore, the driving force of speciation in Hygrobiidae as a whole can be assumed to be isolation by plate tectonics.

8.2 Allopatry vs. sympatry, dispersal vs. vicariance, adaptiveness vs. non-adaptiveness

The five cases that were studied extensively in this dissertation present five different and unique events of speciation, all of which were influenced by different biogeographic settings and environmental factors (Table 10).

Table 10: Summary of the characteristics of speciation in the study groups. Assumed from the results for every group, several factors that influence speciation are given. 'Biogeography' states whether a dispersal or vicariance scenario is seen as the more likely explanation for an allopatric distribution, or whether the species occupy sympatric ranges today. 'Radiation' states whether (as discussed in this chapter) these clades are assumed to have undergone adaptive or non-adaptive radiation.

Study group	**Type**	**Biogeography**	**Radiation**	**Chapter**
Comoran *Paroedura*	reptiles / Comoros	dispersal	non-adaptive	5.1
Comoran *Lycodryas*	reptiles / Comoros	dispersal	non-adaptive	6.2
Hygrobiidae	aquatic beetles / global, Australia	vicariance	?	5.3
Antiporus femoralis	diving beetles / Australia	dispersal / vicariance?	?	6.1
Sternopriscus tarsalis radiation	diving beetles / Australia	broadly sympatric	adaptive	5.2

In any attempt to understand the mechanisms of speciation it is important to gather indications for how the present-day distribution of the study group was formed. For most of the 20th century, speciation was assumed to occur almost exclusively in allopatry (MAYR 1954, 1963, TURELLI et al. 2001). In the last decades sympatric speciation has been shown to be more common than previously thought (FUTUYMA & MAYER 1980, SCHLIEWEN et al. 1994, VIA 2001). It is not always easy to reconstruct the geography of the speciation of extant taxa, as their present-day geographical distribution may differ from their distribution at the time of speciation (LYNCH 1989, BARRACLOUGH & VOGLER 2000). Sympatric speciation has often been assumed for closely related species that live in sympatry today (MATTERN & MCLENNAN 2000, LOSOS & GLOR 2003), but the ranges of such species may have expanded after the speciation stage (HEWITT 2004, RIBERA & VOGLER 2004). Allopatry in turn can be explained by dispersal, i.e., the colonization of habitats after their isolation, or vicariance, i.e., the fragmentation of an ancestral habitat and its population. Evidence for vicariance can often be found in the fossil record, but no fossils are available from the groups I studied here (possibly only for *Hygrobia*, Chapter 5.3). Molecular divergence time estimates are another approach to clarify the question of dispersal vs. vicariance which, again, is difficult if fossil calibration points are rare or absent.

In the cases of the Comoran reptiles, the question is easy to answer. Native terrestrial organisms, including the ancestors of endemic species, have necessarily colonized true oceanic islands via dispersal, since these islands are geologically young and have never had any connection to any other landmass. Speciation has therefore occurred in allopatry (but see exceptions in Table 8). *Lycodryas* snakes present the most straightforward of the example presented here, in which every island is inhabited by a separate species or subspecies of a monophyletic group or species complex (Chapter 6.2). WHITTAKER & FERNÁNDEZ-PALACIOS (2007) use the term 'archipelago speciation' for this mode of speciation. Some other endemic Comoran radiations of reptiles follow this example (Table 8). The case of *Paroedura* geckos does not match this concept of 'archipelago speciation' because this genus colonized the archipelago in two independent events (Chapter 5.1). However, both subsequent speciation events took place in areas isolated from the mainland and from each other, therefore in allopatry.

In Australian aquatic beetles, the scenarios are more complex, as predicted at the beginning of this chapter. The questions of whether speciation has occurred in sympatry or in allopatry, and whether allopatric ranges result from vicariance or dispersal, are not easily answered and rely much more on inference from models of past climate and geology than in the cases of island species. The case of *Antiporus femoralis* and *A. occidentalis* is comparably easy to explain. These two sister species are suggested to represent successors of the formerly connected population of their ancestral species. However, the speciation event was dated to 1.9 to 1.0 mya, so it is not easy to determine whether the two species result from vicariance or from a dispersal event over the forming arid zone. (Chapter 6.1). In my more complex scenario suggested for the *Sternopriscus tarsalis* radiation, speciation occurred through repeated retreats of populations into glacial refuges and subsequent re-expansions (Chapter 5.2). This kind of speciation has an allopatric (refuges) and a sympatric (likely overlapping expanded ranges) component and does not easily fit the typical distinction between these two alternative models. The origin of the Hygrobiidae, with present-day species found in Europe, East Asia, and Australia, is estimated around the breakup of the Mesozoic super-continent Pangea (the speciation between the four species of the Australian clade is not in the focus of this work; Chapter 5.3). The very far disjunct ranges suggest an allopatric scenario. The diversification time estimates do not allow any reliably estimation of whether the family diversified shortly before or after the breakup of Pangea. If the diversification took place after the breakup, the ancestors of the extant clades must have reached their present-day ranges by dispersal between the continents. If the diversification took place earlier, then the extant species are relics of a presumably former Pangean distribution.

Another aspect of speciation is adaptiveness. 'Adaptive radiation' is commonly used as a standing term and goes back to typical examples such as that of the Darwin Finches, whose species show many clear adaptations to ecological factors (GRANT 1984). However, many authors argued that speciation needs not necessarily have an adaptive base: differences between species might be limited to (secondary) sexual traits, or purely genetic mechanisms such as polyploidization or gene duplication might have initiated speciation (GITTENBERGER 1991, SCHLUTER 2000, SCHÖN & MARTENS 2004). With the exception of mechanisms like polyploidization which create an 'instant' reproductive barrier, however, it will always be difficult to decide whether traits that distinguish species are adaptive or not (WHITTAKER & FERNÁNDEZ-PALACIOS 2007). For us to be recognized as such, species necessarily need to be distinguishable in some traits, be they morphological, genetic, genomic, ecological, or behavioral. Consequently, the decision whether a trait is adaptive or not will in many cases be an arbitrary one, especially if the trait is to be non-adaptive: SCHÖN & MARTENS (2004) point out that "one can never demonstrate to satisfaction that no diversification has occurred in any of the niche dimensions". Therefore, I follow these authors in arguing that any claim that a speciation or radiation is non-adaptive has to be a tentative one.

Consequently, I tentatively argue that the speciation in the Comoran reptiles studied is mostly non-adaptive. Most likely, the adaptive step was taken upon the first colonization of the archipelago, when the new island populations adapted to the island environment. There are significant morphological differences between each the Comoran *Lycodryas* snakes and both Comoran *Paroedura* clades and their respective mainland sister taxa. Comoran *Lycodryas* are larger than their mainland counterparts and show a remarkable sexual dimorphism, whereas

Comoran *Paroedura* are smaller and more cryptic both in adult and juvenile coloration. In Chapters 5.1 and 6.2, I present arguments for why these traits are adaptations to the island environment. More remarkably, the adaptive traits are even shared between the not closely related Comoran clades of *Paroedura*, suggesting an example of convergent adaptation to the same environmental constraints. Within the Comoran clades of *Lycodryas* and *Paroedura*, on the other hand, these traits are very conservative, and morphological differences are small and likely attributable to drift or sexual selection. Therefore, I suggest that the speciation in these cases had an initial adaptive and a subsequent non-adaptive component.

Again, the situation is more complex in the continental scenario. The ancient and vicariant Hygrobiidae are morphologically and ecologically relatively conservative, and therefore, they may seem to present a case of non-adaptive speciation (again, disregarding the Australian radiation; Chapter 5.3). However, the ancient origin of the lineage makes it very hard to infer which factors promoted its speciation. Arguably, the speciation of Hygrobiidae likely has an adaptive component because: (1) Today, the species inhabit ecologically very different ranges, and some species appear to have broader ecological amplitudes than others. This suggests divergent adaptations to ecological variables. (2) The global environments underwent massive changes since the estimated split between the Eurasian and Australian lineages of Hygrobiidae in the Mesozoic, and, like almost all organisms that survived these events, Hygrobiids must have a long history of specific adaptation to these changes. This leads to the question whether speciation was followed by adaptation or the other way round (SCHÖN & MARTENS 2004). This question is very hard to answer especially for such ancient diversification events, for which the past diversity and the ecological conditions are hard to reconstruct. But even young speciation events may pose similar problems, such as *Antiporus femoralis* and *A. occidentalis* (Chapter 6.1). While the two species are indistinguishable in all morphological characters studied, they are divergently adapted to the climates of their distributional ranges. Possibly, the adaptation to different climatic backgrounds followed the isolation of the population. However, my data does not allow for correlating the age of speciation with the onset of habitat diversification with sufficient precision. Therefore, divergent adaptation may alternatively have already contributed to the speciation process in this group.

There is one case left that can be argued to represent an example of an adaptive radiation. I showed in Chapter 5.2 that the ten species of the *Sternopriscus tarsalis* radiation show very divergent responses to ecological parameters of climate and habitat. In the groups discussed so far, the adaptive component in speciation appears to be a mere consequence of the different ecological backgrounds in the allopatric ranges occupied by the species. In the *Sternopriscus tarsalis* radiation the adaptation to divergent habitats and climates may have been a driving force of speciation because, according to the scenario presented here, the ranges of the incipient species repeatedly overlapped and do so even today.

This summary of the various case studies presented in this dissertation confirms WALLACE'S (1902) view that the less complex systems of evolution and speciation in islands teach us lessons in that help us to understand the more complex continental scenarios. But island systems can be complex in their own ways, and every new study contributes towards our understanding of island biogeography, evolution, and speciation.

8.3 The species problem in 2013: how DNA-barcoding, cybertaxonomy, and integrative taxonomy may help

This dissertation includes the descriptions of three species and two subspecies new to science. This is a mere drop in the ocean of another 2 to 8 million species remaining to be described on planet Earth (COSTELLO et al. 2013). As already introduced in Chapter 3.2.3, taxonomy in the 21st century faces the following problems: (1) An extremely high number of taxa remain to be described. (2) The amount of taxonomic literature that has to be reviewed for every description is immense and grows with every new description. (3) The availability of new data from, e.g., molecular phylogenies, ecological, and behavioral studies, has shown that arbitrary decisions on the taxonomic status of organisms based on 'taxonomic expertise' alone may lead to confusion and the need of numerous taxonomic revisions (PADIAL et al. 2009, 2010, MIRALLES et al. 2011). Therefore, taxonomists need to be capable of (1) quickly recognizing potentially new taxa, (2) quickly verifying the identifications of taxa with taxonomic data available worldwide, and (3) delimiting taxa as objectively, reliably, and sustainably as possible.

In this chapter, I discuss the following developments in taxonomy that may be helpful in tackling the problems stated above: (1) DNA Barcoding, (2) cybertaxonomy, and (3) integrative taxonomy.

Chapters 4 to 0 of this dissertation demonstrate the importance of DNA barcoding in modern taxonomy, in line with the previous work of many authors (HEBERT et al. 2003, 2004 SAVOLAINEN et al. 2005, HAJIABAEI et al. 2007). All taxa that were newly described in the course of this dissertation were initially detected by DNA barcoding or comparable DNA screening techniques (Table 11). All these newly described taxa are cryptic species or subspecies whose identification was then confirmed by morphological and / or ecological data.

Table 11: The importance of DNA Barcoding for the taxonomic work conducted in this thesis.

Species	Type	Detection	Description
Antiporus occidentalis	diving beetle / Australia	Barcoding auf Australian Dytiscidae (HENDRICH et al. 2010)	Chapter 6.1
Paroedura stellata	reptile / Comoros	DNA screening of Comoran reptiles (see Chapter 4.1)	Chapter 5.1
Comoran *Lycodryas* taxa	reptile / Comoros	DNA screening of Comoran reptiles (see Chapter 4.1)	Chapter 6.2

These cryptic taxa had previously not been recognized most likely because of their morphological similarity to closely related taxa. This is particularly remarkable in *Paroedura stellata*, as discussed in Chapters 5.1 and 8.1.2: This gecko can be distinguished from the other Comoran form *P. sanctijohannis* by meristic characters, but the two species are more similar to each other regarding adult and juvenile coloration than to the mainland sister taxa of *P. stellata*. This similarity may have resulted from convergent adaptation to the island environment and 'hid' the distinct status of *P. stellata*. No morphological character was found that could be used to distinguish the cryptic beetle species *Antiporus occidentalis* from its sister taxon *A. femoralis*. In this case, the DNA barcoding result was supported by further mtDNA, nDNA, and Ecological Niche Modeling. In addition to the cases studied in this work (Table 11), the barcoding analyses of Comoran reptiles (Chapter 4) and Australian diving beetles (HENDRICH et al. 2010) indicate many new directives for further taxonomic studies.

My studies also provide examples for the importance of taxonomic research, here based on initial DNA barcoding and screening, in conservation. With the descriptions of *Antiporus occidentalis* and *Paroedura stellata* I also provided evidence for the ecological distinctness of these taxa from their respective sister species (with which they had previously been considered conspecific; Chapters 4 and 5.1). I could demonstrate, based on habitat data, that *P. stellata* warrants a lower threat status (Vulnerable according to IUCN 2001) than its sister *P. sanctijohannis* (Endangered). This underlines the importance of protection measures for the latter species now that its formerly 'safest' island population was found to belong to a different species (HAWLITSCHEK & GLAW 2011, Chapter 7.2).

DNA barcoding, as also underlined by the work done in this dissertation, is becoming a standard method also in taxonomic and conservation research. Most likely, the establishment of Next-Generation Sequencing methods will further contribute towards this development and considerably accelerate the process of inventorying biodiversity. These methods allow not only the reliable processing of high numbers of samples in very short time, but also completely new ways of sampling, such as environmental sequencing (VALENTINI et al. 2009, HAJIBABAEI et al. 2011).

In my taxonomic work following the initial barcoding step, I relied heavily on historic literature on Comoran reptiles (Chapters 5.1 and 6.2). While I knew that the total available literature on this topic was manageable, I was also aware that insufficient exposition of cryptic nomenclatural acts and availability of old literature may present problems in taxonomic work (see GLAW et al. 2007 and CADLE & INEICH 2008). Therefore I aimed at maximizing the exposition, availability, and impact of my taxonomic results using the following methods that are broadly summarized under the term 'cybertaxonomy'. (a) I requested LSIDs for every taxonomic publication and every taxon. (b) I preferred to publish in open-access journals. (c) I created Species-ID entries that included correctly identified photographs of the species and could be linked to the publications. (d) I uploaded all sequence data to GenBank via EMBL. I hope that this course of action will facilitate the dissemination of my results and make them easily accessible for future researchers relying on my contributions to taxonomic literature.

In the next step following publication accompanied by these 'basic' cybertaxonomic actions, the results – at least those of my research on Comoran reptiles – are to be made available to the broad public. In Chapter 7.3, I introduce SmartHerper Comoros, a project that is still in development but whose publication is planned for the same year as the submission of this dissertation. SmartHerper Comoros will be a mobile application for smartphones that provides users with basic information on the identification and biology of Comoran reptiles, like a traditional field guide. Users will furthermore be able to use the application to contribute data on these species via direct upload of photographs and GPS coordinates. This spans the bridge between professional and citizen science and is hoped to raise public interest in the animals as well as awareness for conservation efforts.

The cybertaxonomic methods (upload to GenBank, creating Species-ID pages etc.) require additional effort from the author of a taxonomic description, but make it easier for readers – among them potential authors of future taxonomic descriptions or revision – to access and filter the total high amount of taxonomic information (HENDRICH et al. 2009, WINTERTON 2009, VENIN et al. 2010, HENDRICH & BALKE 2011). I argue that consequently, if all taxonomists use these methods, the additional effort required for cybertaxonomy will be more than compensated for by the benefits of an easier review of taxonomic data and literature. The same is most likely true for integrative taxonomic approaches: they require additional effort from the author of a taxonomic description, but in the future, less effort may be required for taxonomic revisions conducted by the same or other authors on the same taxa.

All taxonomic descriptions included in this dissertation follow an approach of integrative taxonomy, using analyses of various genetic and morphological characters (Table 12). All descriptions of reptiles (Chapters 5.1 and 6.2) explicitly follow the lines of evidence approach with Integration by Partial Congruence proposed by MIRALLES et al. (2011). The description of the beetle *Antiporus occidentalis* does not nominally follow this approach, but is based on a similar methodology using evidence from different kinds of data (Chapter 6.1). The same is true for the analysis of the *Sternopriscus tarsalis* radiation, in which no new taxa are described, but the delimitation of previously described taxa is re-analyzed (Chapter 5.2).

*Table 12: Integrative taxonomy in this dissertation following the lines of evidence approach (MIRALLES et al. 2011) or a comparable methodology. The table shows which lines of evidence supported species delimitation in which study group. *: Not evaluated because no distinction was expected.*

Group	**Type**	**mtDNA**	**nDNA**	**morphology**	**ecology**	**Chapter**
Antiporus occidentalis	diving beetle / Australia	yes	yes	no	yes	6.1
Sternopriscus tarsalis radiation	diving beetle / Australia	no	no	yes	yes	5.2
Paroedura stellata	reptile / Comoros	yes	yes	yes	yes	5.1
Comoran *Lycodryas* species	reptile / Comoros	yes	no	yes	*	6.2
Comoran *Lycodryas* subspecies	reptile / Comoros	yes	no	no	*	6.2

MIRALLES et al. (2011) proposed the lines of evidence of mtDNA, nDNA, and morphology. In addition to these lines, I used ecological data in most integrative taxonomic analyses. In *Antiporus* and *Sternopriscus* beetles (Chapters 6.1 and 5.2), I used the quantitative data presented by the results of Ecological Niche Modeling. The niche identity and background tests of ENMtools (WARREN et al. 2008, 2010) provide a statistical means of estimating the significance of ecological divergence between species. This allows the use of Ecological Niche Modeling as a tool for objectively testing diversification as an additional line of evidence. In *Sternopriscus* (Chapter 5.2), I additionally used qualitative ecological data (altitudinal range categories and habitat type) to support the results of the Ecological Niche Modeling. In Comoran *Paroedura* geckos (Chapter 5.1), I did not use ecological data as an explicit line of evidence, but I used qualitative ecological data on habitats to support the results of the lines of evidence of mtDNA, nDNA, and morphology. In *Lycodryas* snakes (Chapter 6.2), I did not use ecological data at all because field observations suggested that no ecological diversification between the populations of this group could be detected using available data. *Lycodryas* is the only case in which the lines of evidence approach yielded not only species, but also subspecies.

The addition of further lines of evidence, based on other types of data, is possible. Bioacoustic (SINSCH & SCHNEIDER 2009, GLAW et al. 2010), behavioral (TÖPFER-HOFMANN 2000), genomic (STÖCK et al. 2006), biochemical (REINERT et al. 1997), and many other sources of data have been used in species descriptions. Mostly, the basic directive of MIRALLES et al. (2011) should be followed that divergence in one line of evidence suggests subspecific status, and divergence in two or more lines suggests specific status of the candidate species in question. However, divergence in some characters may present direct evidence that the candidate species is indeed a separate species according to most Operational Criteria of the Unified Species Concept. VIEITES et al. (2009) mainly based their Candidate Species Approach on such traits that mediate reproductive isolation or that are known to provide evidence for lineage separation. I call these types of evidence **'trump traits'** because they 'win' over other lines of evidence and directly suggest specific status, even if the results of other lines of evidence are conflicting. Examples for such trump traits may be genomic characters such as differences in ploidy (as found in, e.g., STÖCK et al. 2006), morphological or other traits clearly suggesting intrinsic reproductive isolation (e.g., USAMI et al. 2006, MIKKOLA 2008), or empirical evidence clearly suggesting reproductive isolation (e.g., RICO-MARTÍNEZ 1997, TÖPFER-HOFMANN 2000, UHART & ALBERTO 2009). Additionally, phylogenetic data may provide evidence that the candidate species in question do not form a monophyletic group. I found this in Chapter 5.1 for two taxa previously considered conspecific, *Paroedura stellata* and *P. sanctijohannis*. Nevertheless I followed an integrative taxonomic approach to gather evidence for the taxonomic status of all species comprised in the clade together with *P. stellata* and *P. sanctijohannis.*

This example illustrates that the finding of trump traits should not make other lines of evidence superfluous. I propose that other lines of evidence should still be investigated as a back-up for errors in the analysis of the trump traits, and for future comparisons with other species for which the trump trait line of evidence does not fit. More likely, trump traits may prove particularly useful in cases in which the other lines of evidence present unclear or conflicting results, and they partly allow overcoming the arbitrary selection of a number of lines of evidence that has to be met to recognize species.

As discussed above, one of the major problems in modern taxonomy is the need of efficiently describing a high number of taxa. In many cases, an integrative approach exploring many lines of evidence may consume more time and effort than can be allocated to taxonomical work. Therefore, I want to point out that integrative taxonomy remains a tool that is mainly helpful in critical cases of species delimitation, in which only the exploration of several lines of evidence will yield satisfactory results. In other cases, formal descriptions for high numbers of species based on few lines of evidence (e.g., DNA barcode + morphological description of key characters) may be rapidly provided and distributed using cybertaxonomic methods (e.g., Riedel et al. 2013a, 2013b). One of the most helpful tools for this rapid taxonomic work may be the use of 'natural language' for species descriptions. There, the morphological data is integrated in a description that is created by software and can also be read by the same software as well as by human readers (DALLWITZ 1980).

Finally, all these tools that are available in modern taxonomy will increase the efficiency and the sustainability of taxon descriptions and other taxonomic work, but will still require the experience and the common sense of the taxonomist.

8.4 Species – apple of discord and currency of biodiversity research

> "It is clear that the arguments [about species concepts] will persist for years to come but equally clear that, like barnacles on a whale, their main effect is to retard slightly the progress of the field. Ultimately, speciation will require less rumination and more perspiration."
>
> Jerry Allan Coyne

> "We neither wish to contribute more fuel to the debate, nor add another barnacle to Coyne's whale."
>
> Loren H. Rieseberg and John M. Burke in "The biological reality of species: gene flow, selection, and collective evolution"

I discussed in the previous chapters that (1) our understanding of the nature and definition of species is becoming more and more precise, but also more and more different from the traditional understanding of species, and (2) modern tools will likely make taxonomy a more and more hypothesis-based, but also automatized science. Both these insights cause considerable discord within the scientific community.

A common agreement on the general definition of species is fundamental for taxonomy, as it allows for the agreement of taxonomists on the delimitation of individual species (HAUSDORF 2011). However, this agreement is still based more on the experience of taxonomic experts, as it was in DARWIN'S times: "No one definition has satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species" (DARWIN 1859). Scientists questioned the sense of the debate on the species problem already decades ago (COYNE 1992), but new species concepts are still being published and heating the debate (DE QUEIROZ 2005, HAUSDORF 2011). The Unified Species Concept (DE QUEIROZ 2007) is an attempt to find a solution by subsuming all previously published species concepts. It was welcomed by many researchers (PADIAL et al. 2009, 2010), most likely in part because it presented a compromise that might de-escalate the debate. However, some authors reject this concept, arguing that it is no more than a compromise and that it does not offer a real solution: this could only be done by a single species concept on which all biologists can agree (HAUSDORF 2011). However, such a 'final species concept' is not in sight. As demonstrated in this dissertation, the Unified Species Concept has proven a good working concept that can be successfully applied in integrative taxonomy.

Integrative taxonomy makes taxonomic work more sustainable, and cybertaxonomy makes taxonomic work more efficient and faster. The ICZN decision of 4th September, 2012 (ICZN 2012), removed a major obstacle to the speed of species descriptions by allowing valid taxon descriptions in online media. These developments accompany exhaustive and vivid discussions on new taxonomic concepts (CANTINO & DE QUEIROZ 2000, NIXON et al. 2003, HEBERT et al. 2003, MALLET & WILLMOTT 2003, EVENHUIS 2007, FLOWERS 2007, MILLER 2007, VIEITES et al. 2009, PADIAL et al. 2009, 2010, HASZPRUNAR 2011, DE QUEIROZ 2012). This 'Taxonomic Renaissance' is bringing taxonomy back into the spotlight of scientific attention as a fundamental discipline of biology and shows the importance of this discipline in modern biodiversity research, and not too early: Taxonomy needs to be a modern, dynamic, and efficient scientific discipline in order to cope with the challenge that the largest part of biodiversity on Earth is still unknown to science and at the same time threatened by human activity (FLOWERS 2007). I believe that taxonomy, as an integral sub-discipline of biodiversity research, bears some responsibility towards biodiversity and its conservation. However, like all disciplines of research, biodiversity research also bears responsibility towards the public. Discovering and naming species, and reporting about named species, is one of the major, even stereotypical, services that the public demands from biology.

Whenever any taxonomic rank is in the focus of para-taxonomists, citizen scientists, or the broad public, it is that of the species. Species, and their names, are important because they are the only taxonomic rank for which any objective definition is possible. Therefore, almost all counts of categories of organisms refer to species. These may be counts of economically important species, species dangerous to man, species available as pets, or threatened species. For this reason, species are the most important concept – the 'currency' – in biodiversity research. For the same reason, I believe that contributions to the debate on the species problem do make sense, and that they will produce many insights that will advance our understanding of speciation, evolution, and biology as a whole.

9 Major conclusions of this dissertation

The following very compressed list sums the most important conclusions drawn from the work of this dissertation. All methods, results, and references are found in the previous chapters. Below the list, a sum of numbers and facts concerning this dissertation is presented.

- ✓ The natural **colonization of the Comoros islands** by various groups of reptiles did not follow any repeating pattern strongly correlated with the geographic positions or the geological age of the islands. Rather, the islands were colonized by **different reptile groups in different ways**, most likely including various **extinction and re-colonization events**.
- ✓ Species in study groups of **Australian aquatic beetles** were shown to be of **very different ages**, from Mesozoic in *Hygrobia* over Neogene in *Antiporus* to Pleistocene in *Sternopriscus*. Neogene speciation events were correlated with **past climate change** as the presumed major trigger.
- ✓ Molecular genetic data of reptiles indicate that at least one island of the Comoros Archipelago, **Grand Comoro**, may be **considerably older than the geological estimates** of its age. Age estimates for the island are 0.01 to 0.5 my, however, the age of the **endemic island clade of *Paroedura* geckos** is estimated to 0.7 to 2.7 my. This is supported by comparably high haplotype diversity in the endemic clades of other reptiles in the same island.
- ✓ Some **morphological traits** of Comoran endemic reptiles were highlighted and discussed as possible **adaptations to the island environment**. These traits are the highly similar, but likely convergent, cryptic adult and juvenile coloration of *Paroedura* geckos, the increased size of *Lycodryas* and *Liophidium* snakes, and the sexual dimorphism in *Lycodryas*. The traits suggest a clearly **adaptive component in the speciation** events following the colonization of the archipelago.
- ✓ Evidence for **ecological niche diversification** between species of Australian *Antiporus* and *Sternopriscus* **diving beetles** was found in categorical ecological data and the results of Ecological Niche Modeling. This also indicates an **adaptive component in these speciation events**.
- ✓ Molecular, morphological, and ecological data indicate that the ***Sternopriscus tarsalis*** **radiation** of Australian diving beetle species originated in one of the **fastest speciation events** known so far among insects. I discussed that this radiation had characters fitting to the 'species flock' concept. This would be the first described **species flock** of aquatic insects.
- ✓ A **molecular clock** study indicates that the disjunct distribution of the **Hygrobiidae** family of aquatic beetles, with species in Europe, East Asia, and Australia, is not the result of recent dispersal, but likely dates back to the **breakup of Pangea**.

- ✓ **Ecological data** was successfully used as a line of evidence for **species delimitation** in the integrative taxonomy of Australian diving beetles. It was used in the forms of qualitative categorical data and quantitative data produced by **Ecological Niche Modeling** for delimiting species of *Antiporus* and *Sternopriscus* diving beetles.
- ✓ **Integrative taxonomy** using mtDNA, nDNA, morphology, and ecological data was successfully applied for species delimitation even when species could **not be distinguished by morphology** (in *Antiporus* diving beetles), or when they could **not be distinguished by any of the molecular genetic markers** studied (in *Sternopriscus* diving beetles).
- ✓ Using the example of Comoran *Lycodryas* snakes, I argued that, in contrast to the arguments of some authors, the taxonomic rank of **subspecies** should not be abolished. Instead, taxonomic entities with **some degree of differentiation**, but **without any clear evidence for lineage separation**, should be described as subspecies.
- ✓ **DNA barcoding** was shown to provide a **reliable preview to phylogenies**. While inferior to the results of multigene phylogenetic studies, DNA barcoding topologies led to the **identification of cryptic species** in Comoran reptiles and Australian beetles that were later confirmed by multigene phylogenies and morphological analyses. Additionally, DNA barcoding proved useful in the identification not only at species level, but also at the **infraspecific level of island populations** of Comoran reptiles.

Numbers and facts:
All numbers refer to this thesis alone and may be higher if side projects that are not directly part of this dissertation are included.

Days from official application to submission of this thesis: **1509**
Total pages: **248**
Scientific articles included: **7**
Total number of words: **115701**
Number of words (text only) in scientific articles: **55481**
Total number of literature references (doubles not counted): **695**
Mentions of Charles Darwin: **27**
Mentions of Kevin de Queiroz: **33**
Highest number of works cited from a single first author: **9** (Sara Rocha)
Total number of collaborators in scientific articles: **11**
Total number of persons acknowledged: **69**
Field survey days: **86**
Reptiles collected: **132**
Samples processed: **339**
DNA-Sequences produced: **945**
Average days from submission to publication of scientific articles: **210**
Financial cost of this thesis (incl. wages for author, EUROS): **90867**
Major computer crashes: **1**

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11 Appendices (CD)

The CD coming with this volume comprises the pdf files of the papers included in this dissertation and the supporting files belonging to these papers.

The following folders and files are included in the CD:

- Chapter 4.1
  - Table_S1.xls
- Chapter 5.1
  - Hawlitschek and Glaw 2012 – Paroedura stellata Comoros.pdf
  - Table S1.xls
  - Table S2.pdf
  - Table S3.xls
- Chapter 5.2
  - Hawlitschek et al 2012 – Sternopriscus pleistocene radiation.pdf
  - Table S1.pdf
  - Table S2.csv
  - Table S3.pdf
- Chapter 5.3
  - Hawlitschek et al 2012 – Hygrobia distribution dating.pdf
  - Appendix_A1.pdf
  - Appendix_A2.pdf
  - Appendix_A3.pdf
  - Appendix_A4.pdf
- Chapter 6.1
  - Hawlitschek et al 2011 – Antiporus ENM Phylo.pdf
  - Figure_S1.tif
  - Figure_S2.tif
  - Figure_S3.tif
  - Table_S1.doc
  - Table_S2.doc
  - Table_S3.doc
  - Text_S1.doc
- Chapter 6.2
  - Hawlitschek et al 2012 – Lycodryas revision.pdf
  - Table S1.xls
  - Table S2.pdf
- Chapter 7.1
  - Hawlitschek et al 2011 – Comoros remote sensing conservation.pdf
  - Appendix I.xls
  - Appendix II.xls

  - Dissertation Hawlitschek 2013.pdf
  - Hajek et al 2010 – Copelatus sibelaemontis.pdf
  - Hendrich et al 2009 – Neobidessodes revision.pdf