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Molecular approaches to the assessment of biodiversity in limnic gastropods (Cerithioidea, Thiaridae) with perspectives on a Gondwanian origin

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SUMMARY

Due to their limited potential of dispersal in combination with their habitat fidelity, limnic gastropods tend to preserve distribution patterns over long periods of time. Thus they are suitable organisms in biogeographical research and the study of the relations between colonization events and speciation. In this thesis intensive investigations into the phylogeography and phylogeny of Australian freshwater snails are provided, presenting the first molecular study of the Thiaridae (Caenogastropoda: Cerithioidea) based on four DNA sequence markers (COI, 16S, H3, 28S) and amplified fragment length polymorphisms (AFLPs). The aim of this study is to determine the historical events that may have influenced the phylogeography of these taxa and their presence on the Australian continent. In general, the origin of Australian freshwater faunal elements and the directionality and timing of colonizations are still controversial. Conventionally, many biotic elements found in Australia today are considered to be recent invaders from the Indo-Malay archipelago but more and more cases have become known that deviate from this standard scenario.

In order to test whether the thiarids represent recent invaders from the north or if they originated on the Australian continent, the evolutionary relationships within the family as well as its phylogenetic position in the superfamily Cerithioidea is analysed. A molecular clock approach is applied subsequently to date the origins of the Thiaridae and their sister families so that the dispersal events can be related to historical tectonical changes. A prerequisite for the reconstruction of past distribution patterns is the delimitation of the Australian species and the analyses of their current distributions. For this purpose, preceding investigations to characterize individual taxa by morphology are complemented with molecular analyses and additional sampling with the objective to obtain confirmation of the differentiated species and the detailed analysis of their present distribution patterns. By comparison of the molecular phylogenies, as well as the distributional data, the fossil record and divergence date estimates in conjunction with the excellent record of Earth history the long-held view that the thiarid fauna is an appendage to the southeast Asian biota can be rejected. Instead, an Australian continental, i.e. East-Gondwanian origin is found to be the most parsimonious explanation of the present distribution. The age of the thiarids dates back to about 50 Ma and coincides with (although not necessarily causally linked to) the separation of Australia from Antarctica. With an ancestral thiarid lineage that originated in Australia, Asia seems to have been colonized a number of times within the period of the collision of the Australian plate with Southeast Asia during the past 20-30 Ma. With their now assumed long history on the continent, Australian thiarids represent an important and realistic model system in speciation research which provides details of the dynamics of the underlying mechanisms of speciation under the influence of climate change.

Although there are still ambiguities to be resolved which concern the relationships among the thiarid taxa, the comparison of the extensive molecular datasets and their resultant phylogenies offer considerable insight into this enigmatic group. It is demonstrated that extreme caution must be used when inferring phylogeny from mtDNA loci in the absence of corresponding multi-locus nuclear data. Nevertheless, the mtDNA data corroborates almost all morphologically described species. In regard to the delimitation of the Australian species, a total of eleven distinct clades confirmed by morphology and molecular data are identified. Furthermore with the compilation of recent distribution maps on drainage based scales, extensive data on extant species is now available which gives new insights into the current dispersion and the degree of endemism. Even a new species, that is *Thiara rudis*, is for the first time recorded and verified as taxa with occurrences in Australia. Moreover, the AFLP analysis reveals a recent diversification between the two endemic species *"Thiara" australis* and *Plotiopsis balonnensis* with possible hybridisation in the newly detected zone of overlap.

Within the scope of this thesis a procedure is developed that makes it possible to contextualize old museum material from malacological collections within biosystematics research in a reliable way. It is based on ancientDNA techniques and comprises the amplification of short DNA fragments which are analysed in phylogenetic analyses. In this context the procedure's application to historic specimens collected over a century ago in Papua New Guinea is relevant. The historical mini-barcodes cluster with *Ripalania queenslandica* sequences from Australia indicating that this thiarid is actually not endemic to the continent. In the face of the increasing biodiversity crisis, the study of the biological diversity on all levels is becoming even more urgent. The possibility of obtaining sequence data from untapped genetic data within archived museum specimens opens up vast new reservoirs of information for future research.

ZUSAMMENFASSUNG

Da limnische Gastropoden sehr eng an ihre oftmals isolierten aquatischen Habitate gebunden sind, bewahren sie Verbreitungsmuster über lange Perioden. Sie stellen somit geeignete Modelle zur Rekonstruktion geographisch und klimatisch bedingter Veränderungen des Lebensraumes dar und können helfen, allgemeine Zusammenhänge zwischen Kolonisierungsereignissen und Artbildungsprozessen aufzuklären. Das Ziel dieser Arbeit besteht in der Rekonstruktion der Besiedlungsgeschichte des australischen Kontinents durch eine Familie viviparer Süßwassergastropoden (Thiaridae) unter Verwendung molekularer Marker (mitochondrialer und im Kerngenom basierter Genfragmente inklusive der AFLP-Technik). Die geographische Herkunft vieler Faunenelemente und die zeitliche Abfolge der Kolonisierung dieses seit dem Beginn des Tertiärs nordwärds wandernden Teilkontinents von Gondwana, das durch Tektonik und globaler Veränderungen u.a. Aridifizierung unterliegt, sind bisher nicht hinreichend geklärt. Viele der heute in Australien vorkommenden biotischen Elemente wurden üblicherweise als junge Einwanderer aus dem indo-malayischen Archipel eingestuft. Es werden jedoch mehr und mehr Fälle bekannt, die von diesem Standardszenario abweichen.

Um zu testen, ob es sich bei den Thiariden tatsächlich um junge Einwanderer aus dem Norden handelt, oder ob diese Familie ihren Ursprung auf dem australischen Kontinent hat, wurden die evolutionären Verwandtschaftsverhältnisse innerhalb der Familie, sowie ihre Position im phylogenetischen Stammbaum der Superfamilie Cerithioidea analysiert. Eine Voraussetzung für die Rekonstruktion vergangener Verbreitungsmuster ist, neben der detailierten Analyse der aktuellen Vorkommnisse, die Abgrenzung der einzelnen Thiariden-Arten. Zu diesem Zwecke wurden vorangegangene morphologische Artbestimmungen mit den molekularen Daten abgeglichen. Mit der Aufdeckung der stammesgeschichtlichen Beziehungen zu nicht-australischen Verwandten, durch Altersbestimmung der phylogenetischen Verzweigungen mittels “molekularer Uhr” sowie der Analyse der rezenten und historischen Areale, konnte ein asiatischer Ursprung der Thiariden widerlegt werden. Die zeitliche Abfolge der Besiedlungen im australisch-asiatischen Raum ist komplexer als bisher angenommen. Ein Abriss hinsichtlich Dispersion und/oder Vikarianz ist hier vor dem Hintergrund der unabhängig durch Geologen erfolgten Rekonstruktion plattentektonischer Ereignisse dargestellt. Demnach hat die Besiedlung Asiens ihren Ausgang ursprünglich im australischen Raum genommen und der Kontinent wurde nicht, wie bislang angenommen, von Asien aus besiedelt. Mit der Aufdeckung des ost-gondwanischen Ursprungs der Familie, repräsentieren australische Thiariden ein wichtiges und vielversprechendes Modellsystem in der Speziationsforschung, welches detailierte Einblicke in die Dynamik der grundlegenden Mechanismen der Artbildung unter dem Einfluss von klimatischen Veränderungen ermöglicht.

Auch wenn die phylogenetischen Beziehungen zwischen den einzelnen Thiariden Arten nicht völlig aufgelöst werden konnten, so haben die molekularen Daten und der Vergleich der ermittelten Phylogenien doch bedeutende Einblicke in diese rätselhaft erscheinende Gruppe ermöglicht. Es konnte dargelegt werden, dass mitochondriale DNA Daten mit äußerster Vorsicht interpretiert werden müssen und es hierbei den Abgleich mit nukleären Multi-Locus Phylogenien bedarf. Jedoch stimmten die mitochondrialen Daten mit den morphologisch beschriebenen Gruppen überein und konnten somit erfolgreich bei der Abgrenzung und Identifizierung der Arten eingesetzt werden. Die molekularen Ergebnisse stellen für australische Thiariden das Vorkommen von insgesamt elf differenzierbaren Linien fest. Es konnten im Rahmen dieser Arbeit neue Erkenntnisse bezüglich des Arteninventars in Australien, der Verbreitungsmuster und des Grades an Endemismus gewonnen werden. Es wurde sogar eine bis dato in Australien unbekannte Art - *Thiara rudis* - erstmalig dort nachgewiesen. Ausserdem deutet die AFLP-basierte Phylogenie darauf hin, dass sich die beiden endemischen Arten *"Thiara" australis* und *Plotiopsis balonnensis* erst kürzlich aufgespalten haben und es in einem neu entdeckten Überlappungsgebiet möglicherweise zu Hybridisierung kommt.

Zusätzlich wurde innerhalb dieser Arbeit ein Verfahren entwickelt, welches ermöglicht historisches Museumsmaterial in phylogenetische Analysen mit einzubinden. Es basiert auf ancientDNA-Techniken und beinhaltet die Amplifikation kurzer DNA Fragmente, sogenannter Mini-Barcodes. In diesem Kontext relevant ist die erfolgreiche Anwendung an Schalenmaterial, das vor über einem Jahrhundert in Papua-Neuguinea gesammelt wurde. Die amplifizierten Fragmente clustern mit *Ripalania queenslandica* Sequenzen aus Australien und belegen damit, dass diese Art nicht endemisch auf dem Kontinent ist. Angesichts der fortschreitenden globalen Biodiversitätskrise wird die Erfassung der Biodiversität auf jeder Ebene immer wichtiger. Die Möglichkeit Sequenzdaten von archiviertem Museumsmaterial zu generieren bietet hierbei eine Fülle an Informationen für zukünftige Forschung.

1 General Introduction

1.1 Biogeography

> "Biogeography, as a topic for discourse or discussion, is in some ways like religion: both topics lend themselves to ever more complicated treatment in the abstract, which is apt to border even on the miraculous, but which is apt to crumble in confrontation with concrete facts of life."
>
> Nelson and Platnick (1981: 375)

Biogeography is the study of the distribution and evolution of organisms through space and time (Ball, 1975). The discipline developed as humans tried to understand how life and earth have evolved together, accounting for current geographic distribution patterns in terms of past events. Already Darwin and Wallace were both very interested in biogeography. For Darwin the distribution of organisms provided evidence that evolution had occurred and Wallace described major patterns of zoogeography (a subdivision of the discipline) that are still valid today (Futuyma, 2005). He famously identified the biogeographic discontinuity between the Australian and Oriental faunas now known as Wallace's line, which will be subject also in this study.
The ideas and methods have changed dramatically over the years (Donoghue, 2013). Besides contemporary factors, the current geographic distribution of a taxon is affected by the three different processes of extinction, dispersal and vicariance. The influence of extinction, that is the death of all individuals in a local population, has been accepted without controversy. But this is not the case for the other two processes, which have been considered to be competing explanations for many years (Crisci et al., 2009). Vicariance refers to the separation of populations of a widespread species by a barrier arising from changes in geology, climate, or habitat (Futuyma, 2005). Here the barrier directly leads to the disjunction and, in the course of time, allows the isolated subpopulations to differentiate into distinct taxa. In contrast, in a dispersal event the barrier is older than the disjunction and the common ancestor occurred in one of the areas, dispersing later into another one. According to Yoder and Nowak (2006), we have reached a state of the art wherein the majority of biogeographers are equally receptive to hypotheses of vicariance and dispersal. The two traditional approaches are both important processes, whereby neither can be assumed to be the sole explanation of a taxon's distribution. In most cases, dispersal, vicariance, and extinction all have played a role in the histories of distributions.

Molecular data offers a plethora of possibilities in biogeography research and with present-day methods predictions about recent biota including distributional patterns, likelihoods of dispersal, and the shapes and timing of phylogenies can potentially be tested (Crisp

et al., 2011). DNA sequence data does not only help to determine the relationships among taxa, furthermore molecular phylogenetics can be used for dating divergences between lineages and for reconstructing distributional change through evolutionary time. These properties of molecular phylogenetics allow the evolutionary histories of co-distributed taxa to be compared spatially and temporally and they permit phylogenetic histories to be mapped on geological reconstructions (Lohman et al., 2011).
This study involves possible links between geographical patterns and processes of speciation with correlated morphological data in order to reconstruct the evolutionary history of a family of freshwater Cerithioideans - the thiarids. Due to their limited potential of dispersal in the face of wide distribution, ubiquity and high abundance, snails tend to preserve distribution patterns over long periods of time and are thus suitable organisms for studying historical biogeography.

1.2 Study species

The Cerithiodean Thiaridae, common name thiarids, is a family of snails distributed throughout the tropics and sub-tropics around the world, ranging from Central and South America, including a few Caribbean islands, to Africa and further into Southeast Asia and Australia, extending onto the western Pacific islands (Glaubrecht et al., 2009; Glaubrecht, 2011). Thiarids inhabit inland freshwaters as well as brackish-water environments at lower, tidally-influenced reaches of coastal rivers and streams. Members of the group play a variety of roles in aquatic ecosystems as primary herbivores, vectors of disease, invasive pests and sometimes substantial contributors to biomass (Kano et al., 2011). Thiarids are recognized by their often large (up to 10 cm), high-spired shells that can be smooth but may also bear grooves, nodules, ribs or spines. Their shells are simply coloured and usually appear dark brown or black due to the presence of an organic periostracum on the shells outer surface. Colour, as well as shell shape and ornamentation patterns often vary considerably within species. Like other groups of freshwater snails, this extensive intraspecific variation has produced widespread confusion concerning species recognition (Kano et al., 2011). As a consequence, it is unknown how many species of freshwater thiarids really exist currently, and there is little consensus in scientific literature regarding the correct names of those that have been documented.
Many species of freshwater snails that are characterized by a turreted shell were originally placed within the genus *Melania* Lamarck, 1799. The genus name *Melania* was demonstrated to be a junior synonym of the genus name *Thiara* Röding, 1798. Consequently, the family had to be renamed Thiaridae. This former Thiaridae sensu lato was found to be polyphyletic containing species from many different groups, which were successively recognized as distinct families, such as the Pachychilidae, Semisulcospiridae, Pleuroceridae, Melanopsidae, and Paludomidae (Glaubrecht, 1996). For a discussion of a more up-to-date concept of the freshwater Cerithioidea see reviews e.g. by Glaubrecht

(1996, 1999, 2006, 2011), supplemented by comparative morphological as well as molecular phylogenetic studies corroborating these earlier findings (Lydeard et al., 2002; Strong et al., 2011). Accordingly, the Thiaridae sensu stricto represents one of the two (or three) independent invasions and colonisations of freshwater habitats in the tropics worldwide.
The most characteristic feature of Thiaridae sensu stricto is that they are viviparous with a special structure - a so-called subhaemocoelic brood pouch which is located in the neck region of the head-foot. Instead of laying eggs they retain them in this special chamber. In some species shelled juveniles hatch (eu-viviparity) and in other species eggs within the pouch develop only into veliger stage before they are released into the water (ovo-viviparity). In case of the first modus all growth stages are present at the same time and a shell can comprise up to 5 or 7 whorls when hatching. The 'marsupial' family comprises several dozens, possibly up to c. 30 - 50 biological species, but many more named taxa with yet a largely unresolved taxonomy as already mentioned. An amazingly high taxonomic redundancy is suspected due to the typological approach of naming every phenotype, i.e. conchological disparity instead of true biological diversity (see Glaubrecht 1993, 1996).
The unresolved taxonomy situation confounds efforts to communicate effectively about these species and hinders efforts to understand global patterns of distribution and diversity. There is also little consensus in scientific literature regarding the reproductive biology. For example, for populations of *Melanoides tuberculata* in Israel Ben-Ami and Heller (2005) reported sexually as well as asexually reproducing individuals, thus contradicting the general assumption that indeed all thiarids reproduce via apomixis. It is said where males are present they have ripe gonads and motile sperm. But according to Dillon (2000) the thiarids have no external clue to their gender and this absence could indicate potential hermaphroditism. Much additional research is needed regarding sex determination. It remains to be seen in how far thiarids are actually prone to parthenogenesis.

1.3 Species biogeography - Why is Australia of special interest?

The Australian continent is one of the world's most ancient landmasses with a high number of endemic species. Due to its long isolation from other large continental masses and the well known geological history Australia provides perfect preconditions for biogeographical studies.
The Australian thiarids are distributed over nearly all parts of the continent, except the Tasmanian Island and the central arid part (Glaubrecht et al., 2009). The occurrences are particularly high in the northern-central region of the Leichhardtian zoogeographic region which is approximately represented by the Timor Sea and Gulf of Carpenteria drainage systems. The rainfall pattern in this region is dominated by the wet monsoon occurring within the period November to March (see fig.1). Most rivers here traverse a flat coastal plain about 15 km wide before reaching the sea. These rivers commonly possess wide

flood plains and low gradients, often contracting to a chain of waterholes during the dry season. Some rivers (Gregory River; Fitzroy to Daly Rivers) have reaches of rapids or very deep gorges (Kailola and Pierce, 1988).

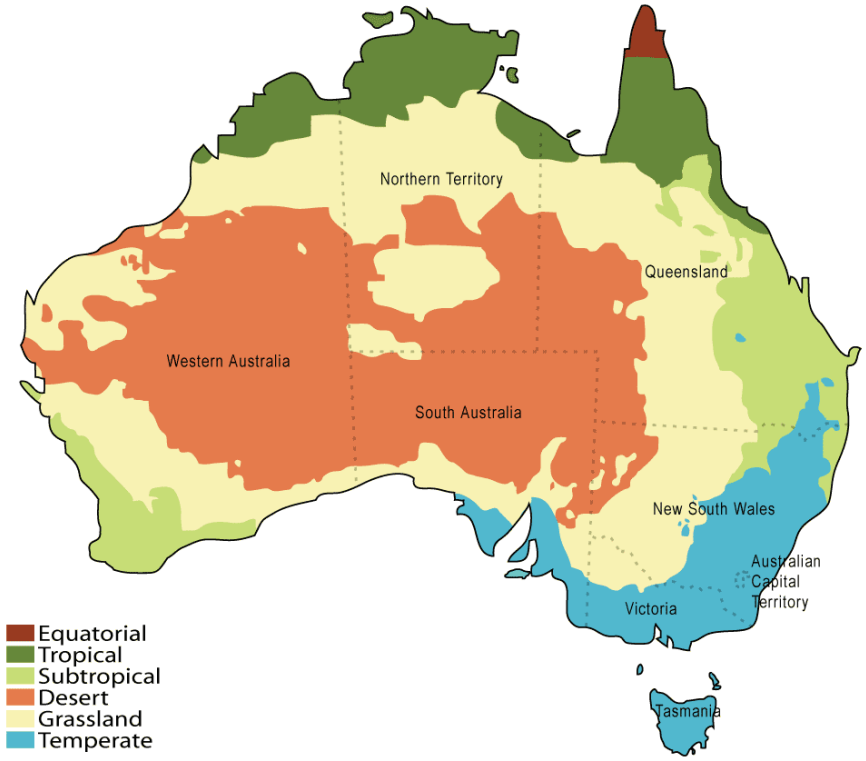

Figure 1 Australia climate map - The Australian Bureau of Meteorology climate classification, a modification of Köppen's classification by Martyman. Data from www.bom.gov.au. Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons.

Due to the size of the continent, Australia experiences a variety of climates. Beside these tropical regions of the north the continent provides five other climatic zones including even arid and semiarid desert regions of the interior as well as temperate regions in the south. (see fig.1). Note the specific adaptations needed to cope with special Australian environments and ecological circumstances, such as e.g. increasing aridity, unpredictable precipitation and ephemerality of freshwater bodies correlated with marked hydrological fluctuations of many rivers and streams and high salinities in temporarily standing waters. Glaubrecht et al. (2009) considered for Australian thiarids that parthenogenesis, in combination with viviparity, might be directly correlated and/or even causally linked with the survival of populations in or rapid (re-)colonizations of new habitats and areas right after or in the course of flooding, possibly being an adaptation to the monsoonal regime of northern Australia.

1.4 Previous work

To reveal the species that are represented in Australia Glaubrecht and colleagues made a systematic evaluation of the Australian thiarids in 2009. In snails the radula is often used for the delimitation of species, but in thiarids, unfortunately, it is of limited use in the distinction because it is relatively constant between thiarid species. No major dentition feature that allows for specific distinction could be found for the Australian taxa. Apparently, all taxa seem to use their radula in the same fashion, as all of them are detritus and algae feeders, mostly occurring on soft substrate with high sandy to muddy components (Glaubrecht et al., 2009). Nevertheless it was suggested at that time to differentiate among the Australian Thiaridae 8 genera with a total of 11 species distinguishable essentially on the basis of their shell morphology. While five species are widely distributed also outside the Australian continent, viz. *Thiara amarula*, *Stenomelania* cf. *aspirans*, *Melanoides tuberculata*, *Pseudoplotia* (=*Plotia*) *scabra* and *Sermyla riqueti*, the other six species were considered to be endemic to Australia with either a wide range over the continent, viz. *"Thiara" australis*, *Plotiopsis balonnensis* and *"Stenomelania" denisoniensis*, or highly restricted ranges to a few drainage divisions, viz. *Ripalania queenslandica*, *Melasma onca* and *Sermyla venustula* (Glaubrecht et al., 2009). In case of *"Thiara" australis* and *"Stenomelania" denisoniensis* the generic allocations are used in quotation marks in order to denote that the phylogenetic placement is doubtful.

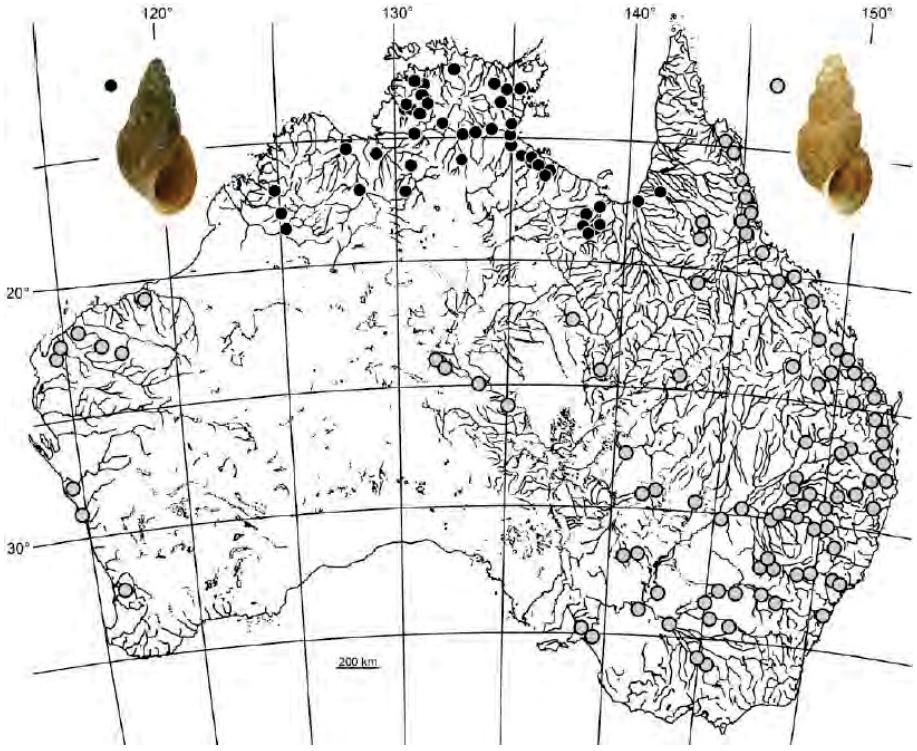

Figure 2 Australia map with occurrences of *"Thiara" australis* (black dots) and *Plotiopsis balonnensis* (grey dots). The two species seem to be distributed mutually exclusively (status of 2009).

Another important finding of the study of 2009 which will be subject in this thesis, is the striking occurrence of the two endemic species *"Thiara" australis* and *Plotiopsis balonnensis* on the Australian continent (see fig.2). The two species seem to have mutually exclusive distributions and are morphologically hard to distinguish. If this observed disjunct distribution represents a fragmentation of a widespread ancestral species it would be expected that the two species turn out to have a sister group relationship.

1.5 Goals and structure of the present thesis

The previously described results of the systematic evaluation in 2009 by Glaubrecht and colleagues are the starting point of this study. By combination of different genetic approaches and augmented sampling the goal is to illuminate the colonization history of the Australian continent and to get a better insight into contemporary relationships between Australian thiarids. This thesis is divided into seven chapters, whereby chapters 3 - 6 represent individual studies each containing a separate introduction, a description of materials and methods, a summary of gained results and a detailed discussion. The specific goals of these chapters are as follows:

Chapter 3 The first aim of this individual study is to examine the molecular phylogeny of the Thiaridae using a large sampling of thiarid taxa including representatives from across the known range aiming for the highest coverage possible and a combination of four genes (COI, 16S rRNA, 28S rRNA, H3). The second aim is to develop a historical biogeographic hypothesis by integrating the fossil record and using it for calibration in a molecular clock approach. It is hypothesized that the long-held view of the thiarid fauna being an appendage to the southeast Asian biota is false. Instead, an Australian continental, i.e. Gondwanian origin is propounded.

Chapter 4 This chapter focusses on the Australian Thiarids and their geographic distribution on the continent. The comprehensive investigations in the work of 2009 to characterize individual taxa are complemented with molecular analyses and additional sampling with the objective of confirmation of the differentiated species and analysis of present distribution patterns.

Chapter 5 Here a technique is shown that allows extracting and analysing DNA from historical material stored up to over a hundred years ago using museum specimens from the Malacological Collection at the NHM Berlin. In five case studies, where identification is needed to clarify specific biogeographical and systematical questions, the effectiveness of this technique is shown. One of these case studies rejects the long-held view that the Thiarid *R. queenslandica* is endemic in Australia.

Chapter 6 In this study the Amplified fragment length polymorphism (AFLP) technique is used with the initial goal to assess genetic variation and population structuring with regard to the different river drainage systems. As additional nuclear data to the sequencing results, which were partly inconsistent, the AFLP data helps to clarify the relationships among closely related species of thiarid taxa with a focus on the two endemic species *"Thiara" australis* and *P. balonnensis*.

Subsequent to the introductory first chapter and before the individual studies the description of the 'General material and methods' is given in **chapter 2**. At the end, in **chapter 7**, a summary of the results and a general discussion of the present thesis including a short outlook is provided.

2 General material and methods

2.1 Sampling

2.1.1 Sampling sites

This study is based on specimens preserved in ethanol, collected during several expeditions in the last twelve years mainly by Matthias and Nora Glaubrecht (née Brinkmann), partly with the help of Thomas von Rintelen, offering a great sample that covers a big part of Australia by more than 1000 records. A list of all sampling localities sorted according to sampling year is attached in the appendix (see p.135). Additional samples were collected in particular during an Arnhem Land expedition by Winston Ponder and Vince Kessner in 2007. Supplementary non-Australian material (mainly from SE Asia) was provided within the framework of current cooperations.

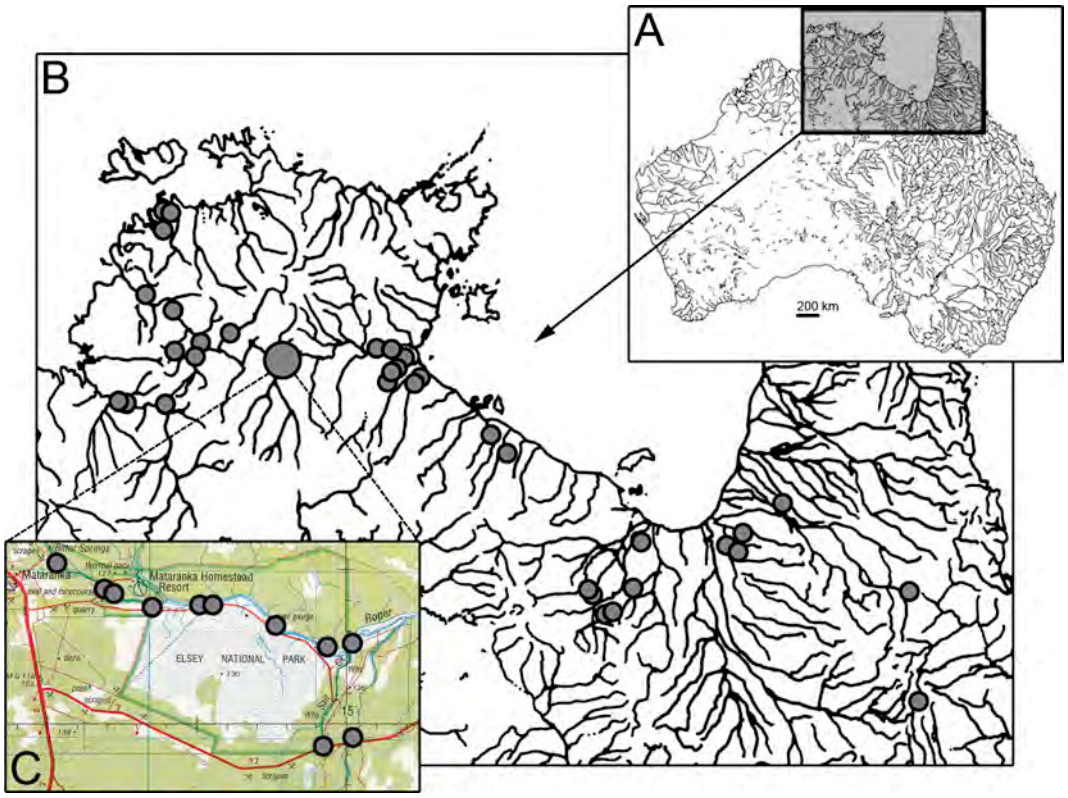

Figure 3 Sampling locations of the collection trip in September and October 2011 in the northern parts of Australia. **A** The continent of Australia. **B** Map extract of the northern part of Australia (Northern Territory and Queensland). **C** Map section of the Roper River with closely adjoining sampling locations (map of Australian Government MAP Katherine SD53-09; Larrimah SD53-13).

In 2011 M. Glaubrecht, N. Glaubrecht, N. Maaß and the author went on an additional field trip in order to collect fresh material for this study. The research group started the tour in Darwin, Northern Territory, did 8176 km through the outback and collected at 46 locations (see fig.3). Google Earth® was used to discover creeks near drivable streets

as locations could mostly only be reached by four wheel drive car. On the Limmen Bight and Towns River accessibility was obtained by boat. Subsequent to the trip covering the North of Australia the author went alone to Western Australia in October 2011 to get samples of *P. balonnensis*. The fresh material was used for molecular analyses especially for the new comprehensive AFLP approach.

2.1.2 Sampling methods

The snails were sampled on mud, rocks or on submerged trees and branches. On each location specimens were placed into plastic whorl bags with water from the sample site to keep them alive for the rest of the day. Each of those bags was labelled with date, location number and GPS data. Every evening the snails from the different locations were sorted and labelled with a preliminary species name. Living snails were fixed in 96% ethanol in the field, with most of the shells cracked the same day to allow the preservative to penetrate the soft bodies. Ethanol was changed once or twice in the field. Samples were finally transported to and shipped by the Museum & Art Gallery of the Northern Territory in Darwin. All voucher material is deposited in the Malacological Department, Museum of Natural History, Berlin (accession numbers with prefix 'ZMB'). A general procedure of sampling treatment is shown in fig. 4.

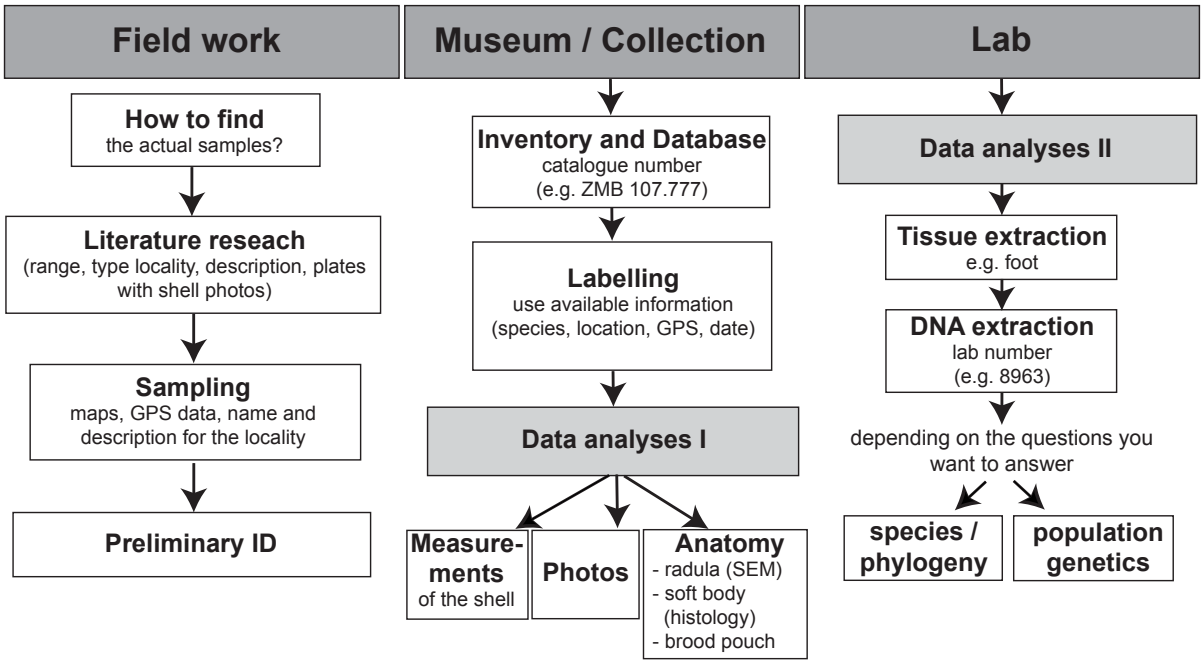

Figure 4 The general procedure of sampling treatment in the Malacological Department, Museum of Natural History, Berlin (accession numbers with prefix 'ZMB').

2.2 Genetic methods

2.2.1 DNA isolation, PCR and sequencing

DNA isolation Total DNA was extracted by application of a modified version of the CTAB extraction protocol for molluscan tissues (Winnepenninckx et al., 1993). About 2 mm^3 of foot muscle (taken from samples preserved in 75-90% ethanol) was dried and cut into small pieces. Tissue pieces were lysed in 300 μl CTAB buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM tris-HCl pH 8.0), 0.6 μl b-mercaptoethanol and 10 μl serine proteinase K and incubated at 55°C and 550 rpm in an Eppendorf® Thermomixer compact overnight. On the next day 300 μl chloroform:isoamyl alcohol (24:1) were added to the lysate, mixed manually for two minutes and centrifuged for 10 min at 15000 rpm in an Eppendorf® centrifuge 5424. Afterwards the top layer was extracted into a new set of tubes. This washing step was repeated (add 300 μl phenol-chloroform, mixing, 10 min centrifuge at 15.000 rpm) and the top layer of the supernatant was placed into tubes preliminarily filled with 600 μl of 95 % ethanol and 25 μl of 3M sodium acetate. The mixture was stored overnight in a freezer at -20°C. On the third day samples were first centrifuged for 10 min at 15.000 rpm building a pellet visible at the bottom of the tube. Ethanol (95 %) was rejected and afterwards 75 % ethanol was added and tubes were shaken vigorously to release the pellet from the bottom. Tubes were then centrifuged 5 min at 15.000 rpm and ethanol was removed. Once the pellet was dried it was dissolved in 30 μl of TE buffer 0.1. The isolated genomic DNA was stored at -20°C.

DNA sequencing DNA sequencing was carried out using the dideoxy method (Sanger et al., 1977). Polymerase chain reaction (PCR) (Saiki, 1985; Mullis, 1986) was used to amplify two mitochondrial gene fragments, a $\sim$ 850 bp region of the 16S ribosomal RNA gene (16S) and a 660 bp fragment of the Cytochrome Oxidase subunit I gene (COI). The 16s rRNA gene was amplified by using the primers 16S_F_Thia2 [5'- CTT YCG CAC TGA TGA TAG CTA G -3' (von Rintelen, unpublished data)] and H3059var [5'- CCG GTY TGA ACT CAG ATC ATG T -3' (Wilson et al., 2004)]. The mitochondrial gene fragment of COI was sequenced using LCO-1490 [5'- GGT CAA CAA ATC ATA AAG ATA TTG G -3' (Folmer et al., 1994)] and HCOvar [5'- TAW ACT TCT GGG TGK CCA AAR AAT -3' (von Rintelen et al., 2004)] as primers. After an initial denaturation step of 3 min at 94 °C, thermal cycling conditions were as follows: 35 cycles (30 sec at 94°C, 45°C (COI) or 50 °C (16S) annealing for 1 min and 72°C extension for 1:30 min) followed by a final extension step at 72°C for 5 min. In cases where this procedure did not lead to sufficient DNA concentration PCR was repeated using the QIAGEN® Multiplex PCR Kit. The used mastermix and PCR profiles for the multiplex approach are attached in the appendix (see tab.24 and tab.25).
For the molecular phylogeny two nuclear gene fragments, 328 bp of H3 and $\sim$ 1070 bp of 28S were amplified using the following primers: 28S Fmod: [5'- ACC CGC TGA ATT

TAA GCA TAT -3' modified from (Van der Auwera et al., 1994)], 28S Rmod: [5'- GCT ATC CTG ACG GAA ACT TC -3' (von Rintelen, unpublished data)], H3 F: [5'- ATG GCT CGT ACC AAG CAG ACV GC -3'] and H3 R: [5'- ATA TCC TTR GGC ATR ATR GTG AC -3' (Colgan et al., 2000)]. For 28S a touchdown PCR was performed with conditions: 94 °C for 3 min, 7 cycles of touchdown PCR (94 °C for 0:30 min, 60-53 °C annealing for 1 min and 72 °C extension for 2 min) followed by 33 cycles (94 °C for 0:30 min, 52 °C annealing for 1 min and 72 °C extension for 2 min) and a final extension step at 72 °C for 8 min. Cycling conditions for the H3 gene were: 94 °C for 3 min, 35 cycles (30 sec at 94°C, 50 °C (16S) annealing for 1 min and 72°C extension for 1:00 min) followed by a final extension step at 72°C for 5 min.
Mastermix was the same for all four genes and is given in the appendix (see tab.23). In each PCR a negative control reaction in which DNA was omitted was included in order to verify the absence of contamination. Success of PCR amplification was controlled by agarose gel electrophoresis.
PCR products were purified using NucleoSpin Extract II Kits (Macherey-Nagel) or ExoSap-IT® (US Biochemicals) following the manufacturers protocols. The same primers were used in PCR and sequencing where gene products were sequenced in both directions. Cycle sequencing reactions were carried out on an ABI 3130xl Genetic Analyser automated DNA sequencer (Applied Biosystems) and accomplished by the company SMB GmbH (Services in Molecular Biology, Berlin).

2.3 Analyses of molecular data

2.3.1 Sequence assembly and alignment

The assemblies of forward and reverse strands were done using CodonCode Aligner (Version 3.7.1; CodonCode Corperation, Dedham, MA, USA) and the correction was done by eye. Fasta files were exported in BioEdit (Hall, 1999) which was used to edit the alignments. For multiple sequence alignments of 16S and 28S sequences the programs MAFFT (Katoh and Toh, 2008) and MUSCLE (Edgar, 2004b,a) were used online by Web Services of the European Bioinformatics Institute (Part of the European Molecular Biology Laboratory, http://www.ebi.ac.uk) (Goujon et al., 2010; McWilliam et al., 2013). COI and H3 sequences were aligned with ClustalW (Thompson et al., 2002) using default parameter settings as implemented in BioEdit.

2.3.2 Phylogenetic analyses

Preparation and tests All data sets were reduced to unique haplotypes using DAMBE 5.3.74 (Xia, 2013) and tested for homogeneity of base frequencies across taxa using PAUP 4.0b10n (Swofford, 2002). Potential saturation in the protein coding genes H3 and COI was assessed by plotting transitions and transversions for each codon position against

genetic distances (F84) and by the test of Xia et al. (2003). Both tests were conducted in Dambe 5.3.74 (Xia, 2013) and both showed that the sequences did not experience severe substitution saturation.

Modeltest The most appropriate model of DNA substitution for each gene fragment was determined by evaluating the corresponding likelihood scores, under the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) using jModeltest0.1 (Posada, 2008). The chosen model was used in the subsequent phylogenetic searches.

Maximum likelihood (ML) NCLconverter (Lewis and Holder, 2008) was used to convert nexus files into phylip format being appropriate for use with RAxML (randomized accelerated maximum likelihood), a program for maximum likelihood-based inference of large phylogenetic trees (Stamatakis, 2006). RAxML analyses were performed under the appropriate model including calculations of bootstrap support values (Felsenstein, 1985). NCLconverter and RAxML BlackBox (Stamatakis et al., 2008) were used on the CIPRES Science Gateway (Miller et al., 2011).

Bayesian inference (BI) The Bayesian Markov chain Monte Carlo simulation was run using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) on the CIPRES web portal (Miller et al., 2011) for 10.000.000 generations based on the previously selected substitution model. In two parallel runs trees were sampled every 200th generation. Excluding the first 35.001 trees of each run as burn-in, a 50% majority-rule consensus tree with posterior probabilities was constructed from the remaining trees.

Maximum Parsimony (MP) Phylogenetic trees were reconstructed by maximum parsimony (MP) using the heuristic search algorithm as implemented in PAUP 4.0b10n (Swofford, 2002), with gaps treated as fifth base. Support for nodes was estimated by bootstrap analysis (10000 replicates).

These three different tree-building methods were examined for each alignment to compare and test the robustness of the results. The final trees were edited using FigTree version 1.3.1 (Rambaut, 2009). For a more concrete description please see the corresponding 'Specific Material and Methods' paragraph of the appropriate chapter.

3 Phylogenetic relationships of Australian thiarids and their biogeographical origin

3.1 Specific introduction

Australia has an outstanding peculiar fauna and flora, sharply distinct in a multitude of ways from that in other parts of the world. The 'fifth continent' represents one of the world's most ancient landmasses and harbours one of the richest biota which stands out due to a high number of endemisms. This high level of endemism is probably a result of Australia's long period of isolation from other continents, since its separation from Gondwana about 45 million years ago (Lohman et al., 2011).
However, many biotic elements found today in Australia were conventionally considered as being recent invaders from the north, as it was expressed by traditional pre-continental drift views e.g. for birds (Mayr and Stein, 1944), amphibians (Darlington Jr, 1965) or other faunal members in particular of inland waters (Williams and Allen, 1987). According to these views, the standard scenario was that this long isolated continent must certainly have been colonized from abroad, in particular by invaders from the Indo-Malay archipelago, once Australia had been within reach from the Oriental region (Glaubrecht et al., 2009). The place of origin of its fauna and the route of entry into Australia has been much discussed and more and more cases are known that deviate from this standard scenario. According to Köhler and Criscione (2013), the biota of the Indo-Australian archipelago is predominantly Southeast Asian in origin, with a comparatively small proportion of taxa of Australian ancestry. In their study they provide the first evidence that for the camaenid land snail lineage *Rhagada* Australia is the area of origin, harbouring ancient Gondwanian lineages that dispersed into Asia. Another example is that of passerine birds as already discussed by Glaubrecht et al. (2009) in the same context. Today, it is well established that oscine passerine birds originated in East Gondwana in the Oligocene and from there spread to Southeast Asia (Jønsson and Fjeldså, 2006; Jønsson et al., 2011; Aggerbeck et al., 2014).

The origin of Australian freshwater faunal elements and the directionality and timing of colonizations are still controversial. In case of the Thiaridae, it has never been tested whether they are recent invaders from the north, or if they originated on the Australian continent in ancient times. The thiarids are limnic members of the Cerithioidea, a large gastropod group with a worldwide distribution which has been successfully employed in testing biogeographical hypotheses (Glaubrecht, 2000; Glaubrecht and von Rintelen, 2008; Glaubrecht, 2009). However, the monophyly and biogeographical origin of the Australian species has remained uncertain. McMichael and Weatherley (1967) commented on the evolutionary relationships of the family, stating that "they must be regarded as relatively recent arrivals in Australia from the north." The same recent origin had been

assumed earlier for unionid bivalves, confirming the traditional biogeographical view of the freshwater fauna of Australia.
Looking at the current distribution, thiarids do occur on southern continents like Africa, Asia and Australia, but are missing in America and Europe (see tab.1). The simplest hypothesis for taxa that have members on different landmasses in the Southern hemisphere is vicariance: the breakup of Gondwana isolated descendants of a common ancestor.

Table 1 The biogeography of freshwater Cerithioidea taxa, listed by family, on a global scale. The six families, with their constituent taxa, as currently conceived, are given here as delimited in Glaubrecht (2011).

region	**America/Caribbean**	**Europe/Palaeartic**	**Africa**	**Asia**	**Australia/NZ**
family	Pachychilidae		Pachychilidae	Pachychilidae	Pachychilidae
	Pleuroceridae				
	Semisulcospiridae			Semisulcospiridae	
		Melanopsidae			Melanopsidae
			Paludomidae	Paludomidae	
			Thiaridae	Thiaridae	Thiaridae
	Hemisinidae*		Hemisinidae		

*only on Caribbean islands and mainland South America. The genus was attributed to Thiaridae before but elevated to an own family, Hemisinidae Fischer & Crosse, 1891 (Bouchet and Rocroi, 2005).

The pattern of a "Gondwanian group" in case of the Thiaridae is also corroborated by modern molecular studies that shed light on phylogenetic relationships wihin the superfamily Cerithioidea (Strong et al., 2011). Here the Thiaridae are monophyletic in almost all analyses, with the genus *Hemisinus* from South America consistently emerging at the base. This sister group relationship between neotropical Hemisinidae & oriental Thiaridae is supported in the morphological data set as well as in the molecular approach and shows a branching pattern that would be expected in case it resulted from the Gondwanian breakup.
The findings of Hamilton-Bruce et al. (2004) are another indication for this scenario. They described a thiarid-like fossil from Early Cretaceous non-marine deposits in northern New South Wales, Australia. The fossil was attributed to the extant genus *Melanoides* and represents the oldest Australian record of the genus and the family. This gastropod fossil which appears quite similar to recent thiarids, suggests the occurrence of Thiaridae in Australia at a time when the continent was far away from what today is Asia, but still closely connected to the Antarctic section of Gondwana (146 Ma to 100 Ma). In addition, Beu et al. (2014) found an uncommon thairid fossil from New Zealand, that lived before the island began to separate from the rest of Gondwana (85 Ma). They positioned the fossil in *Melanoides* (sensu lato), but considered that South American genera of the Hemisininae are possibly related. Based on the age and composition of the probed fauna Beu et al. (2014) suggest that the living freshwater molluscan fauna of the southern landmasses are remnants and evolutionary descendants of a formerly Gondwanian fauna that included Thiaridae.

According to these indications, it is hypothesized here that the long hold view of the thiarid fauna being an appendage to the southeast Asian biota is false. Instead, an Australian continental, i.e. Gondwanian origin of these endemic Thiaridae is propounded as has been discussed earlier by Glaubrecht (1996) and Glaubrecht et al. (2009).

The first aim of this chapter is to examine the molecular phylogeny of the Thiaridae using a large sampling of thiarid taxa , a combination of four genes (COI, 16S rRNA, 28S rRNA, H3) and a range of appropriate Cerithioidean outgroups. The resulting trees represent the most detailed phylogenetic analysis of the thiarids to date and facilitate testing the monophyly of the family and the sister group relationship to the Hemisinidae and the relationships to other cerithioidean freshwater families. The second aim is to integrate the fossil record and geographical distributions, to develop a historical biogeographic hypothesis. The phylogenetic relationships from throughout the Indo-Australian Archipelago are used for a more detailed understanding of the timing and directionality of dipersal events between Asia and Australasia.

3.2 Specific material and methods

3.2.1 Phylogenetic analyses

The working hypotheses i.e. thiarids originated on the Australian continent was tested by phylogenetic analysis of mitochondrial and nuclear genes: 16S rRNA and cytochrome oxidase I as well as histone H3 subunit and 28S rRNA. DNA sequence data was collected for all taxa across the known range aiming for the highest coverage possible. In total 523 16S sequences were obtained, in which 255 are from Australian taxa, 241 from non-Australian taxa and 27 from Cerithioidean outgroups. For the COI gene 430 sequences were analysed (155 Australian, 247 non-Australian and 28 outgroups), for 28S 113 sequences (36 Australian, 101 non-Australian and 12 outgroups) and for H3 210 sequences (57 Australian, 180 non-Australian and 20 outgroups). The alignments were subsequently reduced to 81 nuclear and 83 mitochondrial sequences (see tab. 2) through the elimination of identical or very similar haplotypes of the same species to make it computationally feasible for the phylogenetic analyses. The exploratory phylogenetic reconstructions for the mt-data sets are presented in chapter 4 (see fig. 13) and in the appendix (see fig. 57). Phylogenies were produced both from individual genes and from concatenated sequences (mt and nDNA respectively). The data were analyzed with Bayesian inference (BI), maximum likelihood (ML), maximum parsimony (MP). For details of the conducted analysis see the chapter 'General Material and Methods'. Sequences of *Cerithium eburneum* (Cerithiidae) were chosen as outgroup to root the phylogenies based on the results of Strong et al. (2011).

Table 2 Taxa included in the phylogenetic analyses with sampling locality and sequence information. SEQ: sequenced (bold indicates that extraction/sequencing was conducted by the author); x: no data. For other abbrevations see appendix.

Taxa	Museum-Id	Locality		Lab-Id	28S	H3	COI	16S
Cerithiidae								
Cerithium eburneum	ZMB106323	USA	Florida	1946	SEQ	SEQ	SEQ	SEQ
Pleuroceridae								
Elimia catenaria	ZMB106412	USA	North Carolina	2630	SEQ	**SEQ**	**SEQ**	SEQ
Paludomidae								
Paludomus petrosus	ZMB107881	THA	SW of Phetchaburi	**7336**	**SEQ**	SEQ	**SEQ**	**SEQ**
Paludomus siamensis	ZMB107721	THA	Sai Yok Yai NP	**7334**	SEQ	SEQ	**SEQ**	**SEQ**
Paludomus siamensis	ZMB107726	THA	SE Thong Pha Phum	7196	SEQ	**SEQ**	**SEQ**	**SEQ**
Paludomus siamensis	ZMB107910	THA	SE of Lampang	**7338**	SEQ	SEQ	**SEQ**	**SEQ**
Hemisinidae								
Cubaedomus brevis	ZMB107174	CUB	Pinar del Rio	3493	**SEQ**	SEQ	SEQ	SEQ
Hemisinus spec.	ZMB107126	JAM	Middlesex	2849	SEQ	SEQ	**SEQ**	SEQ
Hemisinus spec.	ZMB113128	COL	Chocó	2999	**SEQ**	SEQ	**SEQ**	SEQ
Pachymelania fusca	ZMB191443	NIG	no detail	2507	**SEQ**	SEQ	SEQ	SEQ
Thiaridae								
Balanocochlis glans	ZMB107366	IDN	Sulawesi	6493	**SEQ**	SEQ	SEQ	**SEQ**
Balanocochlis glans	ZMB191147	IDN	Sulawesi	1806	SEQ	**SEQ**	SEQ	SEQ
Fijidoma maculata	ZMB106379	FIJ	Viti Levu	508	**SEQ**	SEQ	SEQ	SEQ
Melanoides spec.	ZMB107717	IDN	Sumatra	**7346**	**SEQ**	**SEQ**	**SEQ**	**SEQ**
Melanoides spec.	ZMB113598	IDN	Sumatra	**7347**	**SEQ**	**SEQ**	**SEQ**	**SEQ**
Melanoides spec.	ZMB190964	IDN	Sulawesi	2937	SEQ	SEQ	**SEQ**	**SEQ**
Melanoides tuberculata	SUT0210030	THA	Prachuabkirikhan	**8051**	**SEQ**	**SEQ**	**SEQ**	**SEQ**
Melanoides tuberculata	ZMB106726	AUS	Northern Territory	4129	**SEQ**	**SEQ**	**SEQ**	SEQ
Melanoides tuberculata	ZMB107128	JAM	Rio Negro	2857	SEQ	SEQ	x	x
Melanoides tuberculata	ZMB107125	JAM	Trelawny	2860	SEQ	SEQ	**SEQ**	**SEQ**
Melanoides tuberculata	ZMB107129	JAM	Westmorland	2855	**SEQ**	SEQ	**SEQ**	**SEQ**
Melanoides tuberculata	ZMB107193	MAL	Lake Malawi	5134	**SEQ**	**SEQ**	**SEQ**	**SEQ**
Melanoides tuberculata	ZMB127019	MAD	Ihosy	**7867**	x	x	**SEQ**	**SEQ**
Melanoides tuberculata	ZMB200313	IND	Tamil Nadu	2820	SEQ	SEQ	**SEQ**	**SEQ**
Melasma onca	ZMB106636	AUS	Northern Territory	1781	SEQ	SEQ	**SEQ**	SEQ
Neoradina spec.	ZMB107867	THA	W of Ko Nang	**7528**	**SEQ**	SEQ	**SEQ**	**SEQ**
Plotiopsis balonnensis	ZMB106583	AUS	West Australia	1512	SEQ	SEQ	**SEQ**	**SEQ**
Plotiopsis balonnensis	ZMB106686	AUS	Northern Territory	1827	**SEQ**	SEQ	**SEQ**	SEQ
Plotiopsis balonnensis	ZMB106728	AUS	West Australia	2815	SEQ	SEQ	**SEQ**	SEQ
Pseudoplotia acanthica	ZMB191487	IDN	Sulawesi	2885	SEQ	SEQ	**SEQ**	SEQ
Pseudoplotia scabra	ZMB106425	IDN	West Java	1096	SEQ	SEQ	**SEQ**	SEQ
Pseudoplotia scabra	ZMB106679	AUS	Northern Territory	1832	SEQ	SEQ	**SEQ**	SEQ
Pseudoplotia scabra	ZMB107216	AUS	Northern Territory	4781	**SEQ**	SEQ	**SEQ**	SEQ
Pseudoplotia scabra	ZMB107392	IDN	Ambon	6511	**SEQ**	SEQ	SEQ	SEQ
Pseudoplotia scabra	ZMB114990	LAO	Champasak Prov.	**7535**	**SEQ**	SEQ	**SEQ**	**SEQ**
Pseudoplotia scabra	ZMB191498	IDN	Sulawesi	2891	SEQ	SEQ	**SEQ**	SEQ
Ripalania queenslandica	ZMB107214	AUS	Queensland	**7662**	**SEQ**	SEQ	**SEQ**	**SEQ**
Sermyla riquetii	ZMB107883	THA	Puek Tian Beach	**7354**	**SEQ**	**SEQ**	**SEQ**	**SEQ**
Sermyla riquetii	ZMB191388	IDN	Sulawesi	3052	SEQ	SEQ	**SEQ**	SEQ
Sermyla venustula	WAM10048	AUS	West Australia	**7664**	**SEQ**	SEQ	**SEQ**	**SEQ**
Sermyla venustula	ZMB106713	AUS	Queensland	2859	SEQ	SEQ	**SEQ**	SEQ
Sermyla venustula	ZMB192019	AUS	Northern Territory	3799	**SEQ**	SEQ	**SEQ**	SEQ
Stenomelania aspirans	ZMB106391	FIJ	Viti Levu	1448	SEQ	SEQ	**SEQ**	SEQ
Stenomelania aspirans	ZMB107586	AUS	Queensland	**7555**	**SEQ**	**SEQ**	**SEQ**	**SEQ**

Taxa	Museum-Id	Locality		Lab-Id	28S	H3	COI	16S
Stenomelania aspirans	ZMB191208	IDN	Sulawesi	2223	SEQ	SEQ	SEQ	SEQ
Stenomelania aspirans	ZMB191210	IDN	Sulawesi	2175	SEQ	SEQ	**SEQ**	SEQ
Stenomelania aspirans	ZMB191212	IDN	Bali	2176	SEQ	SEQ	**SEQ**	SEQ
Stenomelania denisoniensis	AMS461354	AUS	Northern Territory	6096	**SEQ**	SEQ	**SEQ**	**SEQ**
Stenomelania denisoniensis	ZMB106586	AUS	West Australia	1516	SEQ	SEQ	**SEQ**	**SEQ**
Stenomelania denisoniensis	ZMB107239	AUS	Northern Territory	4897	**SEQ**	SEQ	**SEQ**	SEQ
Stenomelania denisoniensis	ZMB107449	IDN	Ambon	6507	**SEQ**	SEQ	SEQ	SEQ
Stenomelania denisoniensis	ZMB127607	IDN	Timor	**8176**	x	x	**SEQ**	**SEQ**
Stenomelania spec.	ZMB107457	IDN	Seram	6539	**SEQ**	SEQ	SEQ	SEQ
Stenomelania spec.	ZMB107483	IDN	Ambon	6508	**SEQ**	SEQ	SEQ	SEQ
Tarebia granifera	ZMB107396	IDN	Ambon	6521	**SEQ**	SEQ	SEQ	SEQ
Tarebia granifera	ZMB107533	IDN	Flores	6917	**SEQ**	SEQ	**SEQ**	SEQ
Tarebia granifera	ZMB114168	VIE	Lao Cai	6534	x	x	SEQ	**SEQ**
Tarebia granifera	ZMB127609	IDN	Timor	**8180**	**SEQ**	**SEQ**	**SEQ**	**SEQ**
Tarebia granifera	ZMB190883	IDN	Sulawesi	3018	**SEQ**	SEQ	**SEQ**	SEQ
Tarebia granifera	ZMB191454	IDN	Halmahera	6523	**SEQ**	SEQ	SEQ	SEQ
Tarebia granifera	ZMB191458	IDN	Sulawesi	2866	SEQ	SEQ	SEQ	SEQ
Tarebia lineata	ZMB106518	IDN	Bali	1470	**SEQ**	SEQ	**SEQ**	**SEQ**
Tarebia lineata	ZMB191465	IDN	Java	2869	x	x	**SEQ**	**SEQ**
Tarebia lineata	ZMB200325	IND	Karnataka	1469	SEQ	**SEQ**	**SEQ**	SEQ
Thiara amarula	ZMB106354	AUS	Queensland	2870	**SEQ**	SEQ	**SEQ**	SEQ
Thiara amarula	ZMB107220	AUS	Queensland	4785	**SEQ**	SEQ	**SEQ**	SEQ
Thiara amarula	ZMB191489	IDN	Obi	2886	SEQ	**SEQ**	**SEQ**	SEQ
Thiara australis	AMS427964	AUS	West Australia	3076	**SEQ**	SEQ	**SEQ**	SEQ
Thiara australis	ZMB106698	AUS	Northern Territory	1836	SEQ	SEQ	SEQ	SEQ
Thiara australis	ZMB106706	AUS	Queensland	1845	SEQ	SEQ	**SEQ**	SEQ
Thiara australis	ZMB106709	AUS	Northern Territory	1866	SEQ	SEQ	**SEQ**	SEQ
Thiara australis	ZMB107286	AUS	Northern Territory	4878	**SEQ**	SEQ	**SEQ**	**SEQ**
Thiara australis	ZMB107290	AUS	Northern Territory	4916	**SEQ**	SEQ	**SEQ**	SEQ
Thiara australis	ZMB107579	AUS	Queensland	**7990**	**SEQ**	**SEQ**	**SEQ**	**SEQ**
Thiara cancellata	ZMB107489	IDN	Sulawesi	6498	**SEQ**	SEQ	SEQ	SEQ
Thiara cancellata	ZMB191431	IDN	Obi	2817	**SEQ**	SEQ	**SEQ**	SEQ
Thiara mirifica	ZMB191270	IDN	Sulawesi	2881	SEQ	SEQ	**SEQ**	SEQ
Thiara mirifica	ZMB191429	IDN	Obi	2883	SEQ	SEQ	x	x
Thiara rudis	ZMB107280	AUS	Queensland	4867	**SEQ**	SEQ	**SEQ**	SEQ
Thiara rudis	ZMB107377	IDN	Sulawesi	6494	**SEQ**	SEQ	SEQ	SEQ
Thiara rudis	ZMB106472	IDN	Bali	1001	SEQ	SEQ	**SEQ**	SEQ
Thiara rudis	ZMB106704	AUS	Northern Territory	2811	**SEQ**	SEQ	**SEQ**	**SEQ**
Thiara rudis	ZMB191262	IDN	Sulawesi	4561	**SEQ**	SEQ	**SEQ**	SEQ
Thiara rudis	ZMB191279	IDN	Bali	4559	SEQ	SEQ	**SEQ**	SEQ
Thiara winteri	ZMB106554	IDN	Bali	1043	SEQ	SEQ	SEQ	SEQ

3.2.2 Divergence time estimates

Although the Thiaridae have documented fossil records, their interpretation is hampered by the lack of consistent diagnoses of the family itself. Many species of freshwater snails that are characterized by a turreted shell were originally placed within the Melanidae which had to be renamed Thiaridae. This group was found to be polyphyletic, containing many different families. In case of the thiarid fossils from Australia and New Zealand mentioned in the introduction, it remains questionable if they can be attributed to the genus *Melanoides*. In the absence of any comprehensive survey of thiarid fossils, it was attempted to find fossils that can at least be confidently assigned to closely related fam-

ilies. The most reliable study concerning members of the same superfamily is that of Reid et al. (2008). Based on a comprehensive reinterpretation of the fossil record they suggest that the living members of the Potamididae are a monophyletic radiation that has always been closely associated with mangroves and whose earliest certain representatives appeared in the Middle Eocene. In addition, the neotropical Hemisinidae are known to have confidential fossils, known from the early Oligocene and the molecular data was calibrated using fossil records of the two families Potamididae and Hemisinidae (see tab.3). For the molecular clock approach the same alignment was taken as for the phylogeny reconstruction but increased by additional outgroups for the calibration (see tab. 4).

Table 3 Outline of the fossil record of the Potamididae and Hemisinidae. Abbreviations: Cret, Cretaceous (65.5-145.5 Ma); Eoc, Eocene (34-56 Ma); L, Late; M, Middle; Mioc, Miocene (5-23 Ma); Olig, Oligocene (23-34 Ma); Plioc, Pliocene (1.8-5 Ma); Rec, Recent.

	Age	Geographical range	References
Potamididae			
Telescopium telescopium	Middle Mioc	W Pacific	Reid et al. (2008)
Tympanotonos fuscatus	Rec	W Africa	Reid et al. (2008)
Terebralia palustris	Early Mioc	IWP; Italy (L Mioc)	Reid et al. (2008)
Genus *Cerithideopsis*	Late - Middle Eoc	Europe, Peru, Florida (Plioc)	Reid et al. (2008)
Genus *Cerithideopsilla*	Middle Mioc	Indonesia, W Pacific, Mediterranean	Reid et al. (2008)
Genus *Cerithidea*	Mioc-Plic	Saipan, W Pacific, Java	Reid et al. (2008)
Hemisinidae			
Hemisinus cf. *venezuelensis*	Plioc	Venezuela	Jung (1989)
Hemisinus costatus n. sp., *H. bituminifer* n. sp.	Mioc	Cuba	Cooke (1919)
Hemisinus kochi	Middle Mioc	Amazonia	Wesselingh (2006)
Hemisinus cf. *oeciscus*	Olig	Panama	Woodring (1957)
Hemisinus terebriformis n. sp.	Early Olig	Peru	Olsson (1931)

Divergence times were estimated using the log-normal uncorrelated relaxed clock method implemented in BEAST v.1.4.8 (Drummond and Rambaut, 2007; Drummond et al., 2012). The BEAST input file including setting, evolutionary model and options for the MCMC analysis was generated using the program BEAUti (Bayesian Evolutionary Analysis Utility). Choice of nucleotide substitution model followed that for the phylogeny reconstruction (based on MrModeltest). A normally distributed calibration prior with mean 42.9 and standard deviation 2.5 (95% range: 38.8-47.8 ma) was set for the node age of the Potamididae and a second calibration point with mean 31.0 and standard deviation 1.1 (95% range: 33.9-28.1 ma) was set for the node age of the neotropical Hemisinidae. The posterior distribution of divergence times with 95% credibility intervals was obtained by at least two independent MCMC sampling runs of 30.000.000 generations with an initial burn-in of 30.000 during which every 1000th tree was sampled. Analyses were checked for convergence using Tracer v1.4.1 (Rambaut and Drummond, 2008) and ESS values exceeded the recommended threshold of 200 for each parameter. Finally, the logged pa-

Table 4 List of additional sequences of Cerithioidean families for molecular clock approach.

Species	Geographical range used for RASP analyses	GenBank accession or museum numbers (ZMB) COI	16S	28S
Potamididae				
Cerithidea decollata	Africa	AM932764	HE680776	HE680680
Cerithideopsilla djadjariensis	Asia	AM932785	HE680834	HE680753
Cerithideopsis largillierti	Asia, Australia	HE680615	HE680842	HE680759
Telescopium telescopium	Asia	AM932799	AY010318	HE680763
Terebralia palustris	Africa	AM932802	AY010319	AM932742
Tympanotonus fuscatus	Africa	-	-	AM932735
Melanopsidae				
Esperiana esperi	Europe	ZMB107119	ZMB107119	ZMB107119 MS30289
Melanopsis praemorsa	Europe	ZMB191928 ZMB200364	ZMB191928 ZMB200364	HM003674 -
Zemelanopsis trifasciata	New Zealand	ZMB107011 ZMB183350	ZMB107011 ZMB183350	-
Pleuroceridae				
Elimia interrupta	North America	-	-	HM003677
Semisulcospira libertina	Asia	-	-	HM003676
Paludomidae				
Cleopatra bulimoides	Africa	AY791934	ZMB103720	ZMB103720
Cleopatra johnstoni	Africa	AY456536	AY456590	-
Hemisinidae				
Hemisinus cubanianus	South America/Caribbean	-	-	HM003669
Pachymelania byronensis	Africa	ZMB191154	ZMB191154	ZMB191154

rameter values and trees from replicate runs were combined using LogCombiner 1.4.9. The final calibrated chronogram and node estimates were edited using FigTree version 1.3.1 (Rambaut, 2009) and Adobe Illustrator CS5 version 15.1.0.

3.3 Results

3.3.1 Phylogenetic analyses

The Bayesian analyses and RAxML searches converged on nearly identical topologies, which in turn, differed only slightly from the parsimony consensus tree generated with PAUP. Analyses of the combined data set yielded similar relationships to those supported in each of the separate gene trees. Although these topologies differed slightly among terminal taxa, the trees showed similar relationships among deeper nodes. The ML-phylogenetic trees constructed from the combined mt - and nuclear gene data sets are shown in fig. 5 and 6. The Bayesian analyses and parsimony consensus trees are shown in the appendix (see page 149 - 153).

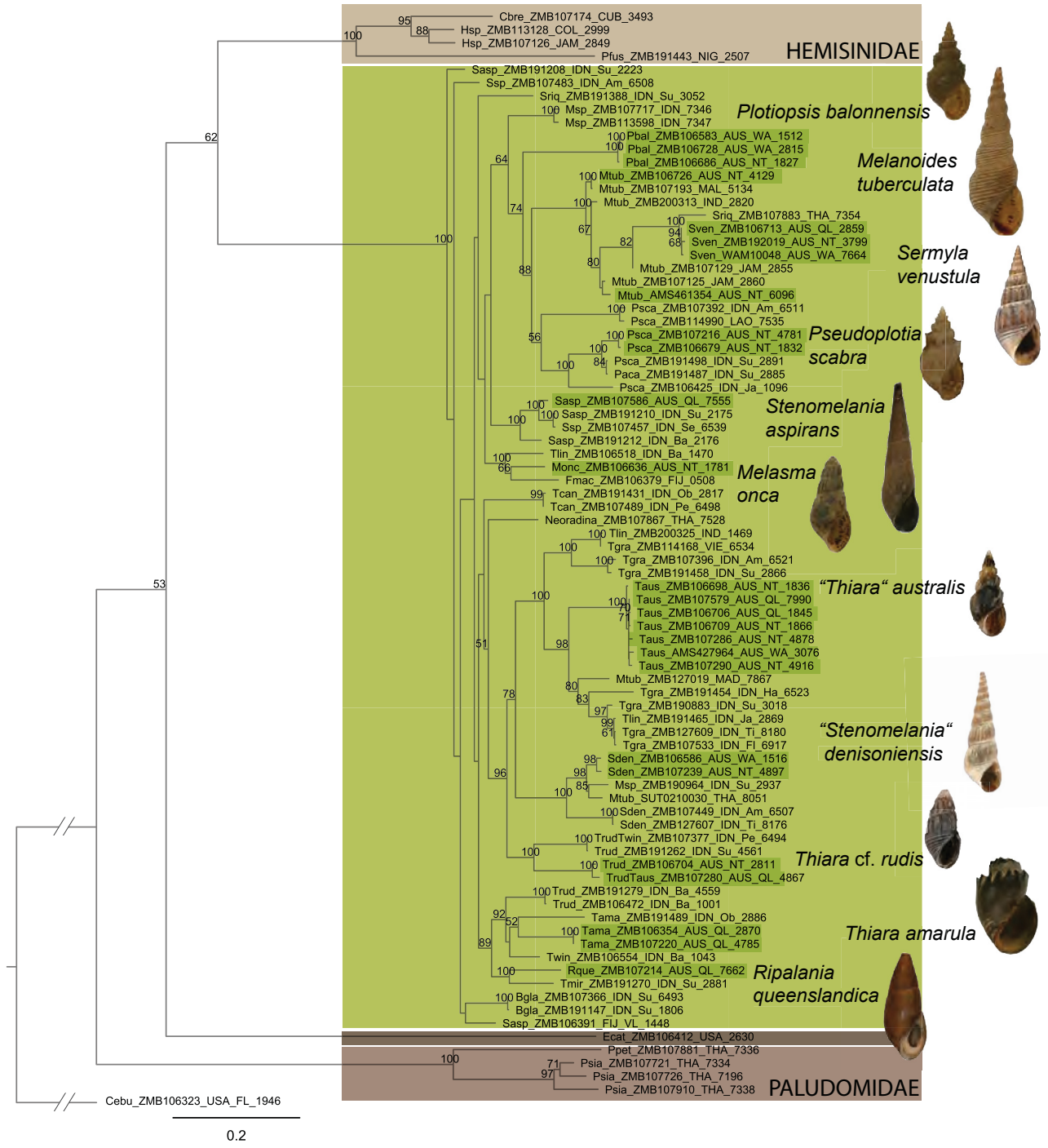

Figure 5 RAxML topology based on combined COI and 16S sequences. Numbers on nodes indicate bootstrap support values of the shown topology. The Australian thiarid taxa are highlighted and not monophyletic. For abbreviations of taxa and locality names see appendix.

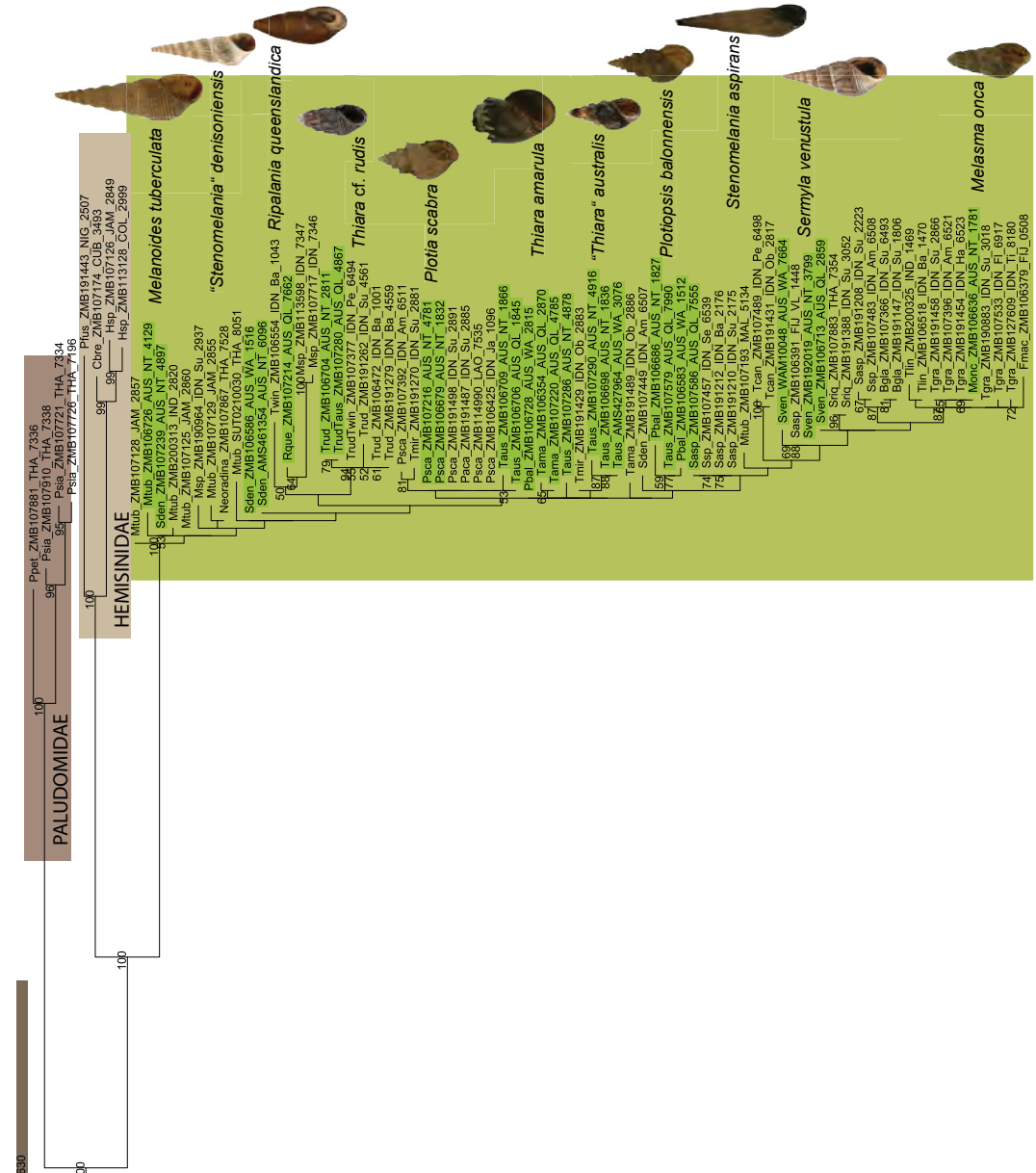

Figure 6 RAxML topology based on combined 28S and H3 sequences. Numbers on nodes indicate bootstrap support values of the shown topology. The Australian thiarid taxa are highlighted and not monophyletic. For abbreviations of taxa and locality names see appendix.

Monophyly of the Thiaridae is highly supported in the trees from individual genes and from concatenated sequences (mt and nDNA respectively). Also the sister group relationship between Thiaridae and Hemisinidae is consistent between the different genes and analyses. The Australian taxa are not monophyletic, species and populations from the continent are nested among their congeners and relatives from outside of Australia. Concerning the relationships within the thiarids the mt-data clearly separates different species (see fig. 5) but the nuclear data does not support the majority of the mtDNA branching orders. Here the relationships among the genera and taxa within the thiarids are mostly poorly resolved providing little phylogenetic signal.

Another difference between mt- and nuclear data can be seen in the position of the Paludomidae. In the mt-RAxML and BI tree the pleurocerid sequence is the sister group to the Hemisinidae/Thiaridae group although with unsupported nodes. By contrast, in the analyses based on nuclear data the Paludomidae are consistently at the base of the clade uniting the Hemisinidae and Thiaridae. This branching order is highly supported (100% bootstrap/posterior probability).

3.3.2 Molecular Dating

The results of molecular dating using the present data and a Bayesian approach with a relaxed clock model are shown in fig.7 and fig.8. Although the nDNA data do not support the majority of the mtDNA phylogenetic relationships, the ages of nodes are in a similar range in both approaches. The divergence-time estimates for major lineages are summarised in table 5. These estimates suggest that the initial evolutionary diversification of thiarids probably occured around 50 Ma. Including the 95% density intervals this covers an extended period between Early Oligocene (24,71 Ma) and Late Cretaceous (83,91 Ma). The age of the Hemisinidae is calculated to be in the same Eocene epoche, with a 95% density interval between 31,02 Ma and 72,87 Ma.

The mt-timetree shows that the divergence between Thiaridae and Hemisinidae probably happened in the Late Cretaceous (83,65 Ma; 95% density interval: 52,3-120,26 Ma). Among Paludomidae, the split between Paludomidae that originated in Asia and the African ones is dated also in that time range (76,17 Ma; 95% density interval: 52,3-120,26 Ma). These two nodes are only represented in the chronogram based on the mt-data set. They seem to be unstable in the 28S time-tree and dependent on the composition of the data set. In a previous approach with only one calibration point and less outgroup sequences the position of the Paludomidae is the same as in the mt based analyses (see appendix fig.52).

A small time difference between the nuclear and the mitochondrial data can only be seen at the split between the clade uniting Hemisinidae and Thiaridae and the Paludomidae. The 28S chronogram gives an age about 69 Ma and the concatenated mt time-tree an age about 100 Ma for this node. This gives an overall confidence interval between 38,7 Ma and 143,09 Ma which corresponds to a range from Eocene to Early Cretaceous.

Table 5 Results of a Bayesion estimation of divergence times. Time estimates are given in millions of years (Ma) for nodes representing the most recent common ancestor of relevant clades. HPD: the 95% highest posterior probability density (equivalent to a confidence interval); TMRCA: time to most recent common ancestor.

TMRCA	**28S**	**16S & COI**
mean [95% HPD Lower/Upper]		
Hemisinidae (HEM)	47,78 [31,02/68,15]	49,92 [33,9/72,87]
Paludomidae (PAM)	/	76,17 [41,1/116,06]
Asian Paludomidae	28,58 [5,68/54,54]	35,34 [16,93/59,43]
Thiaridae (THA)	**52,13** [24,71/83,91]	**50,42** [31,99/73,77]
THA & HEM	/	83,65 [52,3/120,26]
[THA & HEM] & PAL	69,13 [38,7/105,68]	99,29 [63,18/143,09]

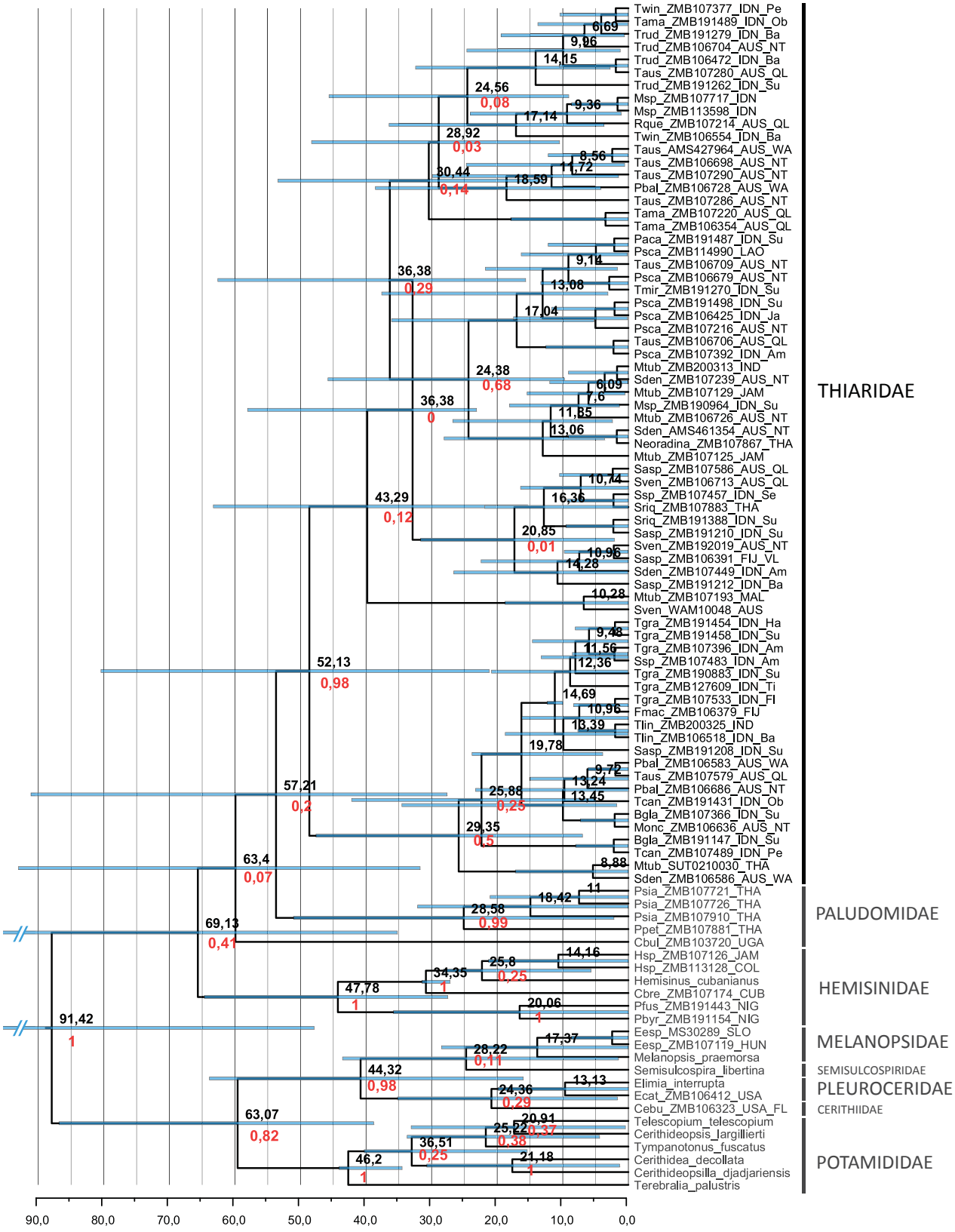

Figure 7 28S BEAST chronogram. Divergence dates (black numbers at nodes) are only displayed if higher than 6 Ma. Blue bars represent 95% highest posterior density intervals and posterior probabilities are highlighted in red. Scale is given in millions of years before present. Note that the tree is unrooted because forced rooting resulted in falsified branch lenght and node ages.

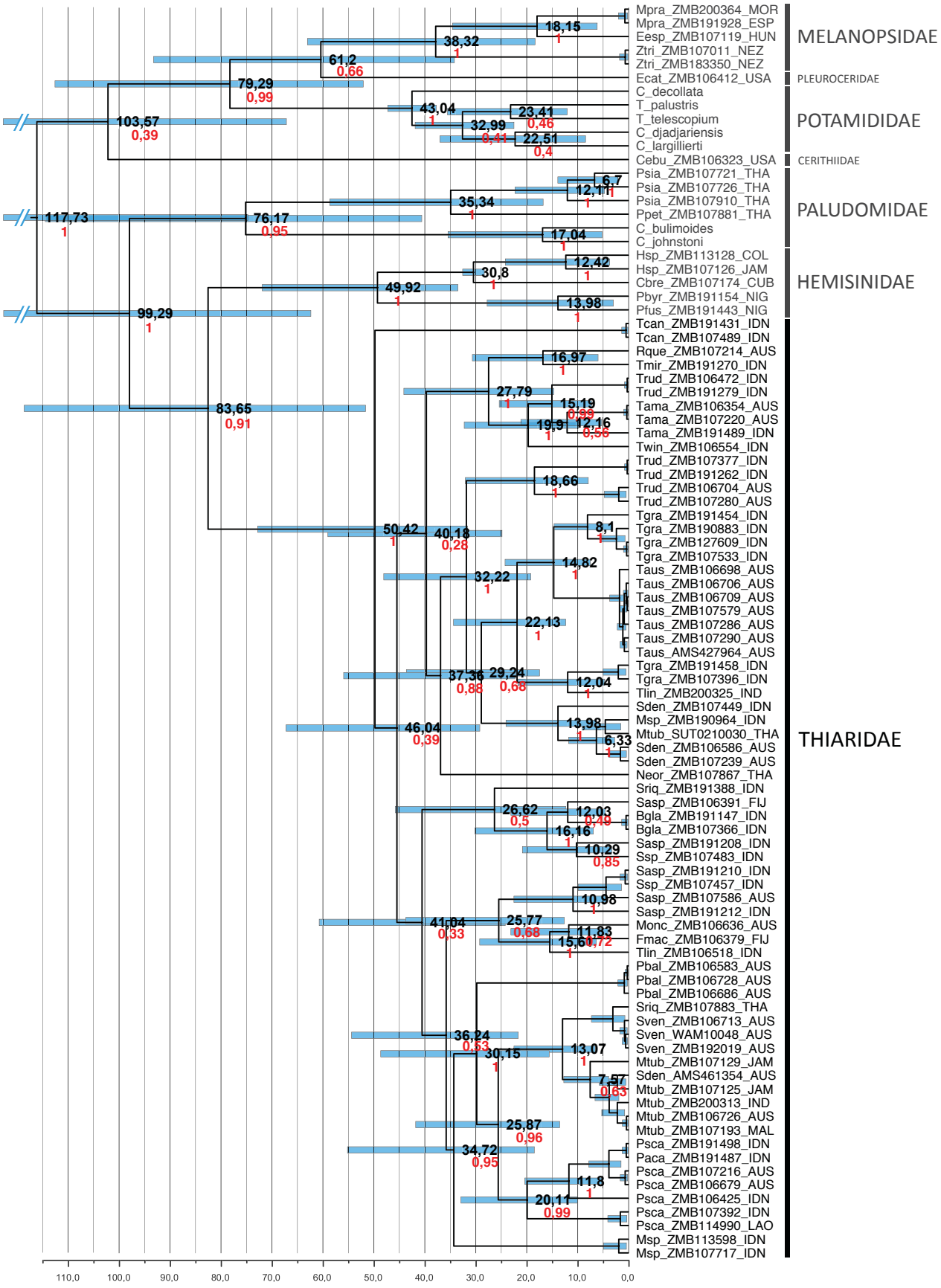

Figure 8 BEAST chronogram based on combined COI and 16S sequences. Divergence dates (black numbers at nodes) are only displayed if higher than 5 Ma. Blue bars represent 95% highest posterior density intervals. Posterior probabilities are highlighted in red (only displayed on nodes older than 5 Ma). Scale is given in millions of years before present. Note that the tree is unrooted because forced rooting resulted in falsified branch lenght and node ages.

3.4 Discussion

The combination of molecular phylogenies with distributional data, the fossil record and divergence date estimates provides the basis for the discussion of the historical biogeography of thiarids and their origin in Australia. Are we dealing with an ancient allopatric divergence by means of tectonic plates, do we have a more recent separation achieved by dispersal or are we faced with a combination of both scenarios?

Above all, the presented results show that the monophyly of the Australian thiarids can be ruled out. Accordingly, the recent distribution cannot simply be explained by a single dispersal event from Asia to Australia neither vice versa. If one of these areas is the center of origin there were multiple dispersals in one or even both directions.

The here shown phylogenies confirm the results within the phylogeny of the superfamily Cerithioidea (Strong et al., 2011): Thiaridae formed a monophyletic group in all analyses, with Hemisinidae consistently emerging at its base. The revealed overall consistent topology [Paludomidae[Hemisinidae,Thiaridae]] is possibly reflecting a Gondwanian vicariant event. A reconstruction of the historical biogeography necessitates a thorough understanding of the gradual breakup of Gondwana (see fig. 9). In the beginning Gondwana was a single contiguous supercontinent comprised of what would become Africa, South America, Antarctica, Australia and India. It is well accepted that approximately 175 Ma ago, rifting between western Gondwana (South America and Africa) and eastern Gondwanan components (India, Antarctica and Australia) commenced (Yoder and Nowak, 2006). The South Atlantic Ocean opened about 130 Ma ago as Africa separated from South America (Macdonald et al., 2003). At about the same time (130 Ma), India, which was still attached to Madagascar, separated from the Antarctica-Australia block, opening the central Indian Ocean (Briggs, 2003). During the Late Cretaceous (80 Ma), India broke away from Madagascar, and Australia slowly drifted away from Antarctica. India collided with Eurasia some 50 million years ago, while the northward-moving Australian plate had just begun its collision with Southeast Asia that is still under progress today.

The phylogenetic history seems to be congruent with this known sequence of vicariant events. The two deep nodes in the overall consistent topology lie in a similar range of time: with the age of split between Thiaridae/Hemisinidae at around 84 Ma and between these and Paludomidae at 100-70 Ma. Matthews et al.'s (2012) investigations of the tectonic and volcanic events that occurred during the period 110-90 Ma reveals that all major plates were affected by plate motion changes at this time and this reorganization event was global in scale affecting oceans and continents. This major plate reorganization certainly led to regional sea-level changes with land-emergence and submergence that could have caused the first splits between the families. The ancestral thiarid/hemisinid lineage would have originated in the block of Antarctica/Australia. Due to the changes the ancestral thiarid lineages went to the eastern side and the ancestral hemisinid lineages to the western

side of Antarctica which was still connected to South America. This distribution would explain the similarity of the mentioned fossil of New Zealand with the South American Hemisinids.

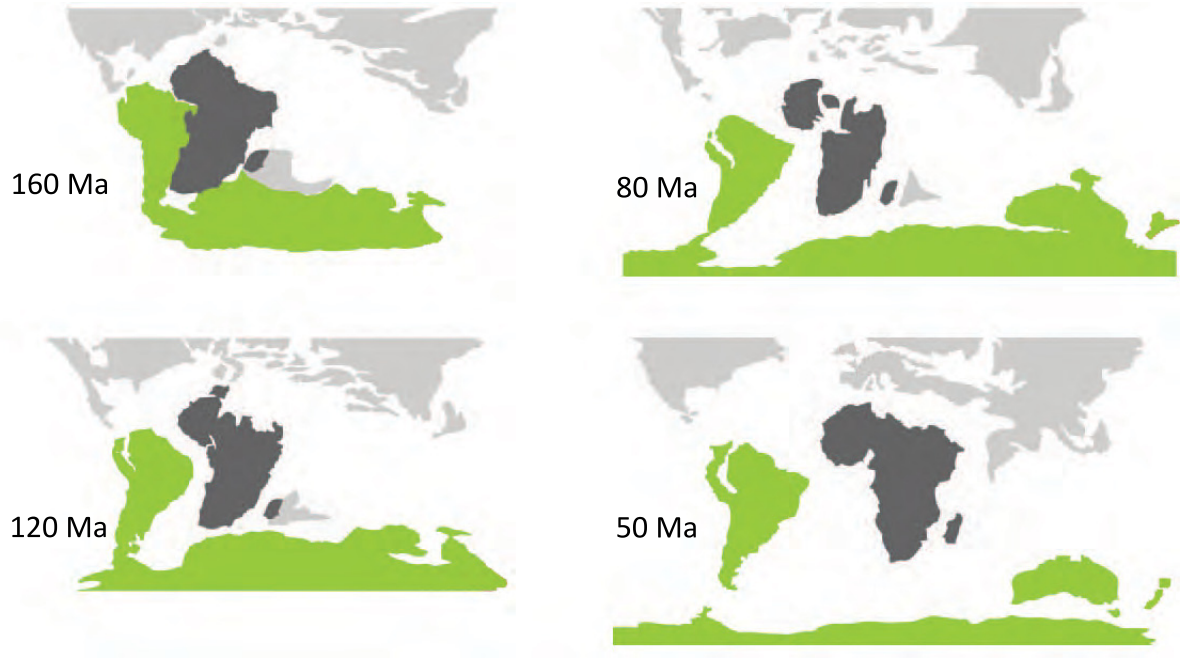

Figure 9 The breakup of Gondwana occurred in stages. Some 160 million years ago, in the Late Jurassic Period, Australia was still within the southerly latitudes and part of eastern Gondwana (Madagascar, India, Australia, and Antarctica). By 120 Ma, the breakup had extended, separating Australia from India and the west coast of Australia faced the Indian Ocean. In the Late Cretaceous Period (80 Ma), a gulf developed between Australia and Antarctica. India had left Gondwana and moved north towards Asia. From aproximately 45 Ma Australia started to be a separate island continent moving north towards Asia. Modified from Mitchell et al. (2014).

The ancestral Paludomidae lineage lived on the continental block associating India and Africa. This scenario could explain the present distribution of Paludomidae in Asia as members of Paludomidae were possibly carried on the Indian plate, following the breakup of eastern Gondwana and subsequent northward drift of India to Asia. As India collided with Asia, there was undoubtedly significant biotic interchange between both landmasses (Rust et al., 2010). Ali and Aitchison (2008) suggest that about 57 Ma was the earliest time such migrations would have been possible for non-volant faunas. Although other critical estimates of initial contact differ to as young as the Eocene-Oligocene boundary 35Ma (Rust et al., 2010). The estimated age of the Asian Paludomidae is about 28 Ma (28S) and 35 Ma (mtDNA), which is consistent with this scenario. However, the age of the Paludomidae is dated to 76 Ma, a time where India and Africa had already been separated for a long period of time as the Indian plate separated from Africa in the Early Cretaceous ca. 130 Ma.

In addition, the estimated divergence between the South American and African Hemisinidae, at about 50 Ma, is inconsistent with the separation of South America from Africa about 130 Ma ago. A possible explanation will be discussed later.

The origin of thiarids If the clock estimates prove correct, then the period of the origin of thiarids coincides with Australia finally being an island continent starting its movement northwards (see fig.9). Based on the results presented here it can be excluded that the family already emerged as such before the breakup of Gondwana. But it is still possible that the ancestral thiarid lineage originated in Australia and that Asia was colonized subsequently.

The plot of Thiaridae lineages through time reveals a pulse of increased diversification within the period of the collision of the Australian plate with Southeast Asia during the past 20-30 Ma. Numerous sister taxa splits have occurred between the Late Oligocene (about 28 Ma) and Early Miocene (about 14 Ma). The region around Wallace's line revealed complex movements of terranes over the past 20-30 Ma. In the Early Miocene (about 23-20 Ma), the Sula Spur, a promontory which was the continuation of the Australian continental margin, collided with the SE Asian margin. Once the Australian terranes have approached Asian land masses the thiarid lineage maybe managed to colonize islands in the Wallacea. This aspect will be discussed in more detail later on. The Wallacean islands are oceanic and have never been connected to either the Indo-Malayan (Sunda) or Australasian (Sahul) continental plates (Andersen et al., 2013). There is the theoretical possibility of a vicariant origin of taxa from both sides across the Wallace Line, that is, from the Indo-Malayan (Sunda) and from New Guinea/Australia (Sahul).

In principle, phylogenetic topologies and area cladograms can be used to distinguish between dispersal and vicariance and to detect the direction of dispersal events or define probable areas of origins (as inferred by dispersal-vicariance analysis). When phylogenies reveal lineages from one geographic area deeply nested within clades from another area, dispersal is typically inferred (Yoder and Nowak, 2006). Conversely, a vicariant origin is expected to result in a spectrum of exclusive sister relationships within the separated populations, reflecting the long-term duration of disconnectedness (Miura et al., 2013). Due to the non-robustness of the trees and inconsistencies between the genes this approach is only shortly discussed and, accordingly, computational analysis was omitted.

The phylogenetic results of the mitochondrial data set identified six pairs of sister groups between Wallacea and Sahul thiarids (see fig.10). The estimated timing of these splits range from 18 to 2 Ma. Of these six, four represent sister relationships within the separated populations/species and two are nested within clades from other areas (that is *S. aspirans* and *P.scabra*). Note that the only two sister groups between Wallacea and Sundaland are built by the invasive species *M. tuberculata* and *P. scabra*, that are known to disperse and colonize easily into new regions.

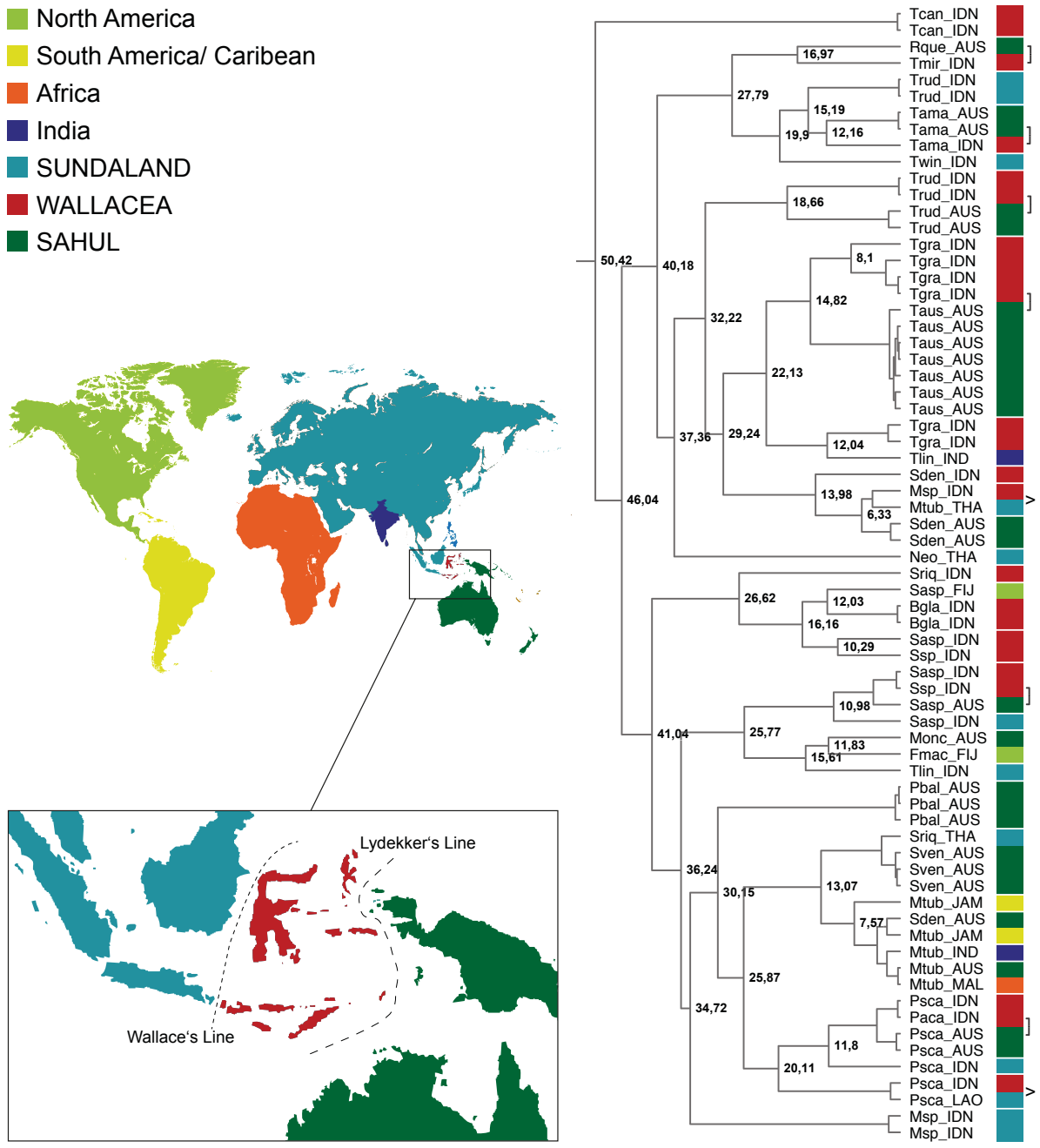

Figure 10 Species - Area cladogram; cut-out of the BEAST mt-phylogeny in which the names of the organisms at tips were replaced by abbreviation of taxa and areas in which they occur. Divergence dates (black numbers at nodes) are only displayed if higher than 5 Ma. Arrows indicate sister groups between Wallacea and Sundaland; square brackets show sister groups between Wallacea and Sahul. The western and eastern limits of Wallacea coincide with Wallace's Line (Wallace, 1863) and Lydekker's Line (Lydekker, 1896). The complex geological history of Wallacea offers the theoretical possibility for a vicariant origin of taxa from across both lines, that is, from Sundaland, and from New Guinea/Australia.

These splittings could be interpreted as a result of vicariance with subsequent dispersal from Australia to Asia. But when looking at early branching clades of the mt-chronogramm, it becomes clear that they are partly formed by species from the Sunda

Shelf which would indicate the probable area of origin. Due to the lack of support and inconsistencies between the genes, the relationships among closely related species of thiarid taxa remain somewhat uncertain. The conflicting patterns between mtDNA and nDNA and the within diversification of the thiarids are discussed in the 'General discussion' chapter on page 109. More robust phylogenies are needed to make a statement.

Dispersal without land connection? As mentioned before, the Wallacean islands are oceanic and have never been connected to either the Indo-Malayan (Sunda) or Australasian (Sahul) continental plates (Andersen et al., 2013). In addition, there is no evidence for the existence of a subaerial link between the Sula Spur and Australia-New Guinea during the Oligocene (33.9 - 23 Ma) to the Early Miocene (23 - 15.97 Ma) (Stelbrink et al., 2012). Such land connections are essential preconditions for matching a vicariance hypothesis in case of species that disperse via land or freshwater. For any freshwater individuals exposure to sea water should inflict mortality owing to physiological stress, but in case of some thiarid species a dispersal stage is present with a free-swimming veliger larvae that may develop in the sea (Strong et al., 2008; Kano et al., 2011). This might also be an explanation for the non-overlap with the separation of Africa and South America. Over a long period of time the continents were only separated by a sea corridor that should be easily bypassed by veligers. Although the release of veligers has been observed, transoceanic dispersal has never been directly confirmed (Kano et al., 2011). And whether the reproductive mode was ovipar or vivipar in the beginning still remains unresolved. However, an alternative hypothesis for the dispersal without land connection could be passive transport via floating debris carried down rivers and swept away by ocean currents.

Sources of Error Several factors, including tree topology, taxon sampling, and fossil assignments can significantly influence clade relationships, age estimates, and diversification rates. As previously mentioned, the relationships within the family lack strong support and the focus was primarly on relationships and estimated ages supported by a combination of molecular (mt and nuclear), morphological, and fossil evidence.
Needless to say, all age estimates should be treated with caution not only because of their large confidence intervals and possible underestimation due to saturational effects (Wilke et al., 2009) but also because fossil data might be imprecise and only give minimum estimates for the age of a group.

Summary All in all, the classical vicariance scenario of dispersal by rafting on a Gondwanian fragment provides the most parsimonious explanation for the present distribution and, at the same time, the results demonstrate that vicariant speciation is seldom an exclusive mechanism as dispersal is an important process at the species and/or population level (Lohman et al., 2011). Crisp et al. (2011) cautioned against the use of simplified

'rafted Gondwanian fragment' interpretations in the biogeographical explanation of formerly wide Southern Hemisphere distributions, pointing out that trans-oceanic dispersal is more likely in most cases. However, in the case of the Thiaridae, the topology of the molecular phylogeny, the timing of events as suggested from a molecular clock were found to be largely congruent with a vicariance scenario within the framework of Gondwanian fragmentation. Although the age of the family is much younger than assumed, it coincides with the separation of Australia from Antarctica. If so, Asia seems to have been colonized a number of times subsequently.

Nevertheless, this hypothesis remains to be fully tested by a more detailed study of the fossil record as the conclusions are based on the absence of definitive fossils on the northern continents. It is an interesting challenge for future research to explore the biogeography of these groups in more detail and the possible role of changes in reproduction strategies and the origin of viviparity.

4 A biogeographical revision of Australian thiarids

4.1 Specific introduction

This chapter focusses on the Australian thiarids and their geographic distribution on the continent. The Thiaridae are widely distributed in all major regions of Australia, with the exception of the arid central and most southern parts. The highest species diversity for thiarids is found in the coastal rivers and inland streams of the wet-dry tropical northern parts. Although in an arid continent like Australia the freshwater fauna should deserve particular attention, appraisals of its components are rare. There were essentially only three studies before the work of Glaubrecht et al. (2009) that dealt in some more detail with the taxonomy of thiarids: the "basic list of freshwater Mollusca of Australia" compiled by Iredale (1943), the contribution of McMichael and Weatherley (1967) based essentially on non-Australian Thiaridae and a catalogue of freshwater taxa with some taxonomic decisions compiled by B.J. Smith (1992). According to Smith (1996) thiarids are third in Australia in terms of number of native genera and species after the Hydrobiidae and the Planorbidae, but the number of species and genera of Australian thiarids remained at best tentative till the comprehensive investigations of Glaubrecht et al. (2009).

Based on their own collections, the study of relevant type material and the comparison with material from major Australian museum collections, Glaubrecht et al. (2009) suggest differentiating 8 genera with a total of 11 species among the Australian Thiaridae. Essentially this classification is based on shell morphology. In addition, they describe and document the radulae and the juvenile's morphology and discuss the taxonomical implications and nomenclatural consequences of all relevant thiarid taxa. Of these 11 species they consider more than half (n = 6 species) as being endemic to Australia, viz. *"Thiara australis"*, *Plotiopsis balonnensis* and *"Stenomelania" denisoniensis* with wider distribution as well as with more restricted ranges *Melasma onca*, *Sermyla venustula* and *Ripalania queenslandica*. In contrast to these endemics, they infer that *Thiara amarula* and *Stenomelania* cf. *aspirans* as well as *Melanoides tuberculata*, *Plotia scabra*, and *Sermyla riqueti* are widely distributed also outside of Australia.

As mentioned, in the work of 2009 primarily morphological features were used to characterize and distinguish individual taxa. These comprehensive investigations are now complemented with molecular analyses and additional sampling with the objective of testing the identity of these taxa as phylogenetic species (i.e. monophyletic units), in the course of a preparation of a systematic revision. The merging of the results provides a more complete picture of all Australian thiarid species and their geographical distribution on the continent with references ranging from continent-wide to drainage-based patterns.

4.2 Specific material and methods

4.2.1 Distribution maps

The distribution maps and material lists used in this study are based on those of Glaubrecht et al. (2009) and complemented, updated and corrected by the molecular results and additional sampling data from four additional surveys conducted between 2009 and 2012. All known localities of material pertaining to the collected thiarids (or consulted collections) were comprehensively compiled in "material examined" tables and distribution maps for each species. Given the limited accessibility to freshwater in remote areas of Australia repeated collections were made at the same spots on different expeditions. The museum numbers of lots from these different sampling years on the same locality are listed one after another in the corresponding table entry. The names of the regions are aligned to correspond to the Australian Bioregionalisation Atlas (ABA), a formalised and comparative system for Australia's biogeographical regions established by Ebach et al. (2013) which is based on the fluvifaunal regions proposed by Iredale and Whitley (1938) (see fig.11).

Figure 11 Freshwater zoogeographical regions as proposed by Ebach et al. (2013) in the Austral Bioregionalisation Atlas, which aims to be a repository of all names and maps used to describe and depict phytogeographical, zoogeographical, freshwater zoogeographical and marine areas within the Austral region.

As the ABA regions represent only a rough division, whenever referring to several parts or drainage systems within one region I apply the names used in McMichael and Hiscock (1958), in combination with those of the major drainage systems as given in Williams and Allen (1987) as proposed by Glaubrecht et al. (2009) (see fig. 12). This is necessary for the Leichhardtian region in particular, which includes the Northwestern and Gulf of Carpentaria coastal drainages extending from the Fitzroy River (Western Australia) to the Torres Straits.

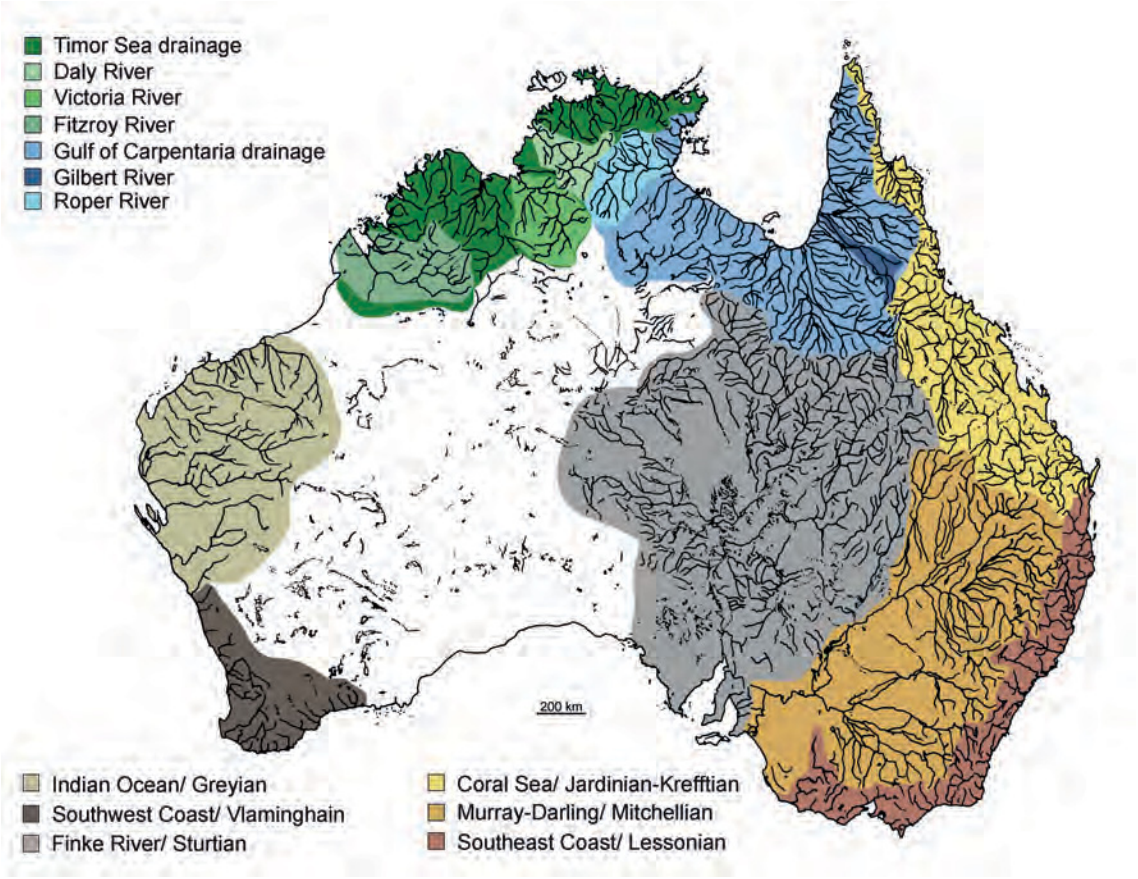

Figure 12 Drainage systems in Australia. The highest species diversity for thiarids is found in the coastal rivers and inland streams of the wet-dry tropical northern parts of Australia viz. the Timor Sea Drainage (shown in green) and the Gulf of Carpentaria drainage (blue).

Note that for the readability of the maps, in cases of analysed multiple samples from closely adjacent localities only one dot is shown to represent the occurrence, that is why the number of dots does not always correspond to the number of list entries. Furthermore, in case of early museum samples usually no geographic coordinates were available (marked by n.d. (= no data) in the corresponding table entry). Different dots are used to distinguish the type and processing status of material: Black dots represent genetically confirmed localities, white dots with an inner black dot stand for ethanol material (that either has not yet been extracted or whose extraction failed) and white dots represent dry shell material.

4.2.2 Photos of shells

Photos of the shells were taken with a digital reflex camera (Canon EOS 350D and 550D). Shells were arranged in a standardized position with the aperture at 90°angle to the camera and the apex forming a horizontal line with the columella. Note that only photos of individuals are shown for which the preliminary species identification was verified or corrected by molecular genetics. The small amount of shell photos is due to the problem that extractions were mostly made of snails with cracked shells. Thiarids can close the aperture of their shell with an operculum, which prevents ethanol from entering and fixing the tissue for DNA preservation. Thus cracking the shell directly after sampling in the field is inevitable to make sure that the ethanol penetrates the complete soft tissue. On account of this problem, in 2011 photos were also taken in the field before cracking, unfortunately individuals could not be handled separately because of logistic issues. Hence, in most cases where sequences are present, no photo of a corresponding intact shell is available. The appearance of shells of *T. amarula*, *S.* cf. *aspirans* and *R. queenslandica* is characteristic of each species. They haven't been confused with another species in any case. For these species a typical shell, that hasn't been explicitly genetically confirmed is displayed beside the corresponding distribution map. For pictures of types see (Glaubrecht et al., 2009).

4.2.3 Genetic data

As the nuclear data did not comprise sufficient genetic disparity for species delimitation, mitochondrial sequences were used for that purpose. At the beginning of the project 16S sequences were partly available and the alignment was complemented reaching 523 sequences for representative thiarids from all over the world and outgroups. From these, 255 16S sequences derive from Australian thiarids. In addition, a fragment of COI was sequenced to check accordance with 16S results, getting 430 sequences in total and 155 from Australian thiarids. Details for sequencing and phylogenetic analyses are given in the 'General Material and Methods' section. Note that in this chapter the resulting mt-phylogenies are only considered for assigning individual sequences (and the corresponding locality) to phylogenetic species (i.e. monophyletic units). A locality or lot is considered to be genetically confirmed for a species if the appertaining sequence clusters in the appropriate branch in the 16S and/or COI tree. If the genetic result is in concordance with morphology and geography the individual is assigned to the species. In cases where the results are in conflict it is checked if there might have been a misassignment in the field. Conflicts are discussed in the comments at the end of each species section.

4.3 Results

As the nuclear data does not comprise sufficient genetic disparity for species delimitation, mitochondrial sequences are used for that purpose. The COI and 16S gene portions were analysed separately and considered for assigning 295 individual sequences to phylogenetic species. Monophyletic clusters of individuals corresponding to the Australian species delimited by Glaubrecht et al. (2009) were recovered in both phylogenies.

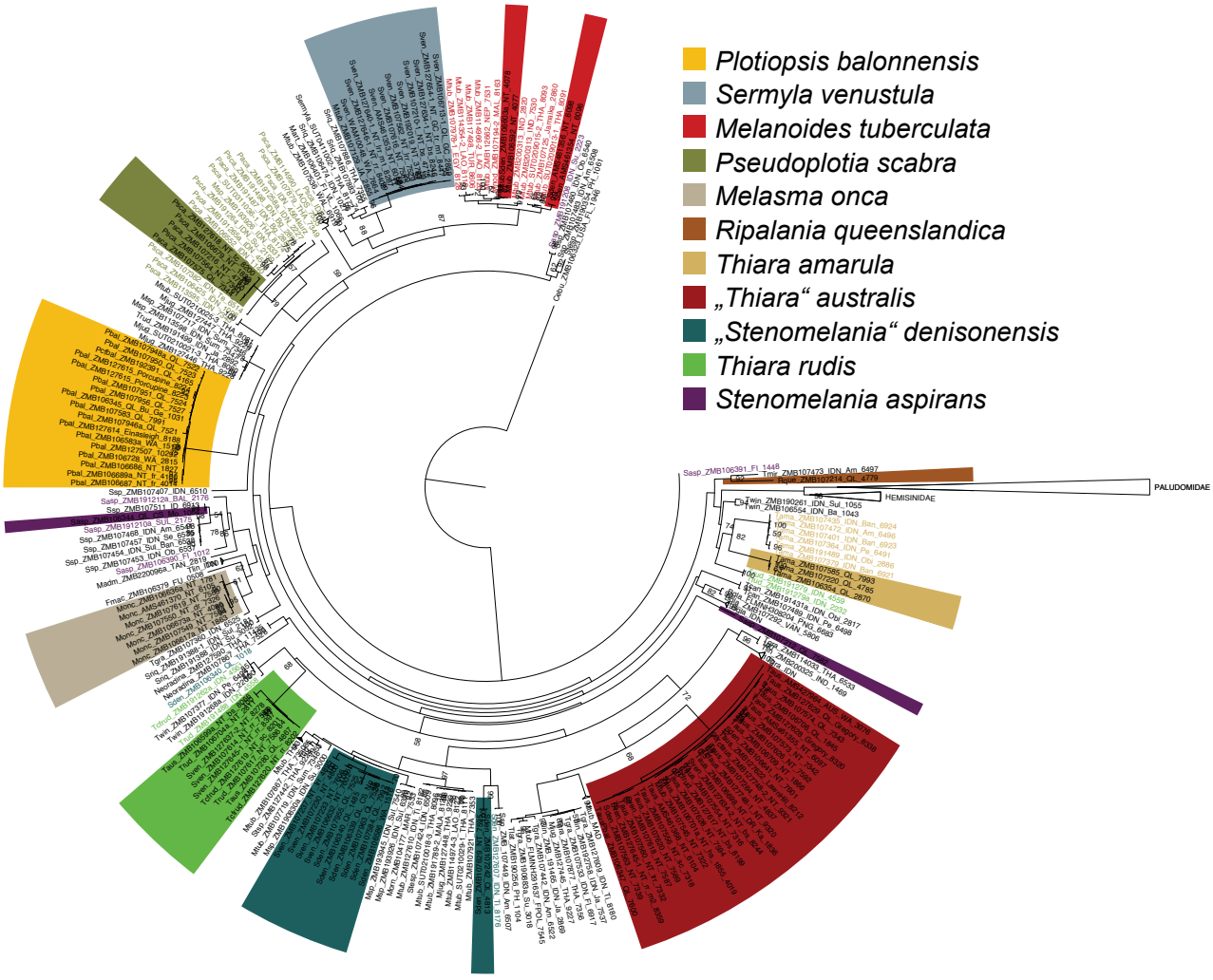

Figure 13 Phylogenetic tree reconstruction by maximum likelihood based on COI sequences of the haplotype reduced dataset conducted with RAxML. Sequences highlighted by coloured areas are from Australia, accordingly conspecifics from outside of the continent have coloured letters. An exception is the genus *Melanoides*, where only the branch with sequences from the type locality is coloured. Numbers on nodes indicate bootstrap support of the shown topology (displayed if higher than 50). Four and five-digit numbers represent extraction numbers, numbers with prefix letter codes museum numbers. Taxa abbreviations in sequence names are uncorrected (see text). Note that not all lots are represented as the alignment was reduced to unique haplotypes before analyses. For abbreviations in taxa names see appendix.

One exception is *Stenomelania denisoniensis*, the Australian sequences of this species fall together with mostly Asian *Melanoides* sequences, which build a cluster separate to a second *Melanoides* cluster which contains the Australian *Melanoides tuberculata* sequences and those from the type locality of the species in India.

Apart from this, species from Australia were shown to possess unique mt-haplotypes by which they could be identified as can be seen in the RAxML tree reconstruction based on COI sequences (see fig. 13). In no case differences in species identification between the COI and the 16S gene were found. The phylogenetic tree reconstruction by maximum likelihood based on 16S sequences is shown in the appendix (see fig. 57).

The genetical confirmation exhibits that many individuals have been erroneously named in the field. Figure 14 shows the rate of successful und unsuccessful pre-identifications (pre-IDs) per taxa, meaning the identifications based on morphology undertaken in the field. Differentiation using only external features was particularly unsuccessful in the case of *Melanoides tuberculata* that was misassigned in almost 60%. The Australian individuals that preliminarily had been labelled as *Sermyla riqueti*, turned out to have typical *Sermyla venustula* haplotypes. Furthermore, the thiarid *Thiara rudis* could be identified by molecular data, clustering with *T. rudis* sequences from Indonesia (see fig.57 in appendix). The specimens have already been collected in field trips since 2004 but misidentified as *"Thiara" australis* and *Sermyla venustula*.

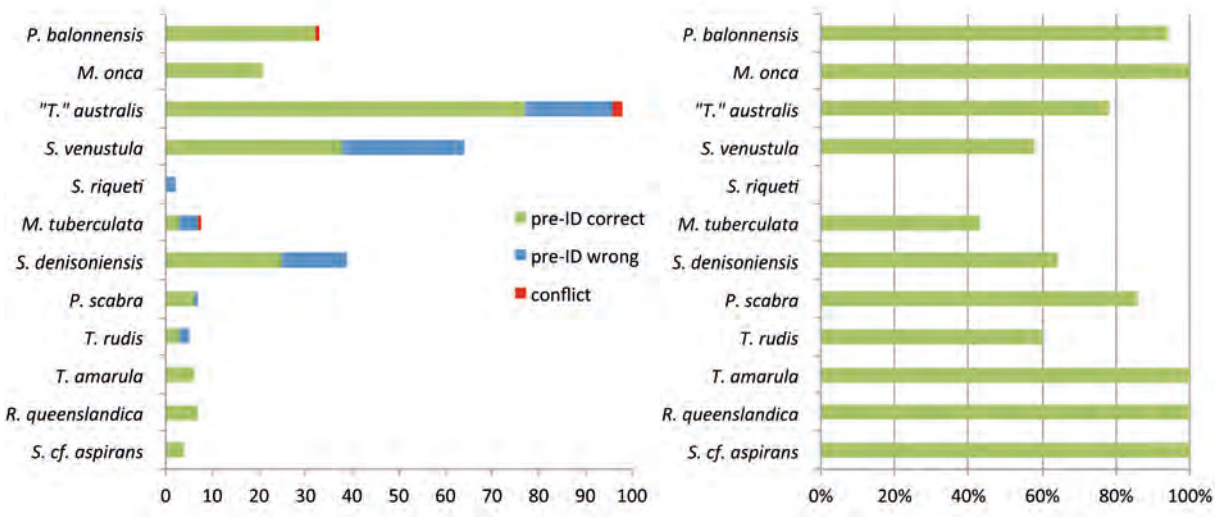

Figure 14 Charts showing the extent of species mis-assignments. In both graphs, the taxa names in the horizontal axis represent the pre-IDs, i.e. the species name to which the individual was assigned in the field. The values on the vertical axis of the left chart represent the number of pre-IDs. In the right-hand chart the percentage of mis-IDs is shown per species.

Table 6 List of wrong and correct pre-IDs per taxa. Conflicts show where specimens were found outside of the known range of the newly assigned species.

pre-ID	correct	wrong	new assignment	conflict
P. balonnensis	32	1:		1x *"T." australis*
M. onca	21	0		0
"T." australis	77	21:	10x *P. scabra*, 6x *M. onca*, 3x *T. rudis*	2x *P. balonnensis*
S. venustula	38	27:	8x *"T." australis* , 7x *T. rudis* , 11x *S. denisoniensis*	0
S. riqueti	0	2:	2x *S. venustula*	0
M. tuberculata	3	5:	3x *S. venustula*, 1x *"T." australis*	1x *S. venustula*
S. denisoniensis	25	14:	8x *M. tuberculata*, 4x *"T." australis* , 2x *S. venustula*	0
P. scabra	6	1:	1x *"T." australis*	0
T. rudis	3	2:	1x *"T." australis* , 1x *P. scabra*	0
T. amarula	6	0		0
R. queenslandica	7	0		0
S. cf. *aspirans*	4	0		0

All in all, the mt based species identification system worked out. In the case of *M. onca*, *R. queenslandica*, *T. amarula* and *S.* cf. *aspirans* the morphological field identifications are 100% identical to the molecular assignments.

Concerning the mis-IDs, 69 out of 73 are excepted as simply mis-identified species in the field. These new assignments can all be confirmed by examining the morphology of the shell and besides they are in accordance with the known geographical range of the appropriate species. Only in four cases the new assignment seems doubtful because the sampling locality lies outside of the known range of the species to which it was attributed by molecular data (see tab.6). Of these conflicts, one sequence originating from a locality in the *P. balonnensis* range and pre-identified to this species, turned out to have typical mt-data of *"Thiara" australis*. Vice versa on two localities near the Gulf of Carpenteria coast (the *"Thiara" australis* range) *P. balonnensis* could be genetically identified although it has never been found in that area before. In these two cases the morphology can't be consulted because the two species are not distinguishable by shell shape. The last conflicting case, a predefined *M. tuberculata* record in West Australia, turned out to be indeed a mis-identification as the molecular assignment to *S. venustula* could be clearly confirmed by morphology.

4.3.1 Systematic revision

In this systematic part taxa are arranged starting with the type species of the Thiaridae, viz. *Thiara amarula*, followed by subsequent taxa in alphabethical order. For more detailed information about taxonomy, description and ecology see Glaubrecht et al. (2009). Differences to the findings of the latter study are pointed out in the comments under the individual species. As suggested in the mentioned paper, the two generic allocations of *"Thiara"* (in case of *australis*) and *"Stenomelania"* (in case of *denisoniensis*) are used in quotation marks only, in order to denote that the phylogenetic placement is doubtful.

It should be noted that here for the first time an additional thiarid, viz. *Thiara rudis*, is documented as taxa with (more or less regular) occurrences in Australia not listed by Glaubrecht et al. (2009) or known to the last available surveys.

***Thiara amarula* (Linnaeus, 1758)**

Type locality: "Asiae fluviis", as given by Linnaeus; i.e. in Asian rivers.

Distribution: The distribution of *Thiara amarula* extends from the south and east coast of Africa west of the Indian Ocean to the Malay Archipelago, the Philippines and further out into the Indo-West Pacific reaching the Solomon and Fiji Islands as well as Samoa, as documented in Schütt and Glaubrecht (1999) and Glaubrecht et al. (2009).
On the Australian continent *T. amarula* is restricted within the Jardinian fluvifaunal province to a small region along the east coast of Queensland, reaching from Bloomfield River south of Cooktown to about a hundred kilometres south of Cairns (see fig. 15). The preferred habitat is just above the brackish water zone of streams and rivers draining to the Coral Sea.

Comments: The present analysis confirms the restricted distribution of Australian specimens in the drainages along the east coast of Queensland. The sequences of Australian *T. amarula* cluster together and build a sister group relationship to sequences obtained from Indonesian samples.

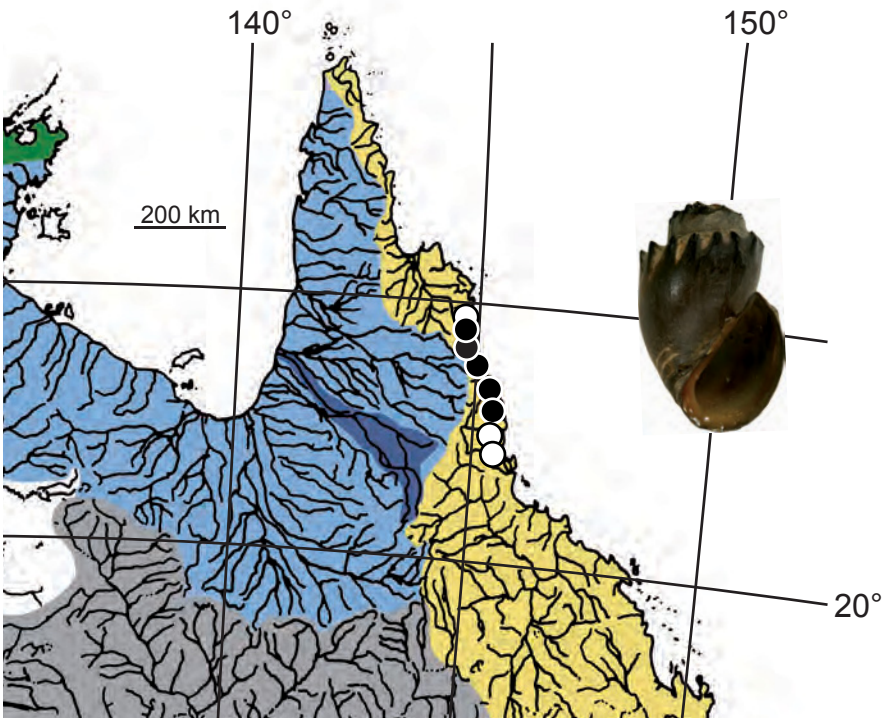

Figure 15 Geographic range of *Thiara amarula* on the Australian continent. Note the restriction within the Jardinian fluvifaunal province. Black dots represent genetically confirmed localities and white ones dry material. Some dots represent multiple nearby localities.

Table 7 Locality data for the material examined of *Thiara amarula*. Bold ZMB numbers are genetically confirmed.

Locality	Coordinates	Museum n°
QL: Bloomfield River	n.d.	ZMB 210026
QL: Granite Creek	15°55.99 S 145°19.54 E	ZMB **107217**
QL: Woobadda River	15°57.35 S 145°21.11 E	ZMB 106348
QL: Douglas Creek, Daintree Riv.	16°16.194 S 145°58.60 E	ZMB 107218, 107594
QL: Cooper Creek	16°10.43 S 145°25.18 E	ZMB 106350
QL: Martins Creek, Daintree Riv.	16°14.163 S 145°18.323 E	ZMB **107599**
QL: Mossman, Daintree Riv.	n.d.	AMS C.109658; ZSM 12430
QL: Mossman River	n.d.	AMS C.93924; CAS 46935
QL: Mowbray River	16°33.87 S 145°27.83 E	AMS C.158117, 158275, 317842; QM 16571, 64459; USNM 854006; ZMB 106353, 210027, **107219**, **107585**, 107590
QL: Barron River	16°52.170 S 145°40.405 E	ZMB **107220**
QL: Barron River	n.d.	BMNH 1922.3.24.9; MCZ 198983
QL: N of Cairns	n.d.	AMS C.117626, C.117617, C.158127
QL: near Cairns	n.d.	AMS C.109659; MCZ 31304, 183317; SMF 108247/2
QL: North Johnstone River	17°30.34 S 145°59.55 E	ZMB 106349, **106354**
QL: Clump Point	n.d.	AMS C.109435
QL: Tully River	n.d.	AMS C.9285
QL: Rockingham Bay	n.d.	BMNH 1879.5.21.405
QL: Cardwell	n.d.	AMS C.109657, syntypes of “amaruloides”

"Thiara" australis (Lea & Lea, 1851)

Type locality: "Victoria River, North Australia"

Distribution: The geographic range of *"Thiara" australis* is limited to the tropical north of the Australian continent. Starting from the westernmost locality at the Fitzroy River of the Kimberley drainages, it ranges through most rivers in the hot-wet zone of Northern Territory to the Gulf of Carpentaria drainages in northwestern Queensland and ends in the region of the Gregory-Einasleigh Range (see fig.16).

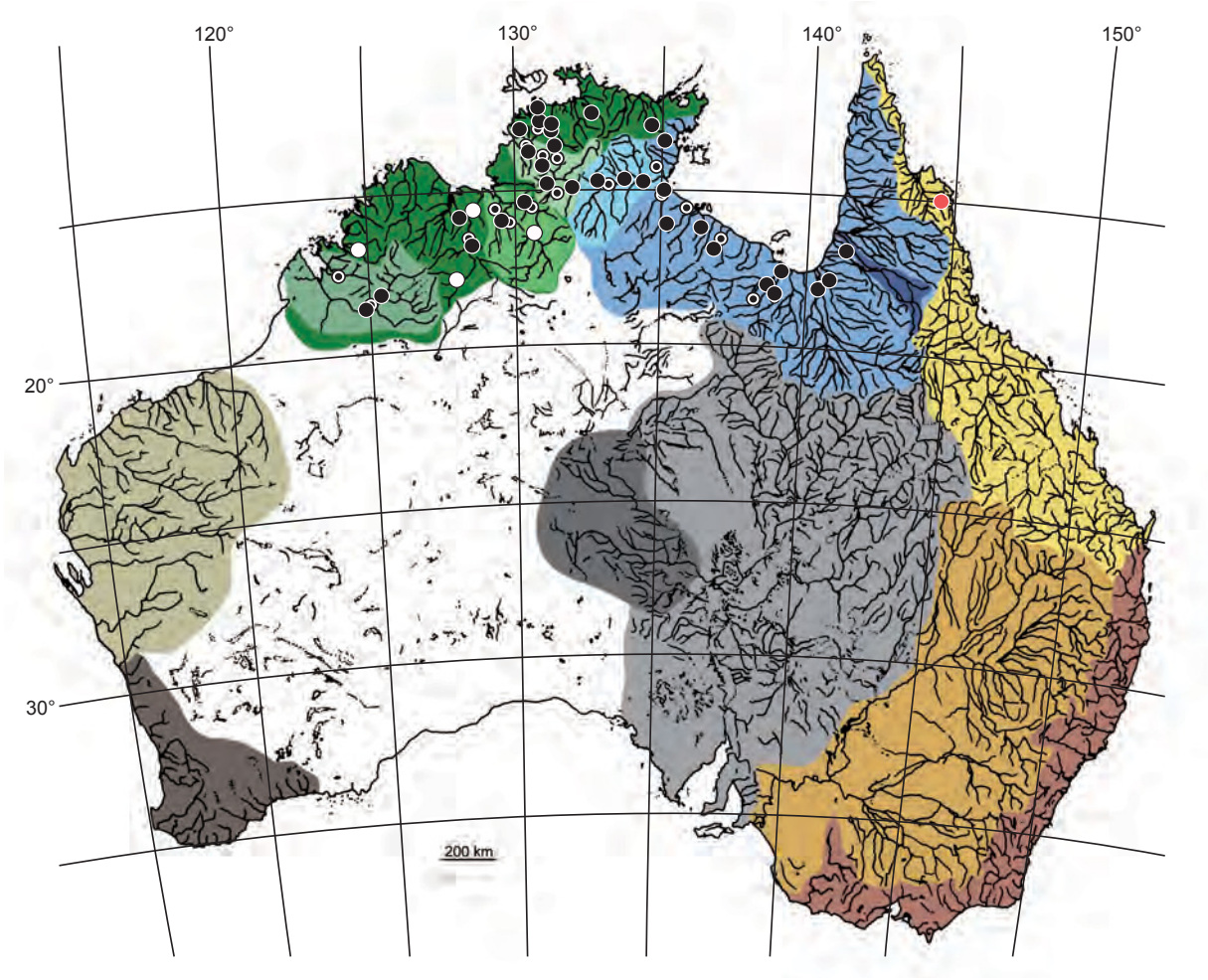

Figure 16 Geographic range of *"Thiara" australis*. Some dots represent multiple nearby localities. Black dots represent genetically confirmed localities, white ones dry material and white ones with a black inner circle wet material. The red dot on the East coast shows the locality in the *P. balonnensis* range from which mt-sequences cluster with *"Thiara" australis* sequences (shell is shown in fig.17d).

Comments: In 2009, the distribution of *"Thiara" australis* in comparison to *Plotiopsis balonnensis* was stated to be "apparently completely exclusive, thus vicariant (allopatric)." The genetic results are inconsistent with this statement, giving hints to

"Thiara" australis occurrences in the Coral Sea drainage. In three cases sequences originating from localities in this *P. balonnensis* range have turned out to have typical mt-data of *"Thiara" australis*. Note that the preliminary identification in the field as *P. balonnensis* being easily misidentified as *"Thiara" australis* was done based on geography only. None of these three extractions and PCRs were conducted by the author but they were repeated. For one (ZMB 107259-1) the second extraction didn't work but for the second (ZMB 107244-2) the result of the rerun showed that there was a mix-up in the lab and that the sequence does not belong to *"Thiara" australis*. As it cannot be ruled out that this is also the case for the sample for which rerunning failed, these two are not discussed further. However, in the third case, i.e. ZMB 106347-2 from Three Mile/Poison Creek (see fig.17d), the second extraction confirmed that the sequence from this *P. balonnensis* range equates to typical mt-data of *"Thiara" australis*. In addition, in 2011 both species were found at the same time and locality (see for more details under *P. balonnensis* section).

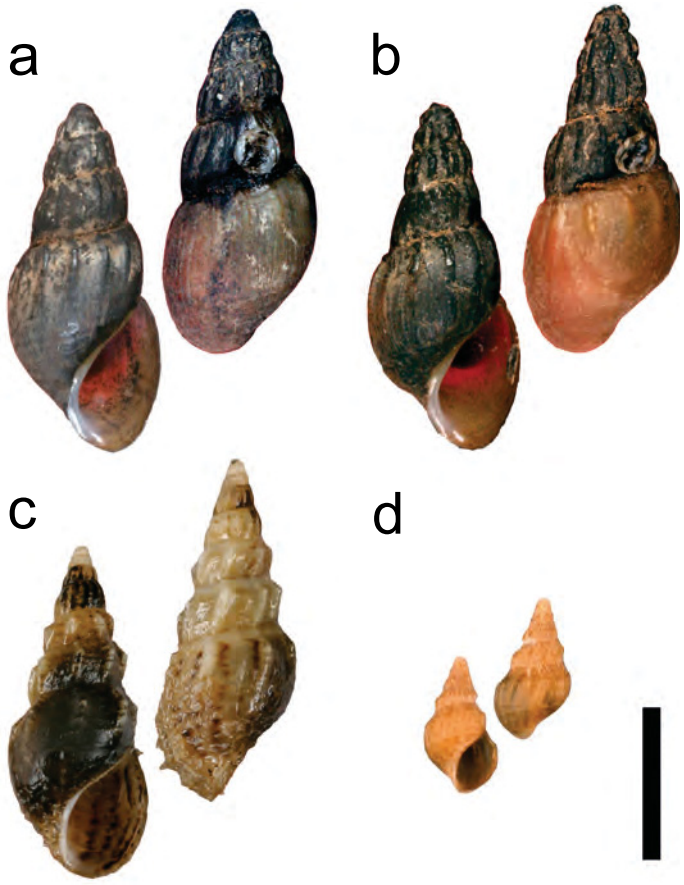

Figure 17 Shells of *"Thiara" australis*. **a./b.** NT: Daly River, Oolloo crossing (ZMB127748), Shells resemble those of *M. onca*, they are partly cracked, the red gleam comes from modeling clay on which shells are fixed for photograhy. **c.** QL: Bynoe River (ZMB 107579-1) sampled in 2009, locality in *"Thiara" australis* range where *P. balonnensis* mt-sequence was found in 2011 (see fig.27) **d.** QL: Three Mile-Poison Creek (ZMB 106347-2), in *Plotiopsis balonnensis* range, mt-sequences cluster with *"Thiara" australis* sequences. Scale: 1 cm

Table 8 Locality data for the material examined of *"Thiara" australis*. Bold ZMB numbers are genetically confirmed. Cr-Creek; Hw-Highway; Riv.-River; Rd- Road

Locality	**Coordinates**	**Museum n°**
WA: Kimberley	n.d.	BMNH
WA: Fitzroy Riv.	n.d.	BMNH 1841.11.74.93.103; AMS
WA: Geike Gorge, Fitzroy Riv.	18°05 S 125°43 E	AMS C.324388; VK 0951
WA: Geiki Gorge	18°06.521 S 125°41.891 E	ZMB 106696, 127510
WA: Fitzroy Riv., Fitzroy crossing township	n.d.	AMS C.**427355**
WA: Bank of Fitzroy Riv. at Sheep Yard Camp	n.d.	WAM 459-80
WA: Fitzroy crossing	18°12.653 S 125°34.74 E	ZMB 106693,127505
WA: Lennard Riv., near Windjana Gorge	17°25 S 124°50 E	ZMB 127506
WA: Isdell Riv.	n.d.	AMS
WA: Charley Riv., 25.3 km WSW of Mt. Blythe	16°22.35 S 125°12.35 E	VK 12.386
WA: Billabong on side of Weber Plains Rd	15°40.35 S 128°44.39 E	AMS
WA: Fowl Yard, Osmand Ck.	17°16 S 128°30 E	AMS
WA: Ord Riv., W of Riv.	15°47.480 S 128°41.580 E	AMS C.**427964**, 114695; ZMB 127512
WA: Ord Riv., Ivanhoe crossing	15°41.22 S 128°41.23 E	AMS; VK 24.180
WA: Kununurra, Lake behind Diversion Dam	15°47.51 S 128°41.94 E	AMS C.324387
WA: Ord Irrigation area, Kununurra	n.d.	WAM 728-77
WA: Farber Beach, Ord Riv., Kununurra	n.d.	AMS
WA: Lake Kununurra	15°47.340 S 128°43.005 E	ZMB **106692**
NT: North Australia	n.d.	BMNH 1857.9.30.9
NT: N.T.	n.d.	AMS C.26019, C.26017
NT: East Baines Riv., at crossing	15°45.737 S 130°1.75 E	ZMB 106697, 127513
NT: Victoria Riv.	n.d.	BMNH 1844.12.27.1.11, 1857.11.18.130, 1857.9.30.10
NT: Victoria Riv., on Victoria Hw	15°36.890 S 131°07.820 E	AMS C.427957, 427657
NT: Victoria Riv., bridge	n.d.	WAM 458-50
NT: Victoria Riv., crossing	14°45.46 S 131°35.68 E	AMS C.22645; VK 24.182
NT: Victoria Riv., Timber Cr	15°38.203 S 130°28.529 E	ZMB 127744
NT: Victoria Riv., Big Horse Cr	15°36.878 S 130°23.7 E	ZMB 127621
NT: Victoria Riv., old crossing	15°34.862 S 131°06.142 E	ZMB 106619, 127735
NT: Victoria Riv., Victoria Riv. Gorge	15°37.79 S 131°08.099 E	ZMB 106621
NT: Victoria Riv., 195 km W of Katherine	n.d.	AMS C.324389
NT: Victoria Riv., Bulita Station	16°07 S 130°25 E	NTM P7843
NT: Victoria Riv., Dashwood crossing	16°20.02 S 131°06.86 E	AMS
NT: Victoria Riv., Top Spring, Timber Rd	n.d.	AMS
NT: Port Essington	n.d.	BMNH
NT: Port Darwin	n.d.	AMS
NT: Howard Springs, 66 km E of Stuart Hw	12°27.5 S 131°30 E	AMS C.324390, 110490, ex 68505
NT: Margins of Howards Springs	12°27.5 S 131°03.0 E	NTM 27451
NT: Howard Springs Cr	12°27.268 S 131°03.108 E	ZMB 106594, **107628**, 127736
NT: Howard Cr, 30 miles from Darwin	n.d.	AMS
NT: Howard Riv., crossing	12°27.752 S 131°05.008 E	ZMB **106596**, **106598**, 106701, **106702**
NT: Berry Springs, S of Darwin	12°42.111 S 130°59.854 E	ZMB **106704**, **107290**, 107592, 127730; AMS
NT: Darwin Riv., 46 Rd miles from Darwin	12°44.56 S 130°57.88 E	NMT P27467, AMS
NT: Darwin Riv., Weed Quad. 1, 2, 4	n.d.	NMT P27469, P27472, P27468, P27470, P27473
NT: Coomalie Cr, Stuart Hw	13°0.88 S 131°07.04 E	AMS C.324138
NT: Stuart Hw	13°01 S 131°07.5 E	NTM P6467
NT: Finnis Riv., NW of Batchelor	13°01.316 S 130°57.093 E	ZMB 106648, 106649, **106664**
NT: Crater Lake, NE of Adelaide Riv.	13°02.76 S 131°05.445 E	ZMB **106659**
NT: Rum Jungle at Litchfield Rd	13°02.604 S 130°59.862 E	ZMB **106661**
NT: Adelaide Riv.	n.d.	BMNH 1891.11.21.153-166,

Locality	Coordinates	Museum n°
		1892.1.29.194
NT: Adelaide Riv., downstream from crossing	13°08.742 S 131°13.14 E	ZMB 106610
NT: Adelaide Riv.	13°10.353 S 131°11.501 E	ZMB **107554**
NT: Adelaide Riv., S at crossing	13°28.975 S 131°5.853 E	ZMB 106611
NT: Scott's Cr, 9 km E of Adelaide R	n.d.	VK 0969
NT: Glass Water Swamp, Litchfield Station	13°19.52 S 130°32.57 E	VK 24.370
NT: Bamboo Cr, 3-10 m from Daly R	13°40.118 S 130°39.501 E	ZMB 106612, 106672, 106674, 107263; VK 24.394
NT: Douglas Riv. crossing	13°40.09 S 130°39.59 E	AMS; VK 24.369
NT: Douglas Riv. crossing, Bond Bridge	13°47.36 S 131°21.185 E	ZMB 106615
NT: Douglas Riv., crossing to Tipperary Station	13°50.22 S 131°09.71 E	VK 24.375
NT: Junction of Douglas and Daly Rs	13°50.26 S 131°08.49 E	NTM P27450
NT: Daly Riv. crossing	13°46.026 S 130°42.688 E	ZMB 106670, 106710, 106715, **107264**
NT: Daly Riv., Oolloo crossing	14°4.24 S 131°15.056 E	ZMB 106666, **107289**, **127748**, 127633
NT: Daly Riv. at Kathleen Falls	14°45.46 S 131°35.65 E	AMS C.21440, 324384
NT: Kathleen Falls, Flora Riv.	14°45.412 S 131°35.791 E	ZMB 106618, 127731; VK 24.179, 24.181
NT: Flora Riv., below Kathleen Falls	14°43.992 S 131°36.487 E	ZMB 127746
NT: Flora Riv., c. 18km from Djarrung campground	14°40.092 S 131°40.963 E	ZMB 127745
NT: Katherine Riv., at Katherine	14°29.441 S 132°14.991 E	ZMB **107288**, 127747
NT: Katherine Riv., downstream Land Bridge	14°29.49 S 132°14.73 E	ZMB **106698**
NT: Lagoon, King Riv., S Katherine	n.d.	AMS
NT: Cr, SW of Katherine, fossil	n.d.	AMS
NT: Cr, SE of Katherine, fossil	n.d.	AMS
NT: Roper Riv.	n.d.	AMS C.109742
NT: Elsey Falls, Roper Riv. on Elsey Station	n.d.	VK 0957
NT: Elsey Cementery, S of Mataranka Springs	15°05.15 S 133°07.44 E	AMS C.339837
NT: Elsey Riv., at Elsey Cementery	n.d.	ZMB 106652
NT: Warloch Ponds on Elsey Cr	16°5.042 S 133°7.258 E	ZMB 192014, 127632
NT: Waterhouse Riv., Stevie‚Äôs Hole	14°55.782 S 133°8.732 E	ZMB 106680, **107287**, 107612
NT: Roper Riv., at Botanic Walk	14°56.126 S 133°8.532 E	ZMB 127727
NT: Little Roper Riv.	14°55.581 S 133°7.176 E	ZMB 106627, **106630**, 106677, **107285**, **107286**, **107560**, 127726, 127730; AMS C.317321
NT: Little Roper Riv., south bank	14°55.59 S 133°7.14 E	ZMB 127516, 127719, 127721, 127735
NT: Mataranka, 1 km of Kowai Roper Ck.	14°55.74 S 133°7.06 E	AMS C. 5776, 324383
NT: Roper Riv. at 4 Mile Point	14°56.137 S 133°10.033 E	ZMB **106625**, **107284**, 107567, 127722, 127742
NT: Wabalarr, Roper Riv.	14°45.028 S 133°10.44 E	ZMB **107620**, 127724, 127743
NT: Mulurark Rapids, Roper Riv.	14°56.68 S 133°12.38 E	AMS C.324385; VK 24.384-386; ZMB 127729, 127741
NT: Roper Riv., at Jalmurark Campground	14°57.158 S 133°13.29 E	ZMB **106675**, **107265**, 107559
NT: Roper Riv., 2km below Jalmurark	14°57.515 S 133°14.275 E	ZMB 127732
NT: Roper Riv., Roper Falls	14°57.401 S 133°15.018 E	ZMB **107283**, **107556**, 127723
NT: Salt Cr near Elsey Cr	15°0.703 S 133°14.417 E	ZMB 106631, 106633, 106683, 106684, **107266**, 127740
NT: Elsey Cr on Roper Hw	15°0.627 S 133°15.096 E	ZMB **106707**, 107267, 127717
NT: Roper Riv. at Roper Bar	14°42.802 S 134°30.474 E	ZMB 106635, 106708, **107268**, 127733; AMS
NT: Roper Riv., Mountain Cr	14°46.543 S 134°48.016 E	ZMB 127739
NT: East Alligator Riv., at crossing to Arnhem Land	12°25.542 S 132°57.882 E	ZMB 106642
NT: Goyder Riv., Arnhem Land	n.d.	AMS
NT: Koolatong Riv., Arnhem Land	13°9.575 S 135°51,839 E	ZMB **107291**
NT: E Goyder Riv. crossing, Arnhem Land	13°02.70 S 134°97.7 E	AMS C.**461368**
NT: Rose Riv. catchment, Arnhem Land	13°43.40 S 135°06.2 E	NTM P8703

Locality	Coordinates	Museum n°
NT: Mumpumapu waterhole, Arnhem Land	14°38.3 S 135°32.6 E	AMS C.**461355**
NT: Wandoo Riv., Arnhem Land	14°14.02 S 135°36.11 E	AMS C.461357
NT: Arnhem Land track to Arthur rd. and Numbulwar	13°10.5 S 135°72.6 E	AMS C.461365
NT: Wilton Riv., Arnhem Land	n.d.	VK 0956
NT: ca 8 km NE of Towns Riv. crossing	14°59.82 S 135°16.28 E	ZMB 192015
NT: ca 3.8 km NE of Towns Riv. crossing	15°1.199 S 135°14.136 E	ZMB **190010**
NT: Towns Riv., at crossing	15°2.57 S 135°12.718 E	ZMB 106640, **107269**, 127623
NT: Towns Riv., at boat ramp	15°2.09 S 135°13.161 E	ZMB 127718
NT: Towns Riv., downstream	14°59.839 S 135°16.262 E	ZMB 127737
NT: Towns Riv., northern bank	14°59.792 S 135°17.156 E	ZMB 127738
NT: Towns Riv., backwater at junction with Cr	14°59.999 S 135°17.03 E	ZMB 127728
NT: Cox Riv. crossing, billabong 2 km SE	15°20.30 S 135°21.15 E	VK 25.912
NT: Limmen Bight Riv., at Rd crossing	15°28.865 S 135°24.054 E	ZMB 107271, AMS
NT: Booroloola, McArthur Riv. crossing	16°04.866 S 136°19.026 E	ZMB **107272**, **107569**; VK 24.374
NT: Borroloola, junction Rocky Cr./McArthur R.	16°05.00 S 136°18.50 E	VK 24.381
NT: Wearyan Riv., along beach at crossing	16°10.02 S 136°45.481 E	ZMB **107273**
NT: Foelsche Riv.	16°12.628 S 136°53.034 E	ZMB 107274
NT: Robinson Riv., at crossing	16°28.27 S 137°02.932 E	ZMB **107275**, **107573**
NT: Calvert Riv., below junction with Bluey Ck	16°56.066 S 137°21.578 E	ZMB **106709**, **107276**; AMS
QL: Gregory Riv., SE of Burketown	17°53.517 S 139°17.209 E	ZMB 107277
QL: Gregory Riv., Beame Brook	17°52.708 S 139°20.576 E	ZMB **127629**, 107578
QL: Gregory Riv. at Gregory Downs	18°38.695 S 139°14.875 E	ZMB **107278**, 107628, **127628**
QL: Lawn Hill Cr at Adels' Grove	18°41.365 S 138°31.81 E	ZMB 106705, **127622**
QL: Lawn Hill Cr, nr the Cascades	18°42.00 S 138°29.00 E	VK 26.353, 26354
QL: Lawn Hill, Boodjamulla Cr	18°42.051 S 138°29.196 E	ZMB **107281**, 127627
QL: Gregory Riv. at Riversleigh	19°01.116 S 138°43.529 E	ZMB **107279**, 107576, 127624-26
QL: Gregory Riv. crossing, Rsleigh Station	19°01.25 S 138°43.22 E	VK 26.357, ZMB **106706**
QL: O Shanassy Riv.	19°01.354 S 138°45.741 E	ZMB **107280**, **107574**
QL: Judy Lagoon, Armraybald Station	17°57.37 S 139°45.12 E	VK 26.360
QL: small stream, 6.5 km N of Almora	18°14.27 S 139°15.38 E	VK 26.355
QL: Bynoe Riv., at crossing	17°51.53 S 140°47.58 E	VK 26.350, ZMB **107579**, **127630**
QL: Norman Riv., at Glenmore	17°51.228 S 141°08.047 E	ZMB **127631**
QL: Gilbert Riv., at Burke Rd crossing	17°10.117 S 141°45.999 E	ZMB **107282**, VK 26.361

Thiara rudis **(Lea, 1850)**

Type locality: "Hab. Amboyna"; i.e. Indonesia, Molukka, Ambon

Distribution: Given the recent discovery of its occurrence in Australia only five localities on the continent have been known till now (see tab.9 and fig.18)). The species was found in the Northern Territory in Berry Springs (close to Darwin) and on three localities along the Roper River. So far the easternmost locality is in Queensland at the O'Shanassy River. Outside of Australia *Thiara rudis* has a very wide distribution in south and southeast Asia as reported from India, Sri Lanka, Myanmar, Cambodia, Indonesia and the Philippines.

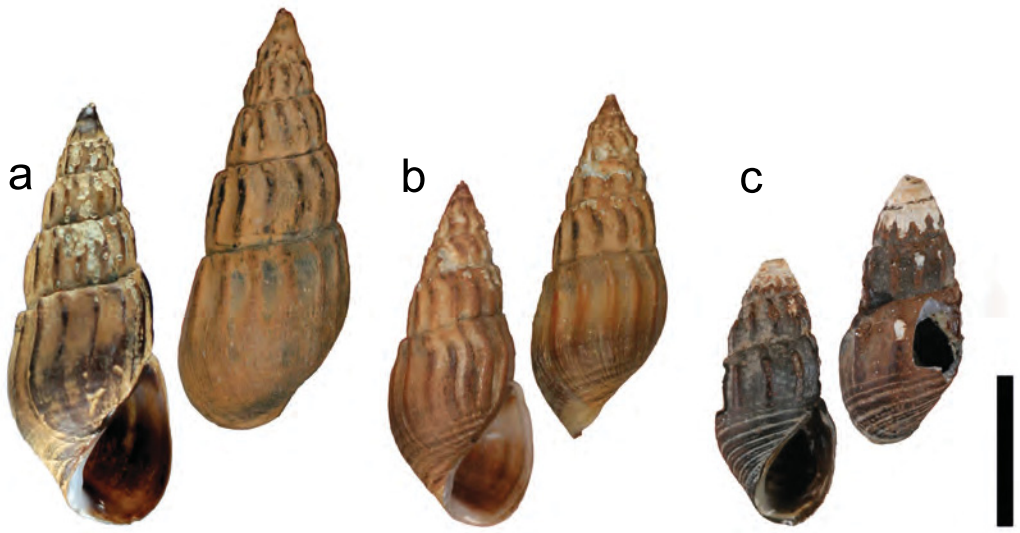

Figure 18 Shell morphology of *Thiara rudis* in Australia. **a./b.** NT: Wabalarr, Roper River (ZMB 107617).**c.** NT: Berry Springs (ZMB 106599).Scale: 1 cm

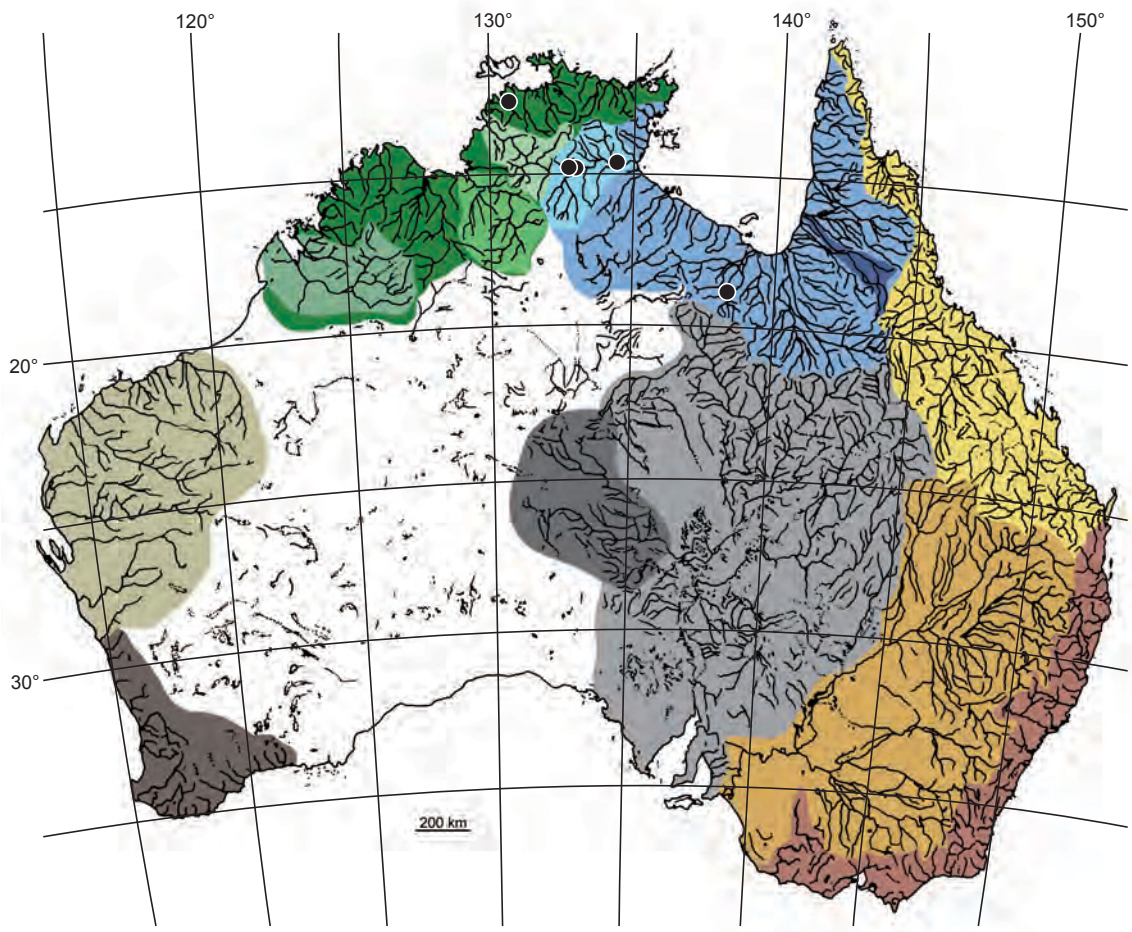

Figure 19 Geographic range of *Thiara rudis* on the Australian continent. Note that here for the first time this species' occurrence in Australia is recorded. Given its recent discovery only five localities on the continent have been known till now which are all genetically confirmed.

Comments: Here for the first time the thiarid *Thiara rudis* is recorded and verified as taxa with occurrences in Australia. It could be identified by molecular data, clustering with *T. rudis* sequences from Indonesia (see fig.57 in appendix). With the knowledge of its occurrence, the species could be selectively and successfully searched for on the field trip in 2011.

Table 9 Locality data for the material examined of *Thiara rudis*. Bold ZMB numbers are genetically confirmed. Asterisk (*) after the museum number indicates that the individual was assigned to a different species in the field.

Locality	Coordinates	Museum n°
NT: Berry Springs, SE of Darwin	12°42.111 S 130°59.875 E	ZMB **106704***, **106599***, **127616**
NT: Salt Creek at junction	14°57.453 S 133°15.095 E	ZMB **127619**, **127636***, **127637***
NT: Wabalarr, Roper River	14°56.028 S 133°10.44 E	ZMB **107614***, **107617***, **127645***
NT: Roper River, Mountain Creek	14°46.543 S 134°48.016 E	ZMB **127620**
QL: O'Shanassy River	19°1.354 S 138°45.741 E	ZMB **107280***

***Melanoides tuberculata* (O.F. Müller, 1774)**

Type locality: "In littore Coromandel"; i.e. India, Coromandel Coast.

Distribution: *Melanoides tuberculata* has a very broad distribution, being found in northern and southern Africa, eastern Mediterranean countries, the Arabian Peninsula, south and southeast Asia, southern China, Japan, Malaysia, and northern Australia. *M. tuberculata* has become widely invasive in the tropics outside of its native range (Facon et al., 2003). In the Neotropical region, it occurs in most countries between the southern states of the USA and Argentina. Its occurrence in Australia is verified for more and more samples. The species is found in the north of Western Australia, in several localities near Darwin and along the east coast of Arnhem Land in Northern Territory. Apparently it is missing in the eastern part of the Gulf of Carpenteria drainage (see fig.21). Its identity on the East Coast, that means all records from Queensland and New South Wales, so far is only based on shell morphology (see shell fig.22). The extensive attempts to get sequences from the eastern localities failed due to the lack of fresh material.

Comments: Because of its spotty occurrences on the continent with vicinity to larger cities, it was assumed that the species is a possible recent introduction to Australia. Now the findings show that it (or at least its typical mt-haplotypes) appears more often than thought. In the field the snails of this species have often been mixed-up with *"S." denisoniensis*. Although co-occurring they look quite different, as can be seen in fig.20. A first idea was, that the records from the East coast might all have been confused

with *"S." denisoniensis* samples, but the appearance of the shell (see fig.22) shows that in fact it seems to belong to *M. tuberculata*.

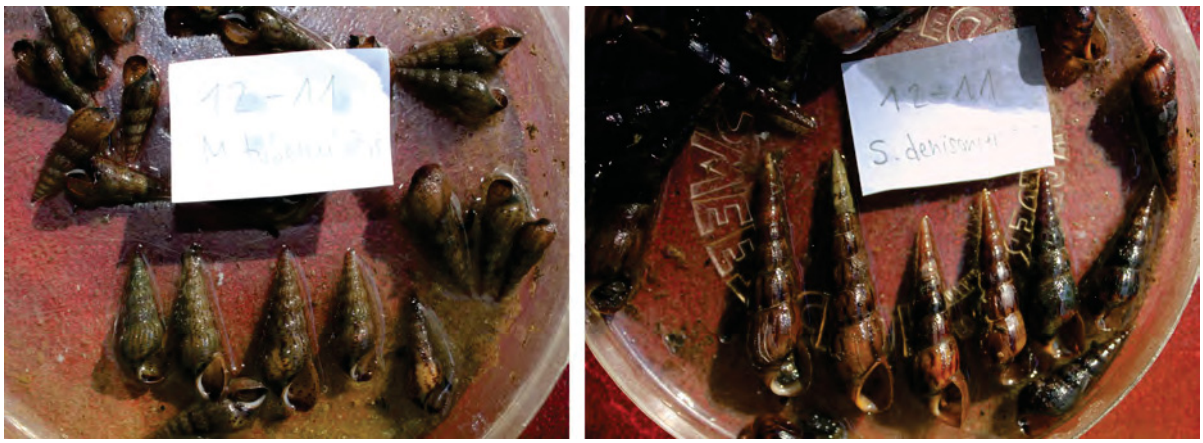

Figure 20 Picture of *Melanoides tuberculata* (left side) and *"Stenomelania" denisoniensis* (right side) from locality where they co-occur. "12-11" is the code for the sampling locality "Bitter Springs".

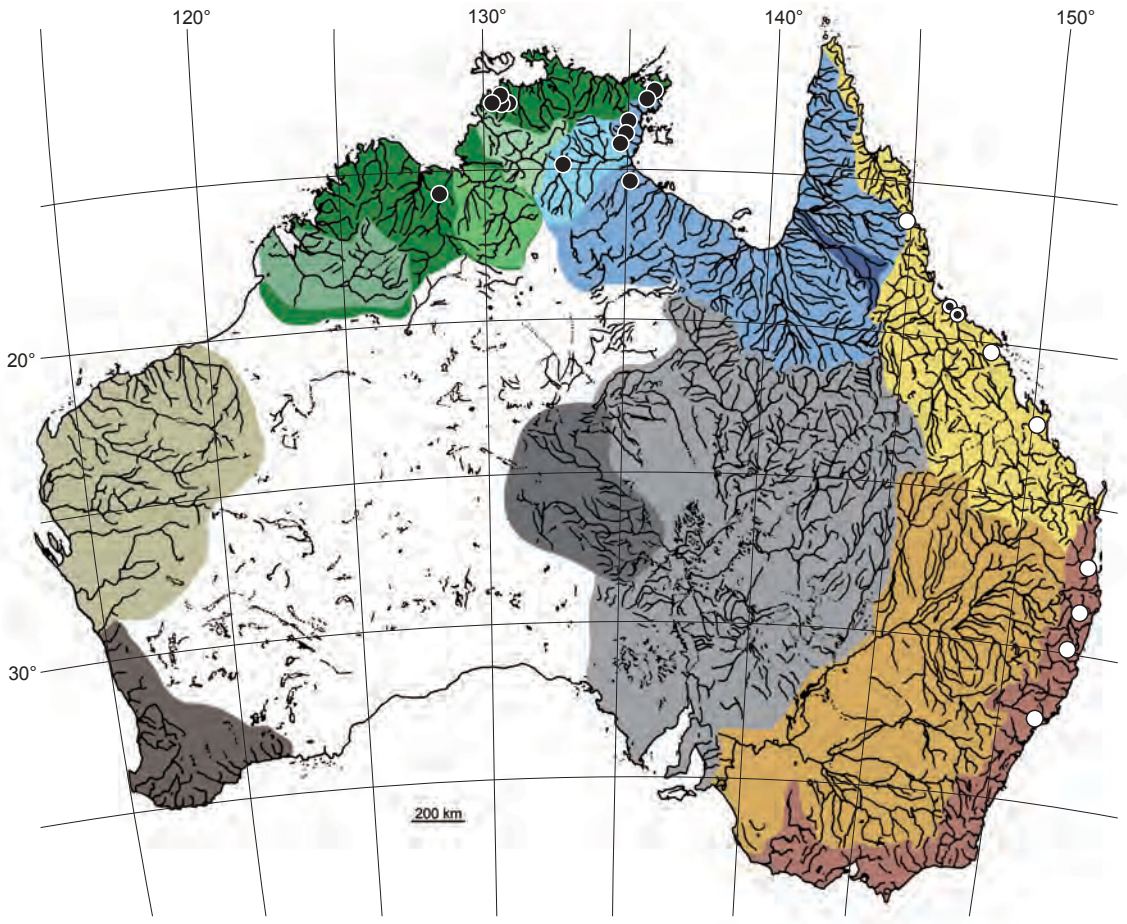

Figure 21 Distribution map of *Melanoides tuberculata* in Australia. Black dots represent genetically confirmed localities, white ones dry material and white ones with a black inner circle wet material. The extensive attempt to get sequences from the east coast failed (see text).

Figure 22 Shell of *Melanoides tuberculata* from the East coast of Australia, i.e. Frenchman Creek, Rockhampton in Queensland (AMS C414973). Extraction failed probably due to age, as it was sampled in 2002. Scale: 1 cm.

Table 10 Locality data for the material examined of *Melanoides tuberculata*. Bold ZMB numbers are genetically confirmed. Asterisk (*) after the museum number indicates that the individual was assigned to a different species in the field.

Locality	**Coordinates**	**Museum n°**
WA: Lilly Lagoon, Kununurra	15°46.825 S 128°44.477 E	ZMB **106690**
WA: Lake Ord, Ord River, at Kununurra	15°47.203 S 128°44.163 E	ZMB 127511
NT: Darwin, George Brown Botanic Garden	12°26.739 S 130°50.179 E	ZMB 103694, **106592**
NT: Howard Springs, Spring creek	12°27.553 S 131°3.069 E	ZMB **127659***
NT: Darwin River	12°44.527 S 130°57.93 E	ZMB **106726***
NT: One Mile Railway Dam, Darwin	12°27.39 S 130°50.44 E	AMS C.322948
NT: Darwin, Ludmilla Creek	12°25 S 130°50.5 E	AMS C.307840
NT: Berry Springs on Stuart Highway	12°42.309 S 131°0.401 E	ZMB 107545
NT: Manton River	12°50.282 S 131°7.998 E	ZMB **106603***
NT: Blacksoil Plan, Adelaide River	12°39.7 S 131°20.3 E	AMS C.322949
NT: Springvale Hmst, Katherine	14°30.09 S 132°13.72 E	AMS C.322950
NT: River, E of Maturanka	14°42.94 S 34°30.44 E	AMS C.317344
NT: Arnhem Land, ''RR road"	14°14.07 S 135°36.18 E	AMS **461367***
NT: Arnhem Land, Gove Peninsula	12°14.94 S 136°53.28 E	AMS **461356***
NT: Arnhem Land	14°9.12 S 135°42.06 E	AMS **461372***
NT: Arnhem Land, Mumpumampu	14°22.98 S 135°19.56 E	AMS **461354***
NT: Arnhem Land Nhulunbry	12°10.86 S 136°47.1 E	AMS **461350***
NT: Bitter Springs	14°54.642 S 133°5.362 E	ZMB **127613**
NT: CoxRiver	15°19.394 S 135°20.699 E	ZMB **107240***
QL: Buchans Pt., N Cairns	n.d.	AMS
QL: Crystal Creek, Paluma, N Townsville	n.d	ZMB 104144
QL: Alice River, 25 km W Townsville	19°20 S 146°40 E	ZMB 104145
QL: Airlie Beach	n.d	AMS
QL: Frenchman Creek, Rockhampton	23°21 S 150°34 E	AMS C.414973
QL: Brisbane	n.d	AMS
NSW: Cudgen Lake, south end	28°19.66 S 153°33.47 E	AMS C.337978
NSW: Yamba, near Grafton	n.d	AMS
NSW: Sydney, St. Peters, Brown Street	n.d	AMS

***Melasma onca* (Adams & Angus, 1864)**

Type locality: "North Australia, tributary of Adelaide River"

Distribution: *Melasma onca* is endemic to the tropical wet northern part of the Northern Territory of Australia. Its geographic range is restricted essentially to the inland areas of the western Leichhardtian fluvifaunal province, including Timor Sea, but also Gulf of Carpentaria drainages. Here the species is found in the river systems of the Daly and the Roper River drainage, from the Adelaide River and South Alligator River eastwards to rivers in Arnhem Land (see fig.23). Indicated by dry shells in the AMS, there is an isolated occurrence at Robinson River, south of the Gulf of Carpentaria. This could not be verified by own collections in 2007, 2009 and 2011 and this survey provides no evidence for a distribution in this area or outside of the Northern Territory.

Comments: It should be noted that *Melasma onca* is (with the exception of the three species only occurring in the Jardinian fluvifaunal province, i.e. *Ripalania queenslandica*, *Thiara amarula* and *Stenomelania* cf. *aspirans*) the only one that can be easily diagnosed and distinguished from other Australian thiarids. Each individual that was assigned to this taxa in the field and molecularly examined, turned out to be, indeed, part of it.

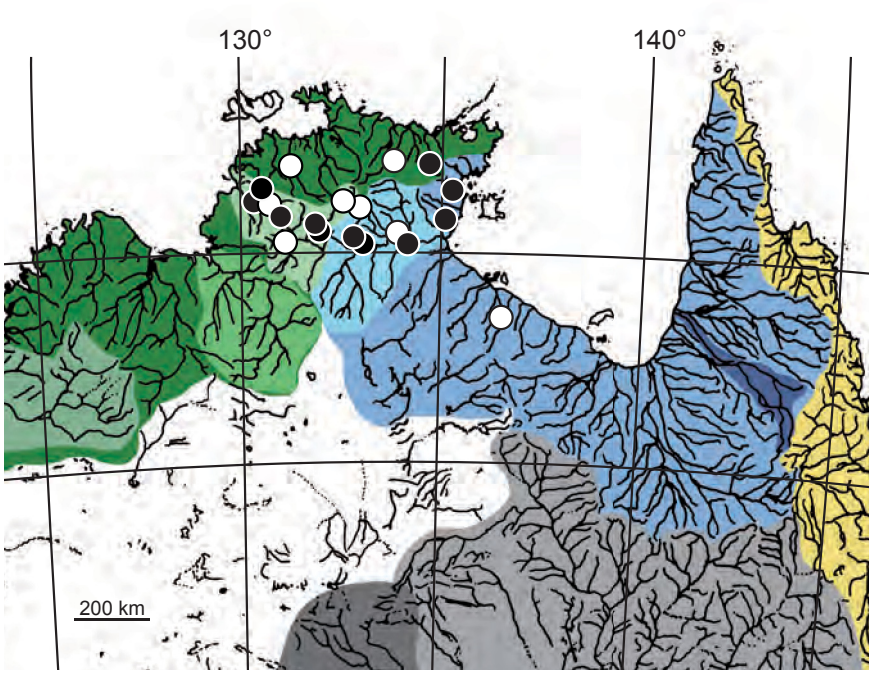

Figure 23 Distribution map of *Melasma onca*. Black dots represent genetically confirmed localities, white ones dry material. Some dots represent multiple nearby localities.

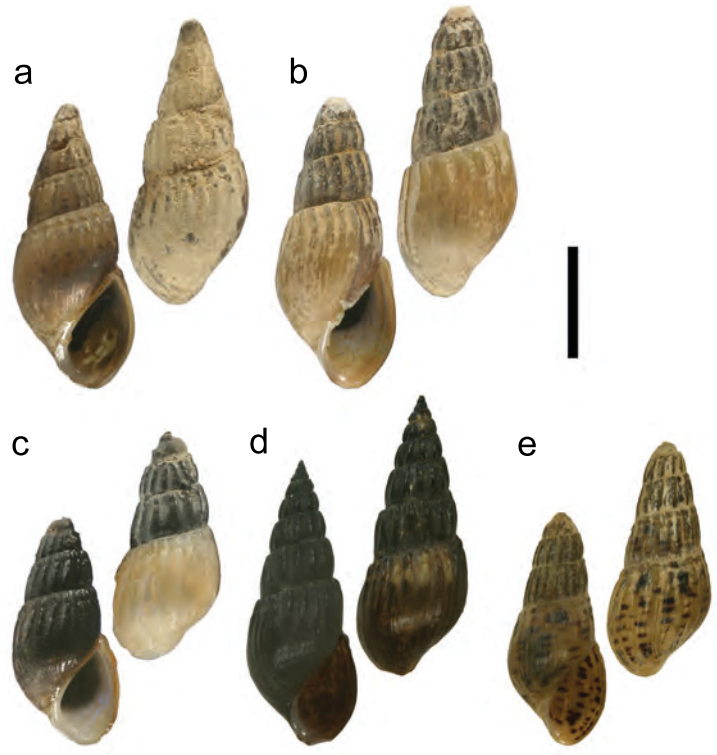

Figure 24 Shells of *Melasma onca* of individuals that were genetically confirmed. **a.** NT: Roper River (ZMB 107223). **b.** NT: Daily River crossing (ZMB 106711). **c./d.** NT: Bamboo Creek (ZMB 106673). **e.** NT: Roper River (ZMB 106636). Scale: 1 cm

Table 11 Locality data for the material examined of *Melasma onca*. Bold ZMB numbers are genetically confirmed. Cr.-Creek; Riv.-River

Locality	Coordinates	Museum n°
NT: Daly Riv. crossing	13°46.026 S 130°42.688 E	ZMB **107549**, **106671**, **106711, 107221**
NT: Bamboo Cr., 3-10 m from Daly R	13°40.118 S 130°39.501 E	ZMB **107550**, **106673**
NT: Oolloo Crossing, Daly Riv.	14°04.24 S 131°15.056 E	ZMB 106667, **107227**, 127749
NT: Kathleen Falls, Flora Riv.	14°45'33" S 131°35'36" E	VK 24.183, ZMB 127753
NT: Flora Riv., below Kathleen Falls	14°43.992 S 131°36.487 E	ZMB 127754
NT: Flora Riv., near junction	14°40.092 S 131°40.963 E	ZMB 127755
NT: Katherine Riv.	13°43 S 132°58 E	NTM P15835
NT: Katherine Riv., at Katherine	14°29.441 S 132°14.991 E	ZMB **106617**, 106699, 127752
NT: South Alligator Riv., Coronation Hill	13°36 S 132°36 E	AMS C.323838
NT: Roper Riv.	n.d.	AMS C.109742
NT: Arnhem Land, Waterhouse	n.d.	ZMB 19084
NT: Waterhouse Riv., E of Mataranka	14°56' S 133°7' E	AMS C.317348
NT: Stevie's Hole at Waterhouse Riv.	14°55.782 S 133°08.732 E	ZMB 106681, 106682, **107226**, 107613; AMS C.317333
NT: Little Roper Riv., at crossing	14°55.581 S 133°7.176 E	ZMB 106628, 127760
NT: Roper Riv., at 4 Mile Point	14°56.137 S 133°10.033 E	ZMB 106626, **107225**, 107565, 127751
NT: Wabalarr, Roper Riv., E of 4 Mile Point	14°56.028 S, 133°10.44 E	ZMB 107587, 107610, **107619**
NT: Roper Riv., Mulurark	14°56.771 S 133°12.609 E	ZMB 106622, **107625**, 127761
NT: Elsey Falls, on Roper Riv., Elsey Station	14°57'15" S 133°15'45" E	VK 25.851, 25.841; ZMB **107555**, 127756
NT: Roper Riv., 2km below Jalmurark	14°57.515 S, 133°14.275 E	ZMB 127757
NT: Roper Riv., at Jalmurark Camp Ground	14°57.158 S 133°13.29 E	ZMB 107222
NT: Roper Riv., Roper Falls	14°57.401 S 133°15.018 E	ZMB 107224; VK 24.387
NT: Elsey Park, near junction	14°57'45" S 133°15'02" E	VK 24.388
NT: Roper Riv. at Roper Bar	14°42.795 S 134°30.575 E	ZMB **106636**, **107223**, 127762; VK 701, 26.351, 25.842
NT: Goyder Riv., Arnhem L.	13°01.68 S 134°58.60 E	NTM P24903; VK 10.126
NT: W Goyder Riv. crossing, Arnhem L.	14°14.02 S 135°36.11 E	AMS C.**461364**
NT: E Goyder Riv. crossing, Arnhem L.	13°01.19 S 134°58.34 E	AMS C.**461366**
NT: E Goyder Riv. crossing, Arnhem L.	13°01.37 S 134°58.37 E	AMS C.**461370**
NT: track to Numbulwar, Arnhem L.	13°35.10 S 135°42.18 E	AMS C.**461352**
NT: Ngukurr-Roper Bar, Arnhem L.	14°40.55 S 134°34.19 E	AMS C.461351
NT: Robinson Riv., at road crossing	16°45.5 S 136°59 E	AMS

***Plotiopsis balonnensis* (Conrad, 1850)**

Type locality: "Balonne River, Australia"; river to the west of Brisbane, Queensland.

Distribution: Apparently the species is restricted thus endemic to the Australian continent where its geographic range includes the Greyian, Vlaminghian, Sturtian, and Mitchellian as well as Northeast and Southeast Coast fluvifaunal provinces, with locations widespread in Western Australia, South Australia, Victoria and along the coast of New South Wales (see fig.25).

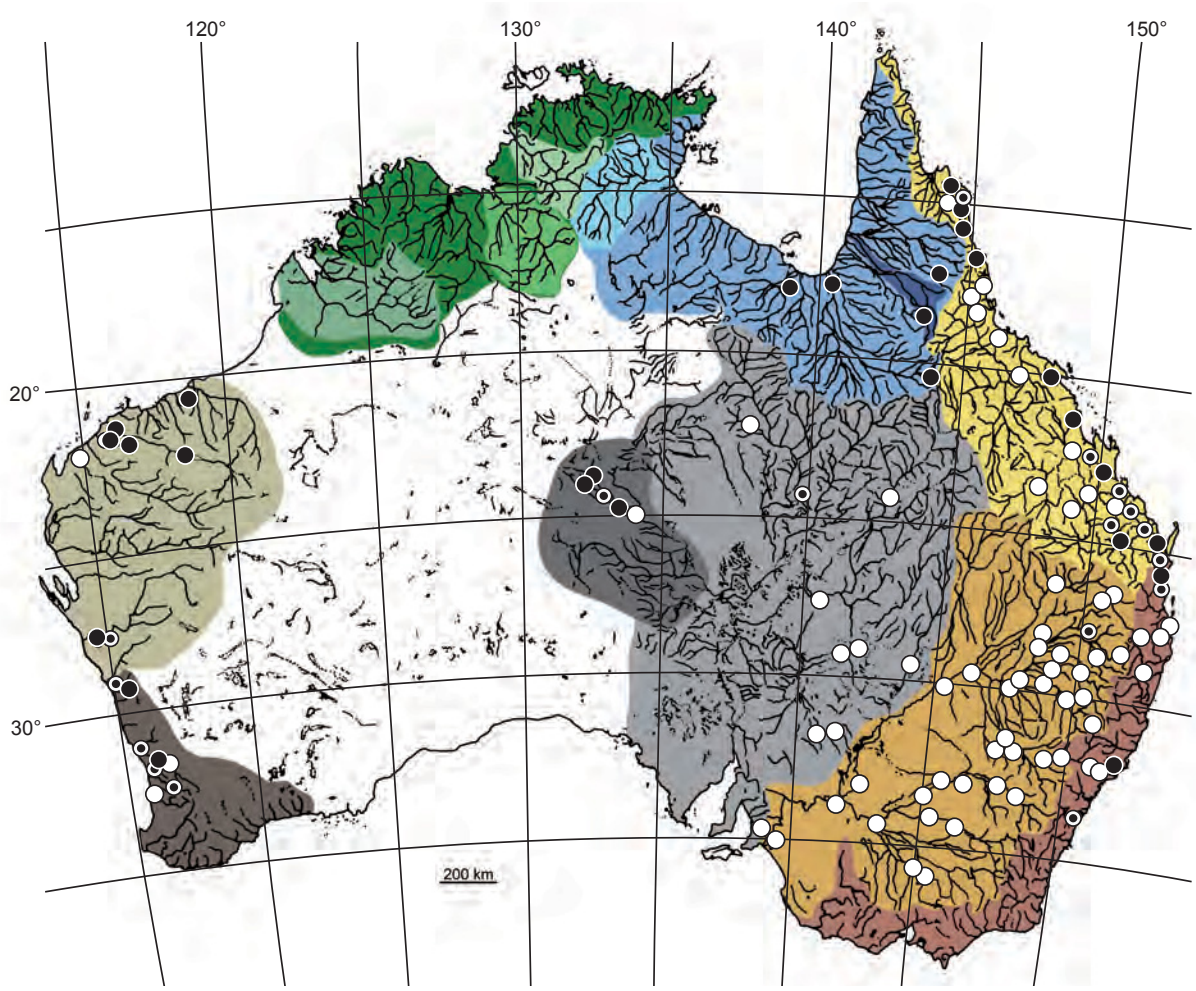

Figure 25 Distribution map of *Plotiopsis balonnensis*. Black dots represent genetically confirmed localities, white ones dry material and white ones with a black inner circle wet material. Some dots represent multiple nearby localities.

Unexpectedly, in the Leichhardtian province, where *"Thiara" australis* clearly dominates and *Plotiopsis balonnensis* apparently is missing, there are two localities near to the Gulf of Carpenteria coast, i.e. Bynoe River and Gregory River where the species could be genetically identified. In addition but known before, in the extreme east of this province in the Great Dividing Range there are locations in the tributaries of the Einasleigh and the

Flinders River. However, *Plotiopsis balonnensis* is notably missing in the entire Timor Sea drainage, where *"Thiara" australis* is widespread. Most remarkable are the isolated occurrences of *P. balonnensis* in the desert central of Australia (i.e. Sturtian or Lake Eyre province), where it could be genetically verified in the Finke River drainage and, in addition, was reported from isolated localities along the Georgina and Diamantina River drainages. These are the only known occurrences in the entire Northern Territory.

Comments: The lacking of *P. balonnensis* in the Leichhardtian province and the apparently completely exclusive distribution of *"Thiara" australis* in this area could be rejected by the molecular study. In addition to the exception of locations in the extreme east of the Leichhardtian province in the Great Dividing Range where *"Thiara" australis* is missing, occurrences at two localities near the Gulf of Carpenteria coast (thus in the *"Thiara" australis* range), i.e. Bynoe River and Gregory River, could be identified for *P. balonnensis*. On the former locality the species could even be found at the same time as *"Thiara" australis* (see fig.27).

It is worth mentioning that on the sampling trip in 2011, no living snails of *P. balonnensis* were found in West Australia. Even on localities where on earlier trips living populations existed (Lake Leschenaultia and Swan River, see tab.12), only empty shells could be collected despite extensive search. One possible explanation is that seasonal fluctuations in water volume might have led to local extinctions of populations by complete desiccation of refugial pools. The bureau of Meteorology of the Australian Government reported that 2009 was the second warmest year on record and that, combined with an annual rainfall very much below average, West Australia suffered from a very serious drought year (http://www.bom.gov.au). Lake Leschenaultia which is a man-made lake, dried up completely in that year, but admittedly along the Swan River (Walyunga National Park) there are a number of large and quite deep permanent freshwater pools which should have the potential as refugial pools.

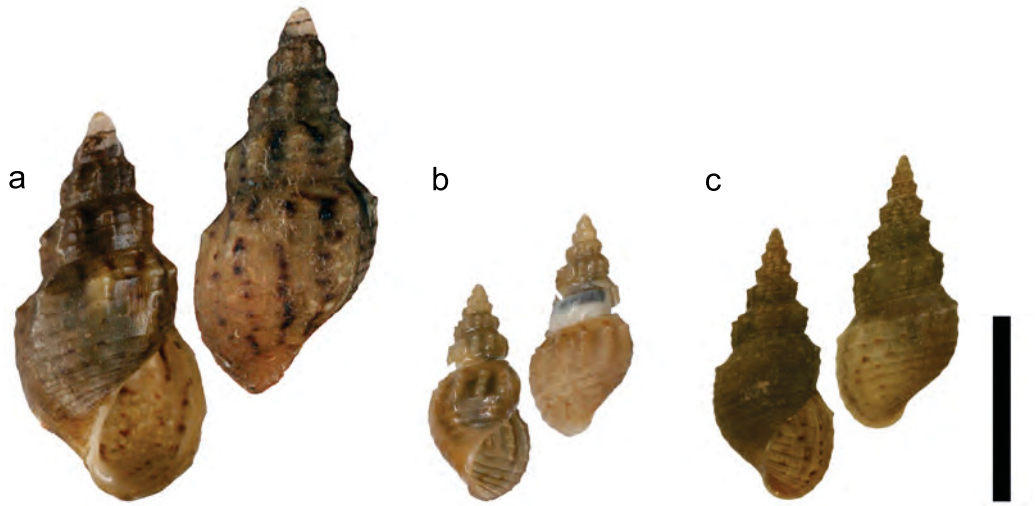

Figure 26 Shells of *Plotiopsis balonnensis* **a.** QL: Mareeba, upper Barron River (ZMB107583-1). **b.** NT: Gregory River (ZMB107277-1), range of *"Thiara" australis*. **c.** NT: Three Mile Point, Finke River (ZMB 106689). Note that in the last case the shown shell belongs to an individual that itself hasn't been genetically confirmed whereas two other individuals from this lot have been confirmed. As the locality and area around is only known for *Plotiopsis balonnensis*, it is quite certain that the shell of which the picture was taken belongs to it, too. Scale: 1 cm

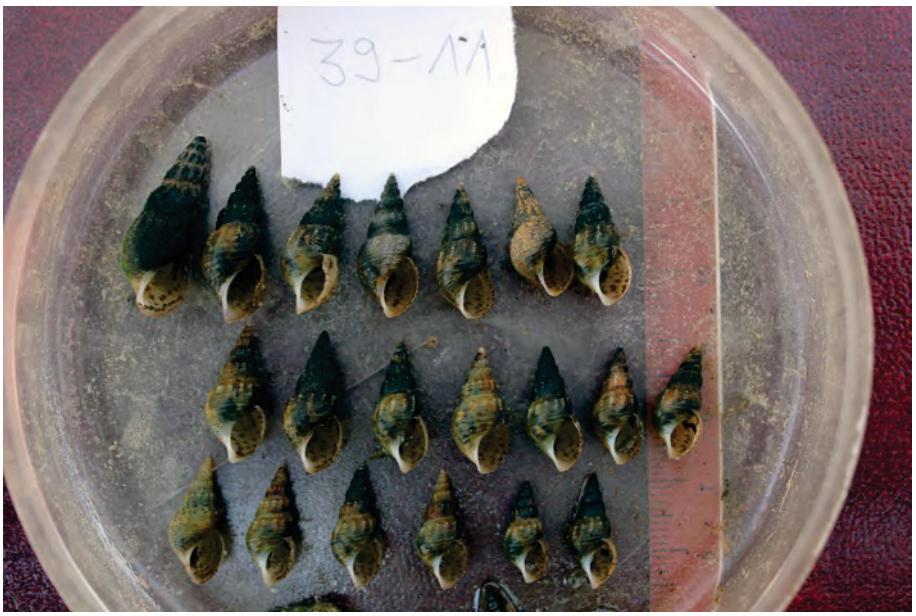

Figure 27 Field picture of location where *P. balonnensis* and *"Thiara" australis* were found simultaneously in 2011. The picture was taken before cracking the shells for preservation with ethanol. Due to this procedure individuals can't be traced back. Out of this lot two individuals were genetically analysed, one turned out to have typical *"Thiara" australis* haplotype and one *P. balonnensis* haplotype."39-11" is the code for the sampling locality "Bynoe River". To be compared with fig.17c., a shell of *"Thiara" australis* from the same locality in 2009.

Table 12 Locality data for the material examined of *Plotiopsis balonnensis*. Bold ZMB numbers are genetically confirmed. Two Asterisks (**) after the museum number show localities where mt-haplotypes of *"Thiara" australis* were found. Cr-Creek; Hwy-Highway; Riv.-River; Rd-Road; NP-National Park

Locality	Coordinates	Museum n°
WA: De Grey Riv.	20°11 S 119°11 E	AMS
WA: De Grey Riv., at crossing of Gt. Nt. Hwy	20°20 S 119°12 E	AMS
WA: De Grey Riv., E of Port Hedland	20°18.665 S 119°15.383 E	ZMB **127507**
WA: Fortescue Riv., at NW Coastal Highway	21°18 S 116°08 E	AMS
WA: Bilanoo Pool, Fortescue Riv., Pilbara Region	21°17.44 N 166°8.4 E	ZMB **107261**
WA: Pilbara Springs, Miaree Pool, S. of Dampier	20°51.15 S 116°36.50 E	AMS
WA: Miaree Pool, Maintland River	20°51.23 S116°36.51 E	ZMB **107262**
WA: Millstream National Park	21°35'40 S 117°04 E	AMS
WA: Fortescue Riv., at Millstream creek	21°35.355 S117°4.33 E	ZMB 127508, 127509, 117731
WA: Hamersley Region, Karijni NP	22°28.521 S 118°33.080 E	ZMB 106727, **106728**
WA: Murchinson Riv., Kalbarri N.P.	27°48.770 S 114°28.540 E	ZMB **106583**
WA: SE of Kalbarri, Murchisson River	27°49 S 114°41 E	ZMB 106584
WA: Murchinson River	27°49 S 114°44 E	AMS
WA: Ellendale Pool, Greennough Riv.	n.d.	WAM 465-80
WA: Ellendale Pool, Greennough Riv.	28°51.63 S 114°58.43 E	ZMB **106582**
WA: 'Perth"	n.d.	BMNH
WA: Swan Riv.	n.d.	BMNH
WA: Avon Riv., NE of Perth, Walyunga	31°44 S 116°4 E	AMS C.60411, 322680, 322655; ZMB 106581; WAM
WA: Walyunga Pool, NE corner	31°44.080 S 116°04.280 E	ZMB 106658, 117732, 117734
WA: Lake Leschenaultia, Chidlow	n.d.	WAM 471-80
WA: Lake Leschenaultia, NE corner	31°51.020 S 116°14.990 E	ZMB 106725
WA: Ashburton Riv., 500m E of NW coastal Hwy	21°58 S 115°01 E	AMS
NT: Finke Creek	n.d.	BMNH 1908.12.8.7-9
NT: Finke Riv., nr Glen Helen	23°25 S 132°16 E	NTM P15965
NT: Glen Helen Reserve	23°25 S 132°16 E	NTM P15858
NT: Finke Riv., at Glen Helen Gorge, nr resort	23°41.322 S 132°40.606 E	ZMB **106687**, 106716
NT: Ormiston Creek, Ormiston Reserve	23°38 S 132°45 E	NTM P15859; AMS
NT: Ormiston Gorge, outlet	23°37.704 S 132°43.375 E	ZMB **106686**, 106717
NT: Hermannsburg	n.d.	BMNH 1909.10.23.9-11
NT: Finke Riv., Hermannsburg Mission	26°20 S 136°00 E	AMS C.322658
NT: Finke Riv., near Hermannsburg	n.d.	AMS
NT: Finke Gorge, SW of Alice Springs	24°03 S 132°43 E	NTM P4250
NT: Finke Riv. at Finke Gorge	n.d.	AMS NT 79-32
NT: Palm Valley, Palm Creek, Krichauff Ranges	24°03 S 132°44 E	NTM P24424
NT: Cycad Gorge, Palm Valley	n.d.	AMS
NT: Palm Valley, 2 km up into valley	24°03.036 S 132°41.680 E	ZMB 106718
NT: Finke Riv., Boggy Hole	24°08.113 S 132°50.233 E	ZMB **106688**, 106719
NT: Boggy Hole	24°08 S 132°53 E	NTM P15861
NT: Three Mile Point, Finke Riv.	24°33.182 S 133°14.355 E	ZMB **106689**
NT: Walker Riv., nr Alice Springs	n.d.	AMS C.2149
QL: "Central Queensland"	n.d.	AMS C.118147
QL: Georgina Riv.	n.d.	AMS
QL: King Creek, 21 km S of Bedourie	24°31.93 S 139°33.9 E	AMS C.322677
QL: Einasleigh Riv., 4 km E of Einasleigh	18°30.938 S 144°06.682 E	ZMB **107260**; AMS
QL: Einasleigh Riv.	18°30.915 S 144°6.645 E	ZMB **127614**
QL: Soda Gorge Spring and Soda Valley Creek	20°36.30 S 144°02.00 E	AMS
QL: Innot Hot Springs	n.d.	AMS
QL: Endeavour Riv. Falls	15°22.270 S 145°01.770 E	ZMB **106346**, **107256**
QL: Bloomfield Riv.	n.d.	AMS C.488
QL: Barron Riv. at Gilies Highway	n.d.	AMS

Locality	Coordinates	Museum n°
QL: Barron Riv. Gorge, half way to hydro station	16°51.632 S 145°39.791 E	ZMB **107258**; AMS
QL: Barron Riv., at Barron Gorge Power Station	n.d.	AMS C.127066, C.322679
QL: Rapids below Lake Placid, Barron Riv. Gorge	16°52.17 S 145°40.405 E	ZMB **107257**; AMS
QL: Mareeba, upper Barron Riv.	16°59.134 S 145°25.158 E	ZMB **107583**
QL: Barron Falls	n.d.	AMS C.9284
QL: Rocky Creek, Atherton Tableland	n.d.	AMS C.158252
QL: Lake Eacham	n.d.	QM 14001
QL: Lake Barrine	17°15 S 145°38 E	AMS
QL: Peterson Creek, Gauging Stn.	n.d.	QM 64462
QL: Bellenden-Ker Range, nr Babinda	n.d.	AMS
QL: Salt Water Creek S of Lynd Riv. Crossing	17°48.985 S 144°25.046 E	ZMB **107603**
QL: Porcupine Creek at Pyramid Pool in Gorge	20°20.752 S 144°27.676 E	ZMB **107611**
QL: Porcupine Creek at Porcupine Gorge	20°21.197 S 144°28.07 E	ZMB **127615**
QL: Bynoe River	17°51.685 S 140°48.231 E	ZMB **127630****
QL: Gregory Riv.	17°53.517 S 139°17.209 E	ZMB **107277****
QL: Fisher's Creek, Palmeston Rock	17°34.167 S 145°53.876 E	ZMB 107259; AMS C.126522
QL: Nr Gregory Falls	17°35.57 S 145°52.29 E	ZMB **106345**
QL: Johnstone Riv.	n.d.	QM 64490
QL: Herbert Riv., N of Ingham	n.d.	AMS
QL: Waterview Creek, Iourama Falls NP	18°52 S 146°07 E	AMS
QL: Ross Riv.	19°21.73 S 146°43.93 E	AMS C.338671
QL: Ross Riv.	19°17 S 146°49 E	AMS C.338666; BMNH 1884.12.27.8-18
QL: Burdekin Riv.	n.d.	BMNH 1846.10.?.26-29
QL: Burdekin Riv., nr Charter Towers	n.d.	AMS
QL: Alice Riv., Townsville	19°19 S 146°35 E	AMS
QL: Hervey Range, 100 km SW of Townsville	19°46 S 146°05 E	AMS
QL: Fletcher Creek, near Charters Towers	n.d.	QM 14196
QL: Charter Towers, NW at Toomba Station	19°58 S 145°34 E	QM 53804
QL: Charter Towers, 51 miles WNW	n.d.	QM 64488
QL: Lolworth Creek, nr Gt Basalt Wall	n.d.	QM 14211
QL: Alligator Creek, Charters Towers	n.d.	QM 64494
QL: Bowen	n.d.	AMS
QL: Bowen, Port Denison	n.d.	AMS
QL: Port Denison	n.d.	BMNH 1879.5.21.489-90
QL: Rolleston Riv., Mount Cooper	n.d.	AMS
QL: Gregory Riv., 10 miles N of Proserpine	n.d.	AMS
QL: Mrytle Creek, 5 miles N of Proserpine	n.d.	AMS C.322654
QL: Digging Crossing, Eungella, upper Burdekin	21°10.419 S 148°28.759 E	ZMB 113636
QL: Broken Riv. trib. to Bowen Riv. at Eungella	21°10.13 S 148°30.129 E	ZMB **107956**
QL: Saltwater Ck., Proserpine	n.d.	QM 4412
QL: Pioneer Riv., 16 miles from Mackay	n.d.	AMS C.118146
QL: Lethe Brook, 5 km S of Proserpine on Bruce Hwy	n.d.	AMS
QL: Cattle Creek W of Mackay	n.d.	AMS
QL: Broken Riv., Eungella Dam	n.d.	AMS
QL: Cedar Creek, tributary of S. Pine Riv.	n.d.	AMS
QL: Euri Creek	20°12.294 S 147°57.613 E	ZMB **107951**
QL: N. Pine Riv., 2 miles S of Dayboro	n.d.	AMS
QL: Denison Creek, on Nebo-Mackay Hwy	n.d.	AMS
QL: 16 km S of Sarina, Sarina-Malborough Rd.	n.d.	AMS
QL: Boyne Riv. at Rosedale, S of Gladstone	24°13.40 S 151°15.30 E	AMS
QL: Barambah Ck., tributary of Boyne River	26°19.983 S 152°11.983 E	AMS
QL: Boyne Riv., Gayndah	n.d.	AMS
QL: Twelve Mile Creek, Boyne Riv., near Gladstone	n.d.	AMS
QL: Eastern Boyne Riv., S of Gladstone	24°18.15 S 151°23.30 E	AMS C.322660, 274Q
QL: Calliope Riv., S of Gladstone, Bruce Hwy	n.d.	AMS
QL: Bobs Creek, Fitzroy Riv., nr Rockhampton	n.d.	AMS
QL: Fairy Bower, 6 miles from Rockhampton	n.d.	AMS

Locality	Coordinates	Museum n°
QL: Rockhampton	n.d.	AMS
QL: Rockhampton, Yeppen Lagoon	n.d.	AMS
QL: Baffle Creek, 4 km E Miriam Vale	n.d.	QM 4274
QL: Woolwash Lagoon, S of Rockhampton	23°23 S 150°29 E	AMS C.**414039**
QL: 15 km N of Miriam Vale	24°16 S 151°29 E	AMS C.322656
QL: Rockhampton, Frogmore Lagoon	n.d.	AMS
QL: creek nr Springsure	n.d.	AMS
QL: Port Curtis	n.d.	AMS C.109649; BMNH 1928.5.5.144-149
QL: Montrose Creek, 163 km S Sarina	22°39 S 149°33 E	AMS
QL: Miriam Vale	n.d.	AMS C.109427
QL: NW of Miriam Vale, Colossem Ck.	24°24.20 S 151°28.30 E	AMS
QL: 18 miles S of Biggenden on Biggenden-Gayndah Rd	n.d.	AMS
QL: Kolan Riv., Bruce Hwy	n.d.	AMS
QL: Prospect Ck., Biloela	n.d.	AMS
QL: Prospect Creek, Dawson Valley	n.d.	AMS
QL: Granite Creek, crossing Bruce Hway, N Gin Gin	24°28 S 151°35 E	QM 43552
QL: Gin Gin	n.d.	AMS C.41983, C.42297
QL: Granite Creek, 36 km S of Miriam Vale	n.d.	AMS
QL: Walkamin, Granite Creek	n.d.	QM 64466
QL: Dawson Riv., 8 miles from Moura	n.d.	AMS
QL: Dawson Riv., Taroom	25°38.7 S 149°47.69 E	AMS C.327313
QL: Taroom, banks of Dawson Riv.	25°39 S 149°47 E	QM 56522
QL: Taroom, NE at Croker Gully	25°27 S 150°09 E	QM 56586
QL: Barambah Creek at Thompson crossing	25°41.08 S 151°46.728 E	ZMB 107816
QL: Burnett Riv., W of Childers	25°13.45 S 152°00.45 E	AMS
QL: Kalliwa Creek, tributary of Burnett Riv.	25°21 S 151°52 E	QM 64489
QL: Mingo Crossing, Burnett Riv.	n.d.	QM 64463
QL: Isaac and Burnett Rivers	n.d.	BMNH 1885.6.12.48-60
QL: Iris Riv., S of Childers	25°14 S 152°22 E	AMS
QL: Munna Creek	25°55.499 S 152°25.941 E	ZMB 107820
QL: Eidsvold	n.d.	AMS C.33773
QL: Mary S Creek, nr Gympie	n.d.	AMS
QL: NW of Gympie, Wide Bay Creek	26°04 S 152°14 E	AMS
QL: Mary Riv.	n.d.	AMS
QL: Borumba Dam, Mary Riv.	n.d.	QM 3028
QL: Mary Riv., at Kenilworth	n.d.	AMS
QL: Deacon S Creek, on Bruce Hwy	n.d.	AMS
QL: Litte Widgee Creek trib. to Mary Riv.	26°12.31 S 152°27.193 E	ZMB **107950**
QL: Isis Riv., Bruce Highway, S of Childers	n.d.	AMS
QL: Isis Riv.	25°13.606 S 152°25.238 E	ZMB 107819
QL: Barambah Ck., Central Burnett Hwy	26°20 S 152°12 E	AMS
QL: Burnett Riv., on Mt. Perry Road, nr Gayndah	n.d.	AMS
QL: Burnett Riv. at Trurich Creek, W of Childers	25°18.40 S 151°56.15 E	AMS
QL: Burnett Riv.	n.d.	ZMB 46270, 104176
QL: Coominga, NW of Ipswich	27°37 S 152°47 E	NTM P27453
QL: Atkinson S Dam, 50 km W of Brisbane	n.d.	NTM P27458
QL: Woody Point, Clontarf, Redcliff Peninsula	n.d.	AMS
QL: Clontarf, Moreton Bay	n.d.	AMS
QL: Sandgate, Moreton Bay	n.d.	AMS
QL: Brisbane	n.d.	AMS C.109648 spinose forms
QL: Brisbane Riv., 60 miles from bay	n.d.	AMS
QL: Obi Creek, near Maleny, Landsborough Shire	26°43 S 152°53 E	NTM P27456
QL: Wide Bay Creek nr Brooyar	26°0.992 S 152°38.104 E	ZMB 107821
QL: South Maroochy Riv. at Yandina	26°33.626 S 152°56.629 E	ZMB **107948**
QL: Moggill Creek, Brisbane	27°29 S 152°54 E	NTM P27459; AMS
QL: Moggill Creek, Brookfield	27°30 S 152°30 E	QM 64461

Locality	Coordinates	Museum n°
QL: Kilcoy	n.d.	AMS
QL: Dalby	n.d.	AMS
QL: South Pine Riv. at Samford	27°21.319 S 152°52.912 E	ZMB 107957
QL: Camp Hill, Brisbane	27°30 S 153°05 E	QM 54747
QL: Bulimba Creek at Stackpole Street	27°33.196 S 153°6.723 E	ZMB 107817
QL: Mt Crosby Weir & Pumping Station, Brisbane	27°32 S 152°48 E	NTM P27455
QL: Candamine Riv., near Cecil Plains	n.d.	AMS
QL: Brisbane Riv.	n.d.	AMS
QL: Pullen Creek, Brisbane Riv.	n.d.	QM 64460
QL: tributaries of the Brisbane Riv.	n.d.	WAM 463-80
QL: upper Brisbane Riv.	27°26.226 S 152°38.056 E	ZMB **107946**
QL: Riv. Brisbane, near Ipswich	n.d.	BMNH 1886.4.26.168-77
QL: Ipswich	n.d.	AMS
QL: Ipswich, Swanbank Powerstation	n.d.	AMS
QL: Brisbane, Walton Bridge, Recreation Reserve	27°26 S 152°57 E	QM 47184
QL: Stream flowing out of Enoggera Reservoir	27°27 S 152°55 E	NTM P27457
QL: Coomera Riv., near Canungra	n.d.	AMS C.109645
QL: Tartar S Ck., Macpherson Ranges	28°28 S 152°50 E	AMS C.129350
QL: Hoffman S Ck., tributary Hewtsons Hill	28°26 S 152°24 E	AMS C.128691; QM 10366
QL: Balonne Riv.	n.d.	AMS; BMNH 1859.10.24.2
QL: Dalrympie Creek, 10.8 km from Goomburra	28°00 S 152°15 E	AMS C.129351; QM 10534
QL: Moonie Riv.	n.d.	AMS C.33009
QL: Ban Ban	n.d.	AMS
QL: Caboolture, Burpengary Creek	n.d.	QM 64491
QL: Condamine Riv., weir at Chinchilla	n.d.	QM 5162
QL: Chowey Creek, 15 km W of Biggenden	n.d.	QM 14182
QL: Chinchilla Weir	n.d.	QM 19443, 19457
QL: Marlborough, S at Princhester Creek	22°53 S 150°01 E	QM 19932
QL: Marlborough Creek, banks Godhelp	n.d.	QM 64493
QL: Barcaldine	n.d.	QM 35222
QL: Carnarvon Gorge, Carnarvon NP	n.d.	QM 44144, 64498
QL: Dawson Riv. at Theodore	24°57.417 S 150°4.443 E	ZMB 107818, 192770, **192391**
QL: Banana, 6 km S Banana - Biloela	24°28 S 150°25 E	QM 50643
QL: Blackall, W at Noonbah Stn	24°07 S 143°11 E	QM 53110
QL: Springsure, Minerva Creek, nr Marmadilla Stn	24°00 S 148°08 E	QM 60714
QL: Charleville, Warrego Riv.	n.d.	QM 64455
QL: Roma, Bungil Creek	n.d.	QM 64456
QL: Taloona Homestead, Roma	n.d.	QM 64487
QL: Kings Creek, Darling S Down, Post-Tertiary fossil	n.d.	AMS C.109774
QL: N. Pine Riv., at Young S Riv. crossing	n.d.	AMS
QL: Howard Ck., 12 km from Mt Tambourine	n.d.	AMS C.128695; QM 10370
QL: Innis Creek	n.d.	ZMB 103713
NSW: Mole Riv., below Bonshaw Weir	28°44 S 152°57 E	AMS C.167652
NSW: Richmond Riv., Woodburn	n.d.	AMS
NSW: Upper Richmond Riv.	n.d.	AMS
NSW: Richmond Riv., upstream from Irving Bridge	28°52 S 153°03 E	AMS C.31555, 167443
NSW: Murray Swamps	n.d.	AMS
NSW: Upper Clarence Riv.	n.d.	BMNH 1879.5.21.482-4
NSW: Clarence Riv., nr Baryulgil crossing	29°13 S 152°33 E	AMS
NSW: Little Riv., Bawdens Bridge, Grafton	n.d.	AMS
NSW: Wollombi Riv., Bulga, W of Singleston	n.d.	AMS
NSW: Macquarie Riv.	n.d.	AMS
NSW: Lake Lidell, Hunter Valley, nr Musswelbrook	n.d.	AMS C.110529; WAM 460-80
NSW: 25 miles S of Forbes, on Newell Hwy	n.d.	AMS
NSW: Peel Riv., Tamworth	n.d.	AMS
NSW: Halls Creek, Goulburn Riv., Denman	n.d.	AMS
NSW: Worondi Riverlet, nr Gouburn Riv.,	n.d.	AMS
NSW: Dalwood, Hunter Riv.,	n.d.	AMS

Locality	Coordinates	Museum n°
NSW: Hunter Riv.	n.d.	AMS
NSW: Williams Riv.	32°23.790 S 151°45.750 E	AMS C.421943
NSW: Lake Burragorang	34°0 S 150°26 E	AMS C.311883
NSW: Wah Wah Main, Murrumbidgee Irrigation Area	n.d.	AMS C.110529
NSW: Hay, Murrumbidgee Riv.	n.d.	BMNH 1836.7.26.183-92; AMS
NSW: Spring Creek, Backmead	n.d.	AMS
NSW: Narrandera	n.d.	AMS
NSW: Narrandera	n.d.	AMS
NSW: Yanco, Agricultural High School	n.d.	AMS C.57886
NSW: Lachlan Riv.	n.d.	AMS C.109644
NSW: Gooloogong, Lachlan Riv.	n.d.	AMS C.57038
NSW: Lachlan Riv., 8 miles from Lake Cargelligo	n.d.	AMS
NSW: Lake Cargelligo, Willow Dam	n.d.	AMS
NSW: Leeton	n.d.	AMS C.109646
NSW: 3 miles W of Hillston	n.d.	AMS
NSW: 25 miles S of Forbes, on Newell Hwy	n.d.	AMS
NSW: Cudgegong Riv., nr Mudgee, Twelve Mile	n.d.	AMS
NSW: Bogan Riv., Brewarrine	n.d.	AMS C.109865
NSW: Rochs, Barwon Riv., Brewarrina	n.d.	AMS
NSW: Macquarie Riv., nr Carinda	n.d.	AMS
NSW: Marthaguy Creek, tributary of Macquarie Riv.	n.d.	AMS C.100836
NSW: Macquarie Riv., nr Dubbo	n.d.	AMS
NSW: Dubbo, Western Plains Zoo	n.d.	AMS
NSW: Crunningbar Creek, Warren	n.d.	AMS
NSW: Gouburn Riv.	32°03 S 150°10 E	AMS
NSW: Namoi Riv., Narrabri	n.d.	AMS C.263; BMNH 1894.6.5.194-197
NSW: Junction of Bibba Creek and Namoi Riv.	n.d.	AMS
NSW: Namoi Riv. at Tarriaro Bridge	n.d.	AMS C.101257
NSW: Namoi Riv., nr Gunnedah	n.d.	AMS
NSW: Namoi Riv., 6 miles N of Boggabri	n.d.	AMS
NSW: Poncaree, Darling Riv.	n.d.	AMS
NSW: N bank of Darling Riv., 37 miles SW of Bourke	n.d.	AMS
NSW: Bourke	n.d.	AMS C.100624 syntypes of oncoides
NSW: Menindee, Darling Riv., Krinchega NP	32°24 S 142°23 E	AMS C.322676
NSW: E Bank of Lake Menindee	32°20 S 142°20 E	AMS
NSW: Barraba, Gizzard of Black Duck	n.d.	AMS
NSW: Lowry Creek, Warrabah NP	30°33 S 150°54 E	AMS
NSW: Cobbadah Creek, near Barraba	n.d.	AMS
NSW: Myall Greek, Bingara	n.d.	AMS C.109781; QM 64486
NSW: Riv. nr Bundarra	n.d.	AMS
NSW: Gwyder Riv., Moree	n.d.	AMS
NSW: Gwyder Riv., Anderson Creek, SE of Moree	n.d.	AMS
NSW: Gwyder Riv., bridge at Bingara	29°51.74 S 150°34.65 E	AMS C.322678
NSW: Moree	n.d.	AMS C.51670
NSW: 'Yurunga" Warialda, fossil in lacustrine deposit	n.d.	AMS C.87453
NSW: MacIntyre Riv., 3 m N Inverell	n.d.	AMS
NSW: Kings Creek, off MacIntyre Riv.	n.d.	AMS C.146292
NSW: N of Inverell, 8 miles NW of Graman	n.d.	AMS
NSW: MacIntyre Riv., Inverell, Stannifer Rd.	n.d.	AMS
NSW: Moonie Riv., 40 miles NW of Collarenebri	n.d.	AMS C.33009, 109647
NSW: Barwon Riv., downstream from Walgett	n.d.	AMS
NSW: Barwon Riv., Brewarrina	n.d.	AMS
NSW: Nenegara Waterhole, Paroo Riv.	n.d.	AMS
NSW: Bulloo Riv., Tibooburra	n.d.	AMS C.139443 syntypes of thrascia
NSW: Menindee, 27 ft. below surface, fossil	n.d.	AMS

Locality	Coordinates	Museum n°
NSW: Reservoir nr Silverton, W. of Broken Hill	n.d.	AMS
NSW: Darling Riv. at Bourke	n.d.	AMS
NSW: Pocucaree, Darling Riv.	n.d.	AMS
VIC: Victoria	n.d.	BMNH 1879.1.21.10
VIC: Echuca	n.d.	AMS C.1927
VIC: Lake Cullulerraine	n.d.	AMS
VIC: Goulburn Riv., Shepparton	n.d.	AMS
SA: Coopers Creek, at Innamincka	n.d.	AMS
SA: Cullymurra Waterhole, 11 km E of Innamincka	27°42.1 S 140°50.11 E	AMS
SA: Cullymurra Waterhole, E of Innamincka	27°42.58 S 140°53.1 E	AMS
SA: Adelaide	n.d.	AMS C.109708
SA: Murray Riv.	n.d.	BMNH 1879.5.21.461-4
SA: Murray Bridge	n.d.	AMS C.109404
SA: Mundalla, nr Bordertown	n.d.	AMS
SA: Tailem Band, Murray Riv.	n.d.	AMS C.43663
SA: Morgon, Murray Riv., fossil from Post-Pleistocene	n.d.	AMS C.109780

Pseudoplotia scabra (O.F. Müller, 1774)

Type locality: "In paludosis littoris Coromandel Tranquebari Danorum maxime vulgare; centena & ultra benevoleutia D. Spengler"; i.e. India: Tranquebar, Coromandel Coast.

Distribution: Outside of Australia *Pseudoplotia scabra* (formerly known as *Thiara scabra*) exhibits a wide distribution from the east coast of South Africa to India and Sri Lanka across the Southeast Asian mainland and the islands of the western Indo-West Pacific, where *P. scabra* can be found from the Sunda Islands and the Philippines to New Guinea including the Bismark Archipelago, the Solomons, the New Hebrides and Fiji (Glaubrecht et al., 2009). In Australia *P. scabra* occurs in the Leichhardtian (thus the Timor Sea and Gulf of Carpentaria drainage systems) and in the Jardinian fluvifaunal provinces of the East Coast. More detailedly, in the Northern Territory it is found in Berry Springs, the Victoria, Daly and Roper River systems, as well as in Queensland in the Gregory and O'Shanassy River and in at least three locations along the Coral Sea coast (see fig.28).

Comments: *Pseudoplotia scabra* was formerly placed in the genus *Plotia* by Glaubrecht et al. (2009) but corrected into *Pseudoplotia* by Mienis (2012), followed here.
In 2009, Glaubrecht and colleagues assumed that the Australian populations of this species have either been introduced, or represent a hitherto cryptic species for the continent. With 28 occurrences (as listed in tab.13) the results in this study show that the species is much more common than thought at that time when only eight localities were known.

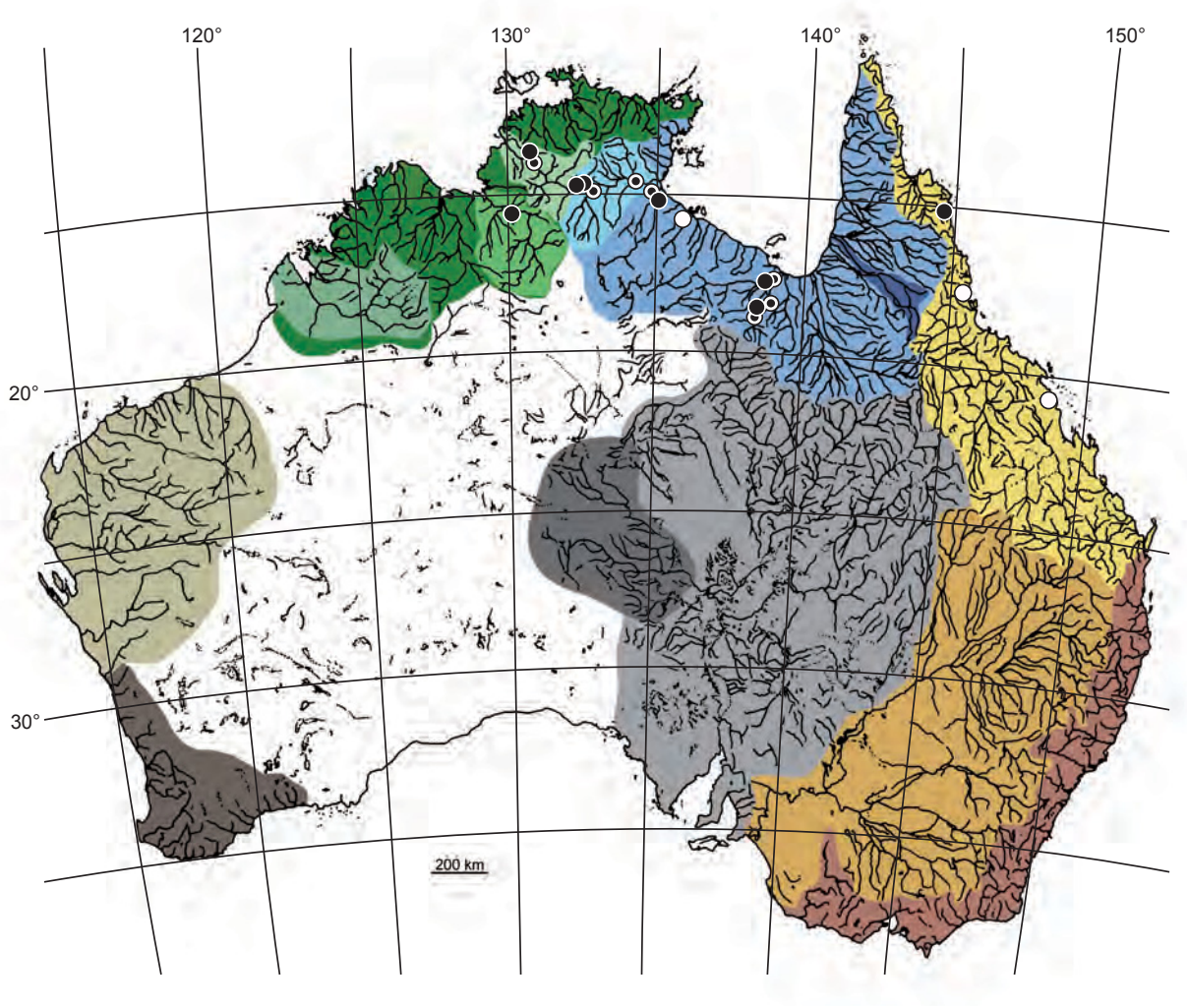

Figure 28 Distribution map of *Pseudoplotia scabra* in Australia. Black dots represent genetically confirmed localities, white ones dry material and white ones with a black inner circle wet material. Some dots represent multiple nearby localities.

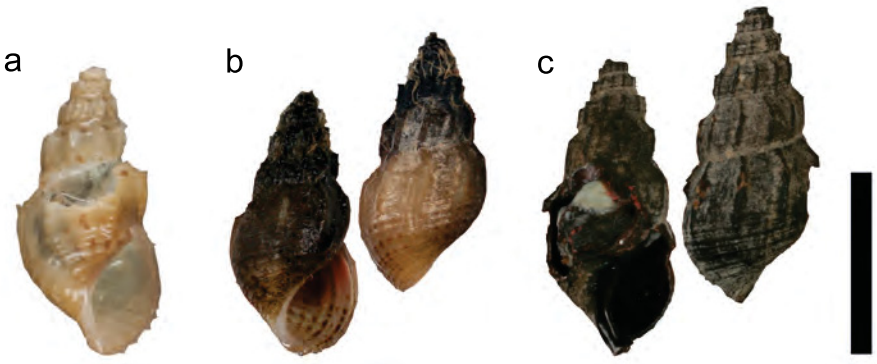

Figure 29 Shells of *Pseudoplotia scabra* of individuals that were genetically confirmed. **a.** QL: Little Roper River (ZMB 107564-1). **b./c.** NT: Limmen Bight River (ZMB 107271), both were initially named *"Thiara" australis* but mt-data confirmes that individuals belong to *P. scabra*. Scale bar: 1 cm

Table 13 Locality data for the material examined of *Pseudoplotia scabra*. Bold ZMB numbers are genetically confirmed. Asterisk (*) after the museum number indicates that the individual was assigned to a different species in the field. ZMB numbers starting with digits 127 represent material that was sampled in 2011.

Locality	**Coordinates**	**Museum n°**
NT: Berry Springs	12°42.153 S 130°59.875 E	ZMB 106599
NT: Bamboo Creek, 3-10m from Daly River	13°40.118 S 130°39.501 E	ZMB **107215**, **107263***, 107551
NT: Daly River Crossing	13°46.142 S 130°42.874 E	ZMB 107546
NT: Oolloo Crossing, Daly River	14°04.24 S 131°15.056 E	ZMB **107216**, 127774
NT: Cox River	15°19.394 S 135°20.699 E	ZMB **107270***
NT: Limmen Bight River	15°28.865 S 135°24.054 E	ZMB **107271***
NT: Little Roper River, north bank	14°55,63 S 133°7,105 E	ZMB **107564**, 127778
NT: Little Roper River, south bank	14°55.589 S 133°07.137 E	ZMB **106679**, 127514
NT: Stevie s Hole at Waterhouse River, Elsey N.P.	14°55.782 S 133°8.732 E	ZMB **107287***
NT: Roper River, at Botanic Walk	14°56.126 S 133°8.532 E	ZMB 127780
NT: Roper River, at 4 Mile Point	14°56.137 S 133°10.033 E	ZMB **107284***, 127770
NT: Wabalarr, at Roper River	14°56.028 S 133°10.444 E	ZMB 127772
NT: Mulurark, at Roper River	14°56.763 S 133°12.614 E	ZMB 127769
NT: Salt Creek at junction to Roper River	14°57.453 S 133°15.095 E	ZMB 127779
NT: Salt Creek, near Elsey Creek	15°0.703 S 133°14.417 E	ZMB 106634, **107266***, 127766
NT: Elsey Creek, at Warloch ponds	15°05.083 S 133°07.439 E	ZMB 127767
NT: Roper River, at Roper Bar	14°42.802 S 134°30.474 E	ZMB 127768
NT: Roper River, Mountain Creek	14°46.543 S 134°48.016 E	ZMB 127771
NT: Timber Creek, above junction of Victoria River	15°38.203 S 130°28.529 E	ZMB **127618***
NT: Victoria River, Big Horse Creek	15°36.878 S 130°23.7 E	ZMB 127763
QL: Gregory River, SE of Burketown	17°53.517 S 139°17.209 E	ZMB **107277***
QL: Gregory River at Riversleigh	19°1.116 S 138°43.529 E	ZMB **107279**, **107575**, 127775
QL: O Shanassy River, at crossing	19°1.378 S 138°45.73 E	ZMB 127764
QL: Gregory River, at crossing, Gregory Downs	18°38.829 S 139°14.912 E	ZMB 127765, 127773
QL: Gregory River, Beame Brook, at crossing	17°52.708 S 139°20.576 E	ZMB 127777
QL: Three Mile, Poison Creek	15°25.81 S 145°7.05 E	ZMB **106351**
QL: Daintree River, Martins Creek	16°14.163 S 145°18.323 E	ZMB 107596
QL: Broken River, Eungella	21°07.9 S 148°29.6 E	ZMB 103714

***Ripalania queenslandica* (E.A. Smith, 1882)**

Type locality: "Saltwater Creek next to the coast, Cardwell", in "Queensland, Australia". Note that the second location "Paroo River" given by E.A. Smith (1882) was declared to be an error by Glaubrecht et al. (2009).

Distribution: The geographic range of *Ripalania queenslandica* was reported to be restricted to Australia with isolated occurrences in few rivers and streams along the tropical coast of NE Queensland within the Jardinian fluvifaunal region. Accordingly, there are also isolated occurrences of the species in the Iron Range and Lockhart River area, and again from the Daintree River drainage south to Cardwell (see fig.30). Now, however, we also have a record outside of the Australian continent in Papua New Guinea.

Comments: The species was thought to be endemic to Australia, but now we have positive indication of conspecifics in other than the Australian region. As described in Chapter 5 a new record in Papua New Guinea could be genetically confirmed.

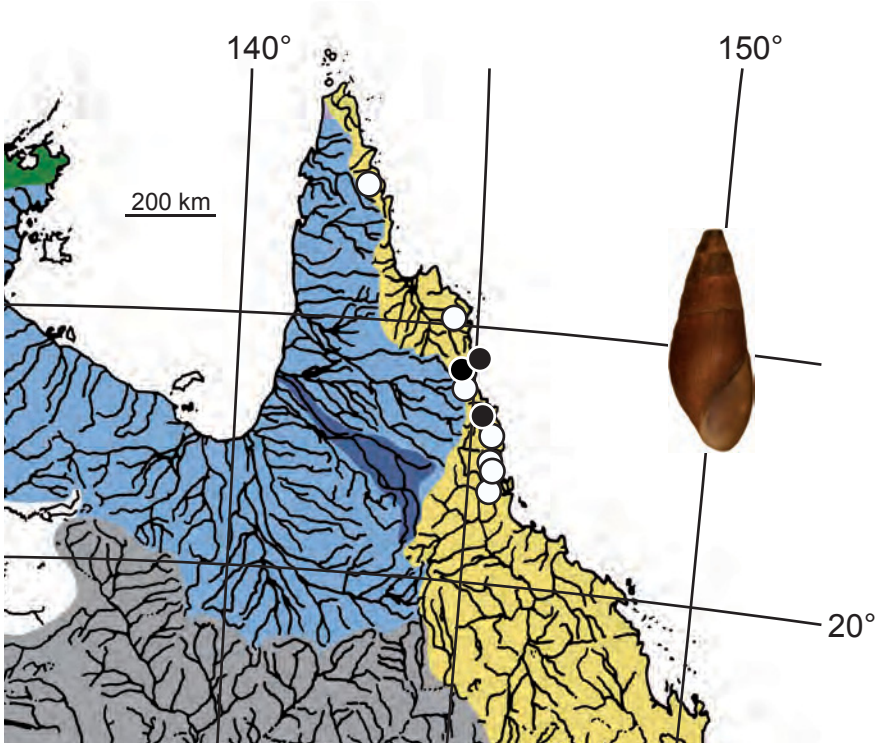

Figure 30 Distribution map of *Ripalania queenslandica* in Australia. Black dots represent genetically confirmed localities and white ones dry material. Some dots represent multiple nearby localities. Note the restriction within the Jardinian region.

Table 14 Locality data for the material examined of *Ripalania queenslandica*. Bold ZMB numbers are genetically confirmed.

Locality	Coordinates	Museum n°
QL: "Australia"	n.d.	ZMB coll. Paetal 1885
QL: Line Hill, Iron Range	12°45' S 143°25' E	QM 64464
QL: Lockhart River, SW of airfield	12°48' S 143°17' E	QM 21407
QL: Daintree River	16°18' S 145°17' E	AMS C.317982, C.100354; NHMB 10.751
QL: Daintree River, above Allanton Hill	16°14' S 145°19' E	AMS C.317986, C.125802; QM 16272
QL: Daintree River, near Daintree	n.d.	QM 13483
QL: Douglas Creek, at crossing	16°16.194 S 145°58.60 E	ZMB **107213**
QL: Martins Creek, Daintree River	16°14.163'S 145°18.323'E	ZMB **107595**
QL: Stewart Creek, junction with Daintree Riv.	16°18' S 145°19' E	AMS C.317984
QL: Low Isles, near Port Douglas	n.d.	AMS
QL: Barron River	n.d.	AMS C.109650
QL: Cairns	n.d.	AMS C.1334; ZMB 61751
QL: near Innisfail	n.d.	AMS C.51805
QL: Innisfail, Blackfellow Creek	n.d.	AMS
QL: North Johnstone River	17°30.34 S 145°59.55 E	ZMB 106355, **107214**, **192474**
QL: Tully River	n.d.	AMS C.9282
QL: Cardwell	n.d.	AMS

Sermyla riqueti (Grateloup, 1840)

Type locality: "Bombay" was mentioned as locus typicus by Grateloup (1840: 433); but, as stated by Glaubrecht et al. (2009), this is not in line with the label information of the type material "Batavia [1. line], Samarang [2. line]", which is today Jakarta, Java.

Distribution: Individuals of *Sermyla riqueti* are widely distributed from India and Southeast Asia including Thailand, Vietnam the Philippines and the Sunda Islands in Indonesia, into the Indo-West Pacific. Glaubrecht et al. (2009) also reported records from the Bismarck Archipelago and the Solomon Islands. Reports about the occurrence of this species in Australia are only based on dry shells in museum collections and any attempt to find them recently has failed.

Comments: All individuals from the conducted field trips that preliminarily had been labelled as *Sermyla riqueti*, turned out to belong to a different species. As living snails could not be found until the present date, no final statement of the recent existence of *Sermyla riqueti* in Australia can be made. At least a regular occurrence can be ruled out.

Sermyla venustula (Brot, 1877)

Type locality: "Port Denison, Nov. Holl. [Novae Hollandia]", given by Brot (1877) and B.J. Smith (1992: 77), which today is Bowen, south of Townsville in Queensland (see star in fig. 31). According to Glaubrecht et al. (2009) and the results of this study, the species does not occur in drainages of the Jardinian province.

Distribution: *Sermyla venustula* is endemic in Australia and its geographic range is mainly restricted to inland rivers of the Northern Territory and Queensland. Next to several rivers along the southern coast of the Gulf of Carpentaria, the records comprise in particular localities from the Roper River drainage and its tributaries, where the species can be found in brackish, in some cases almost saline environments.

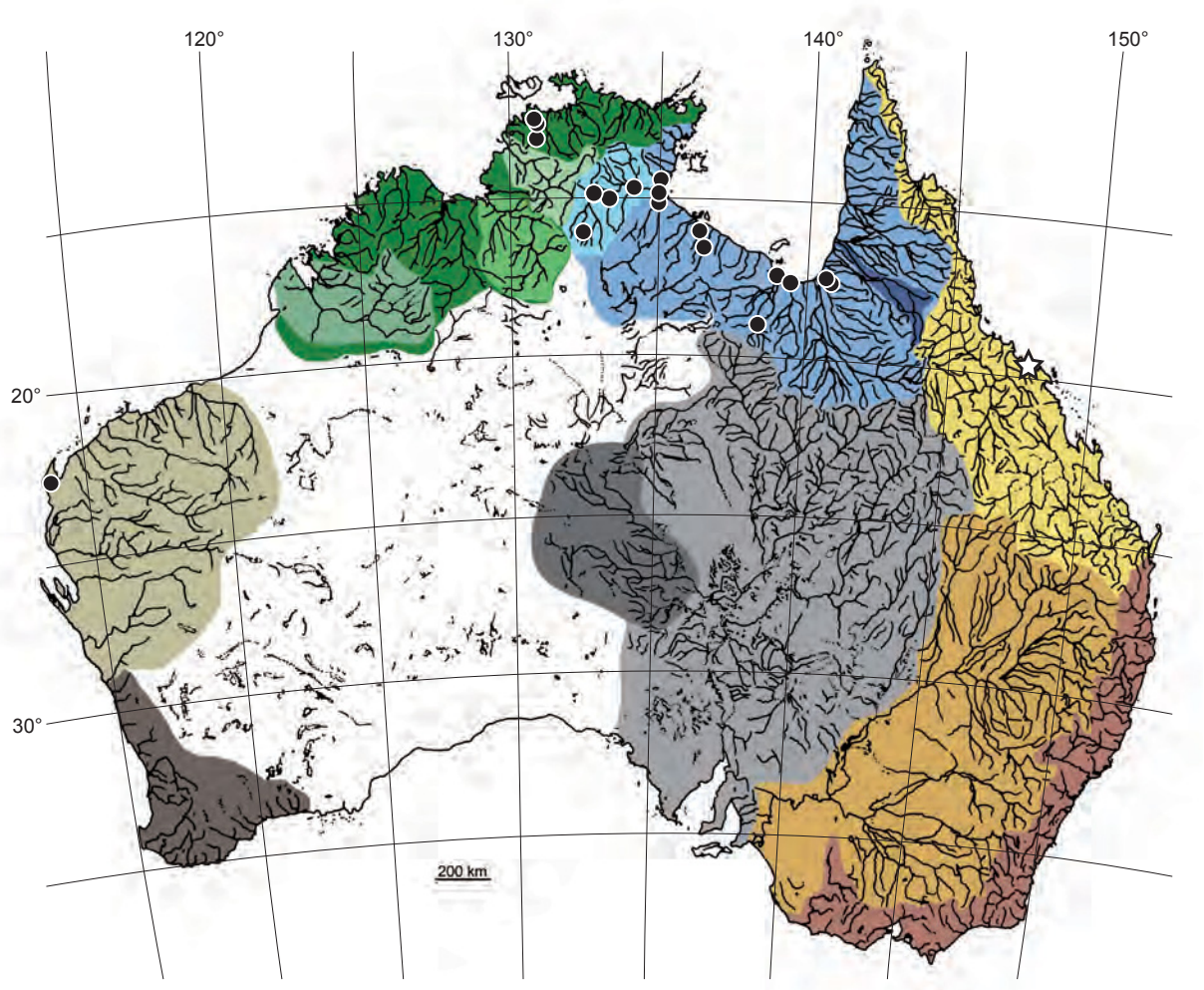

Figure 31 Distribution map of *Sermyla venustula*. Star: Representing the type locality, which is probably incorrect (see text). Note the highly isolated location of Bundara Sinkhole - a cave with an underground connection to the ocean - in West Australia. All localities are genetically confirmed.

The locations Howard Springs and Berry Springs near Darwin are remarkable, because these creeks are highly isolated in northwestern Northern Territory. But it is most remarkable that the occurrence of *S. venustula* is extended exceedingly through a genetically confirmed new locality in West Australia, i.e. Bundara Sinkhole in the Greyian region, far away from the common northern range.

Comments: Two occurrences of *S. venustula* worth mentioning are reported here for the first time: Berry Springs (southwest from Darwin) and the most western record in Bundara Sinkhole, West Australia. The latter is an anchialine cave - an inland cave fluctuating with marine tides - which is about 1.7 km from the sea and with salinity stratified with about 50% seawater (pers. communication Bill Humphreys). Hence, although this locality is far away from the common range, its habitat fits well to the preferences of *S. venustula*, which is tolerant of moderately brackish conditions.
Note that the large and less sculptured specimens from Howard Springs (see fig. 32b), referred to as "carbonata" (Reeve, 1859) by Glaubrecht et al. (2009), do not differ genetically from *S. venustula* and therefore are not treated separately any longer.

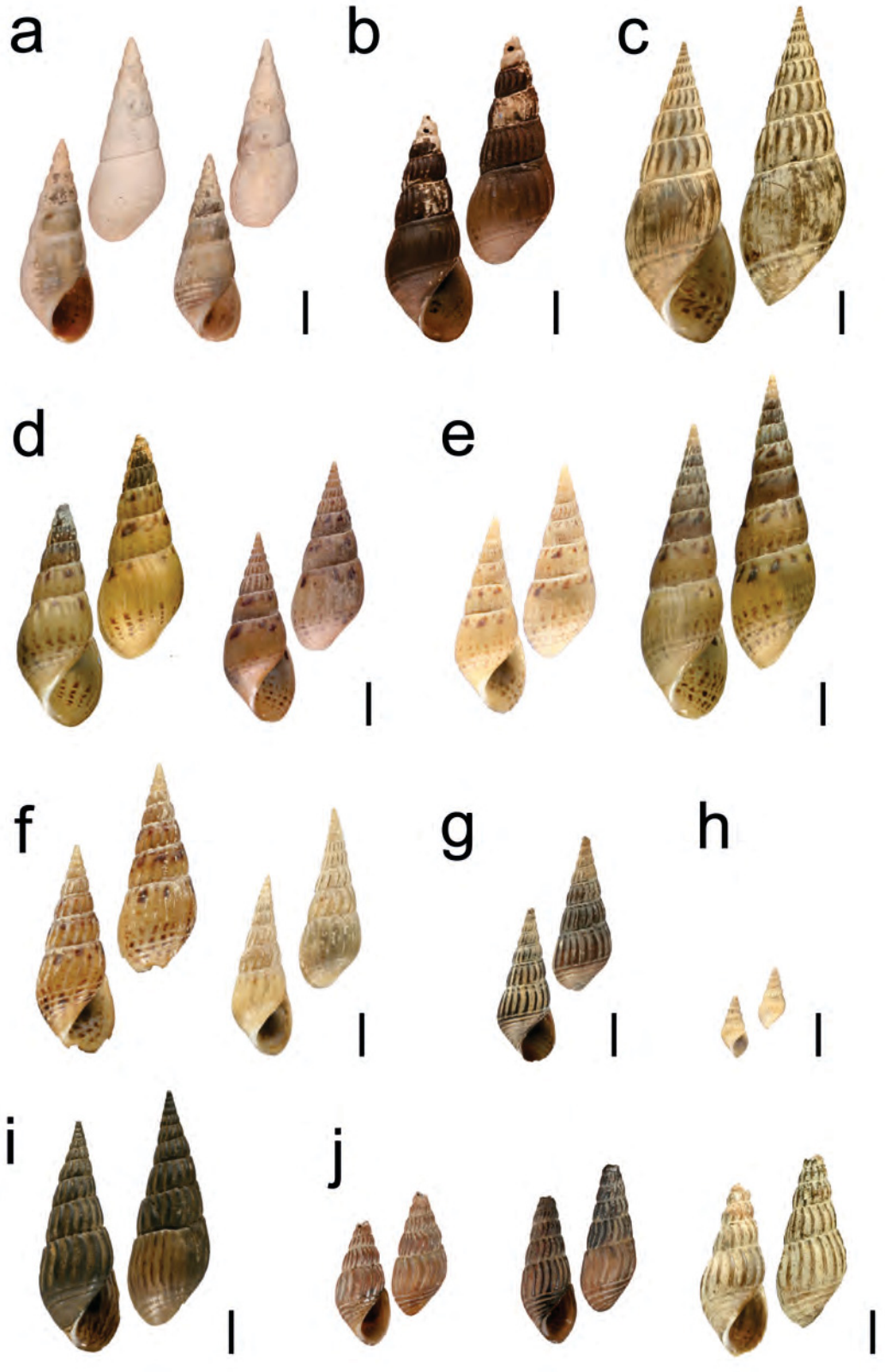

Figure 32 Shell variability of *Sermyla venustula*. As this species was the subject of a master thesis (conducted within the scope of this study), comprehensive shell pictures of genetically confirmed individuals are present (Maaß, 2012). **a.** WA: Bundara Sinkhole (WAM 10048). **b.** NT: Howard Springs (ZMB 107630). **c.** NT: Little Roper River (ZMB 107561). **d.-g.** NT: Roper River: **d.** Mulurark (ZMB 107621). **e.** Jalmurark (ZMB 106676, 107557). **f.** Elsey creek (ZMB 107231). **g.** Warloch ponds (ZMB 192019). **h.** NT: Towns River (ZMB 192018). **i.** QL: Foelsche River (ZMB 107232). **j.** QL: Norman River (ZMB 107209, 107235). Scale bar: 0.5 cm.

Table 15 Locality data for the material examined of *Sermyla venustula*. Bold ZMB numbers are genetically confirmed.

Locality	Coordinates	Museum n°
WA: Bundara Sinkhole	22°25 S 113°46 E	WAM **10048**, 10049, 10050, 13049, 13050
NT: Howard Springs	12°27.345 S 131°03.146 E	ZMB **106593**, **106700**, 127660, **107228**
NT: Howard Springs Creek	12°27.268 S 131°03.108 E	ZMB **106595**, **107627**, **107630**; AMS C.427959
NT: Spring creek at Howard Springs	12°27.553 S 131°03.069 E	ZMB **127660**
NT: Berry Springs, SE of Darwin	12°42.111 S 130°59.875 E	ZMB **107544**, **107545**, **127634**, **107210**
NT: Little Roper River, at crossing	14°55.581 S 133°07.176 E	ZMB 106629, 106678, 107236, **107561**, **107562**
NT: Little Roper River, south bank	14°55.589 S 133°07.137 E	ZMB **127639**
NT: Little Roper River, north bank	14°55.630 S 133°07.105 E	ZMB **127640**, 127641
NT: Stevie's Hole at Waterhouse River	14°55.782 S 133°08.732 E	ZMB **106682**
NT: Roper River, at Botanic Walk	14°56.126 S 133°08.532 E	ZMB 127642, 127643
NT: Roper River, at 4Mile Point	14°56.120 S 133°10.069 E	ZMB 127644
NT: Wabalarr, at Roper River	14°56.028 S 133°10.444 E	ZMB **107616**, 127645
NT: Mulurark, at Roper River	14°56.763 S 133°12.614 E	ZMB **107615**, **107621**, **127646**
NT: Roper River, at Jalmurark Camp	14°57.158 S 133°13.29 E	ZMB **106676**, **107229**, **107557**, **107558**
NT: Roper River, 2km below Jalmurark	14°57.515 S 133°14.275 E	ZMB 127638
NT: Salt Creek, junction to Roper River	14°57.453 S 133°15.095 E	ZMB 127635, 127636, 127637
NT: Elsey Creek on Roper Highway	15°00.627 S 133°14.417 E	ZMB **107231**, 127649, 127650
NT: Salt Creek, nr Elsey Creek,	15°0.703 S 133°14.417 E	ZMB **107230**, **127647**, 127648
NT: Warloch Ponds on Elsey Creek	15°05.042 S 133°07.258 E	ZMB **192019**, 127657, 127658
NT: Roper Bar, Roper River	14°42.816 S 134°30.501 E	ZMB **192017**
NT: Mumpumapu waterhole, Arnhem Land	14°22.59 S 135°19.34 E	AMS C.**461353**
NT: 8 km NE of Towns River Crossing	14°59.82 S 135°16.28 E	ZMB 192016, **192018**
NT: Towns River, at crossing	15°02.570 S 135°12.718 E	ZMB **127651**
NT: Towns River, at junction with creek	14°59.999 S 135°17.030 E	ZMB **127654**, **127655**
NT: Foelsche River	16°12.628 S 136°53.034 E	ZMB **107232**
QL: Bynoe River	17°12.967 S 150°40.433E	ZMB 106712
QL: 4.5 km NW of Normanton	17°39.43 S 141°06.03 E	VK 26.356; ZMB **106713**
QL: Norman River, 1km N of Normanton	17°39.712 S 141°06.154 E	ZMB **107209**, **107235**, 127656

Stenomelania cf. *aspirans* (Hinds, 1844)

Type locality: "in rivers of Feejee Islands"; Fiji.

Distribution: *Stenomelania* cf. *aspirans*, is reported to be widespread in the Australasian region, with known occurrences, in the Bismark Archipelago, the Solomon Islands, Vanuatu, New Caledonia, Fiji and Samoa. On the Australian continent the occurrence of *Stenomelania* cf. *aspirans* is restricted to the Jardinian region, where it occurs in few streams along the tropical coast of Queensland (see fig. 33). Here it is known only from highly isolated locations in the Iron Range and Lockhart River area, the Bloomfield to Barron River region with the southernmost locality at Clump Point.

Comments: The species was not listed in any faunal survey of Australian freshwater molluscs (e.g. E.A. Smith 1882; Iredale 1943; B.J. Smith 1992, 1996), and was recorded for the first time by Glaubrecht et al. (2009).

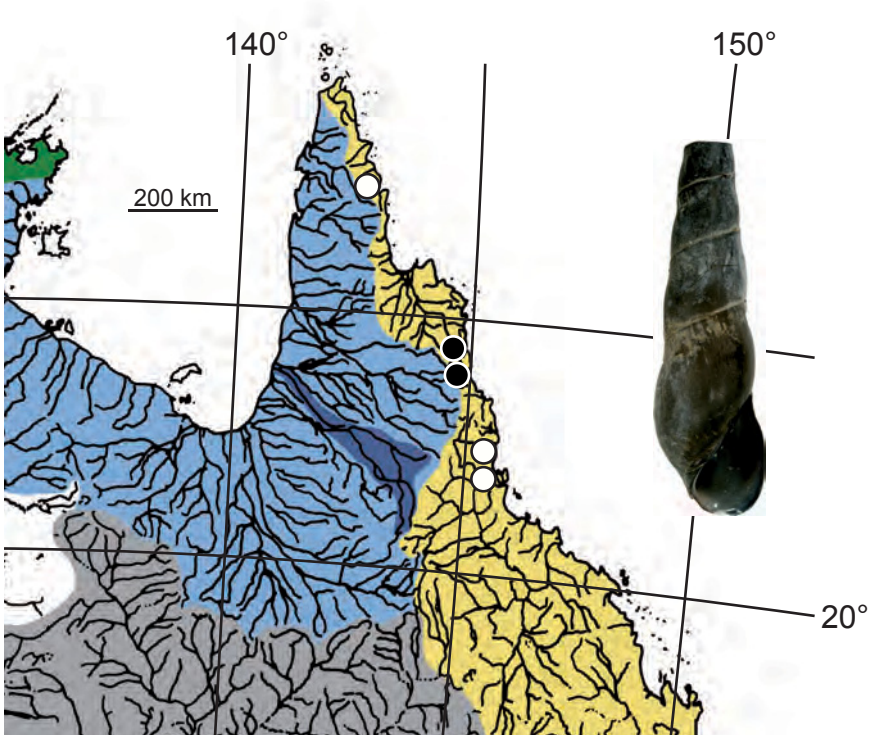

Figure 33 Distribution map of *Stenomelania* cf. *aspirans* in Australia. Some dots represent multiple nearby localities. Like *Thiara amarula* and *Ripalania queenslandica*, the species is restricted within the Jardinian region.

Table 16 Locality data for the material examined of *Stenomelania* cf. *aspirans*. Bold ZMB numbers are genetically confirmed and represent own collections. Note that the coordinates given by the AMS for the Locality Rocky River are out in the Coral Sea.

Locality	Coordinates	Museum n°
QL: "Cape York Peninsula"	n.d.	AMS C.109655
QL: Rocky River	13°49 S 145°28 E	AMS C.317983
QL: Creek into West Claudie River	12°47 S 143°19 E	AMS C.317327
QL: Granite Creek, W of Bloomfield	15°55.99 S 145°19.54 E	ZMB **107211**
QL: Mowbray River	16°33.87 S 145°27.83 E	ZMB 106171, **106344**, **107212**, **107586**; AMS C.115338
QL: Barron River, N of Cairns	n.d.	AMS C.105172
QL: Hartley's Creek, N of Cairns	n.d.	AMS C.109654
QL: Froma Creek, N of Cairns	n.d.	AMS C.158276
QL: Clump Point	n.d.	AMS C.30757

***"Stenomelania" denisoniensis* (Brot, 1877)**

Type locality: "Port Denison", Queensland, as given originally by Brot (1877); today this corresponds to Bowen, S Townsville.

Distribution: In contrast to most Australian thiarids, *"Stenomelania" denisoniensis* is widely distributed throughout the Australian continent, with occurrences ranging from the Greyian across the Leichhardtian region (with Timor Sea and Gulf drainages to the North) and the fluvifaunal provinces Jardinean, Kreftian and northern Lessonian on the East Coast (see fig. 34). While the species occurs in rivers along the southern coast of the Gulf of Carpentaria and in many streams and rivers of the Northern Territory including the Daly and Roper River systems, as well as in the Victoria River, its main occurrences are in the coastal rivers of Queensland draining east to the Coral Sea. On the East coast it reaches its southernmost known occurrence near the border to New South Wales and on the West coast, located at nearly the same latitude, at the Greenough River south of Geraldton. In addition, in Australia's North West there are a few records from isolated populations in the Kimberley and Pilbara regions. Remarkably (as compared e.g. to *P. balonnensis*), this otherwise widely distributed species is lacking in the Sturtian, the Mitchellian and the Vlaminghian provinces. *S. denisoniensis* has been considered as being endemic to Australia. The findings presented here show that the species is actually widespread in the Australasian area, as it could be verified in Indonesia and on Timor.

Comments: *"Stenomelania" denisoniensis* is not endemic to Australia as considered before. Sequences from Timor and Indonesia cluster with those of Australian *"Stenomelania" denisoniensis*. Note that the mt-data is quite inconsistent in the case of *"Stenomelania" denisoniensis*, as well as for *M. tuberculatus*, and that these two species are often mixed-up on account of their similarity. Another source of error is that snails appearing like *S. venustula* turned out to be *"Stenomelania" denisoniensis* (see fig.35 a).

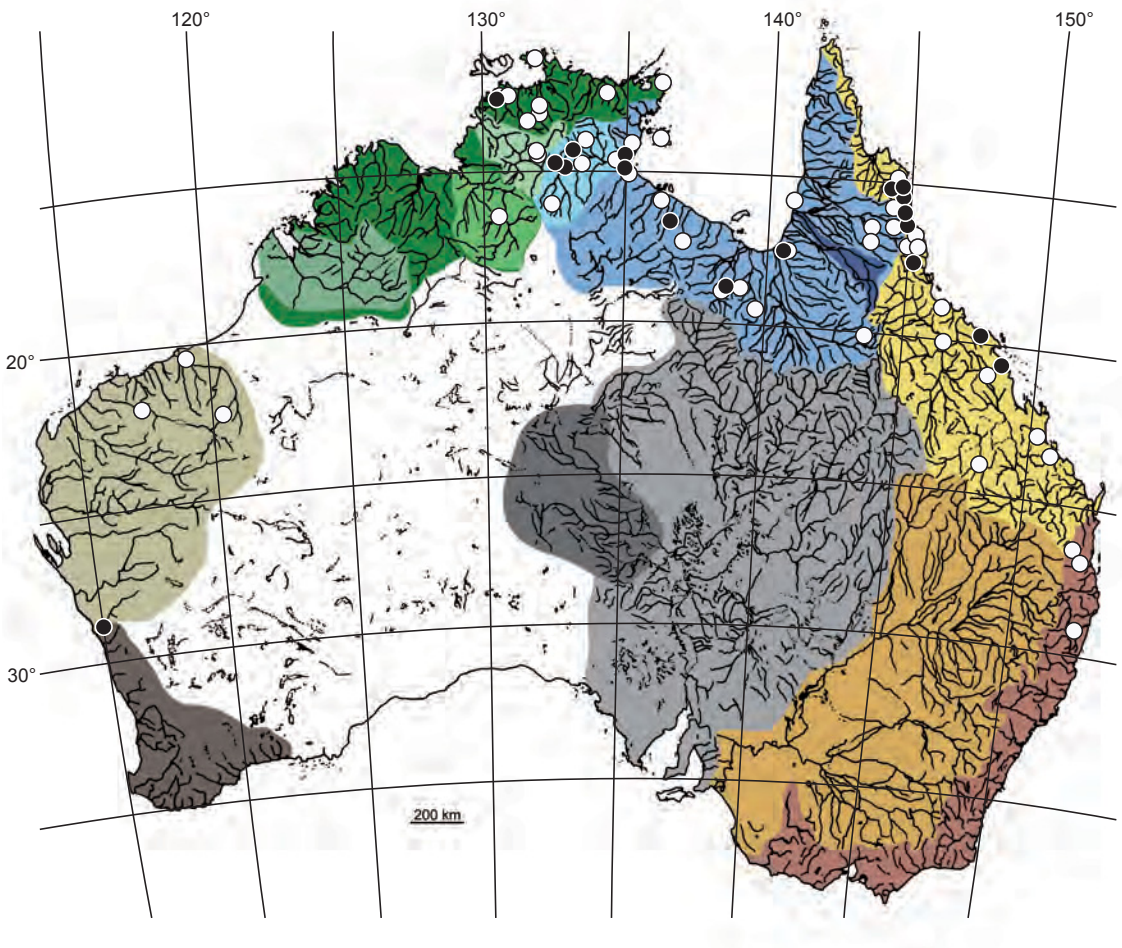

Figure 34 Distribution map of *"Stenomelania" denisoniensis* on the Australian continent. In contrast to most Australian thiarids, the species is widely distributed throughout the Australian continent. Black dots represent genetically confirmed localities and white ones dry material. Some dots represent multiple nearby localities.

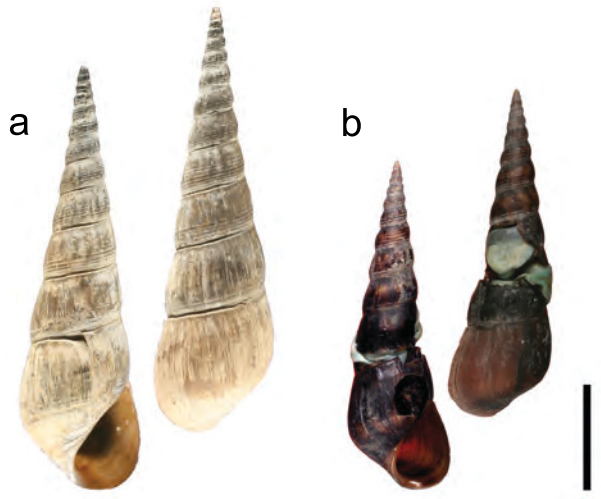

Figure 35 Shells of *"Stenomelania" denisoniensis*. **a.** QL: Gregory River (ZMB 107234-1), initially named *S. venustula* but mt-data confimes that the individual belongs to *"Stenomelania" denisoniensis*. **b.** NT: Bitter Springs (ZMB 127783-1), cracked in the field, found simultaneously with *M. tuberculatus* (see fig.20). Scale bar: 1 cm.

Table 17 Locality data for the material examined of *Stenomelania denisonensis*. Bold ZMB number are genetically confirmed. Asterisk (*) after the museum number indicates that the individual was assigned to a different species in the field. Abbreviations: Riv. - River; Rd - Road; Hwy - Highway; Cr. - Creek; NP - National Park; L. -Land; Stn - Station

Locality	Coordinates	Museum n°
WA: Ellendale Pool at Greenough Riv.	28°51.630 S 114°58.430 E	ZMB **106586**
WA: Pilbara Springs, S of Dampier	20°51'15' S 116°36'50' E	AMS
WA: Pilbara Springs, pool of spring	21°37 S 117°06'20' E	AMS
WA: Millstream, Millstream NP	21°35.5 S 118°4 E	AMS C.324141
WA: Pardoo Station, NE of Port Hedland	20°02 S 199°41 E	AMS stn P27
WA: 150 km E Nullagine	21°40'45' S 121°08 E	AMS C.324137
WA: Kimberleys: Mt. Mathew, Mt. Hart Station	n.d.	VK 12387
NT: "North Australia"	n.d.	BMNH 1857.9.30.18
NT: Black Point Lagoon, Cobourg Peninsula	n.d.	AMS
NT: Lagoon behind Ranger Station, Cobourg Peninsula	n.d.	AMS
NT: Holmes Cr., 12 miles NE of Darwin	n.d.	AMS
NT: Darwin Riv., crossing with Hwy to Cox Peninsula	12°44.527 S 130°57. 930 E	ZMB 106704
NT: Howard Springs, S Darwin	12°27.5 S 131°03.0 E	NTM P1013, P27464, P27466; AMS C.110450; QM 5132; ZMB **107629**, 127798, 127781
NT: Howard Riv., crossing	12°27.752 S 131°05.008 E	ZMB 106597
NT: Berry Springs, S of Darwin	12°42.111 S 130°59.854 E	ZMB 106600, 107255, 127789; QM 5624
NT: Foggy Dam, Humpty Doo	n.d.	AMS C.110490; VK 2523
NT: Middle Point jungle, near Fogg Dam	n.d.	VK 7724
NT: Manton Riv., Weed Quad 3	n.d.	NTM P27465
NT: Manton Riv. at Stuart Hwy, 66 km S of Darwin	n.d.	AMS
NT: Coomalie Cr., at Rd crossing	13°00.602 S 131°06.850 E	ZMB 106645
NT: Coomalie Cr., at rest area on Stuart Hwy	13°0.88 S 131°7.4 E	AMS C. 324138; VK 973; NTM P6468
NT: Crater Lake, 7 km W of Batchelor	13°02.760 S 131°05.445 E	ZMB 106660; VK 24531
NT: Adelaide Riv.	n.d.	BMNH 1891.11.21.151-152; 1892.1.29.193
NT: Rum Jungle at Litchfield Rd	13°02.604 S 130°59.862 E	ZMB 106662
NT: Black Jungle Spring, Kakadu NP	13°02.898 S 132°09.889 E	ZMB 106644; AMS C.32413; VK 25909
NT: Bamboo Cr. at the junction with Daly Riv.	13°40.05 S 130°39.29 E	VK 24378
NT: Daly Riv. Crossing	13°46.02 S 130°42.61 E	AMS C.323809
NT: Victoria Riv.	n.d.	BMNH 1857.9.30.17
NT: Upper Victoria Riv., North Australia	17°18 S	BMNH 1857.11.18.26
NT: Victoria Riv., 195 km W of Katherine	n.d.	AMS C.324142
NT: Kathrine Riv., downstream lower level	14°29.5 S 132°14.847 E	ZMB 127785
NT: Bitter Springs, at Mataranka	14°54.642 S 133°5.362 E	ZMB 127783
NT: Roper Riv., old bridge crossing at Mataranka Homestead	14°55.5 S 133°6.5 E	AMS C.317320
NT: Little Roper Riv., at crossing	14°55.581 S 133°07.176 E	ZMB **107253**, 107563, 127792, 127794
NT: Roper Riv., just below crossing at Mataranka	14°56 S 133°7 E	AMS C.151987
NT: Stevie's Hole at Waterhouse Riv., Elsey NP	14°55.782 S 133°08.732 E	ZMB **107254**
NT: Roper Riv., at Botanic Walk	14°56.126 S 133°8.532 E	ZMB 127791
NT: Waterhouse Riv., Mantaranka tourist resort	n.d.	QM 5156
NT: Roper Riv., Roper Falls, 4 km E of Jamurak	14°57.401 S 133°15.018 E	ZMB **107252**, 127784
NT: Mataranka Falls, Roper Riv., Elsey Park	14°57.30 S 133°15.00 E	VK 24.377
NT: Elsey Cemetery, 11 km of Mataranka Springs	15°5.15 S 133°7.44 E	AMS
NT: Elsey Riv., Elsey Cemetery	n.d.	ZMB 106651

Locality	Coordinates	Museum n°
NT: Salt Cr., near Elsey Cr., at crossing of Roper Hwy	15°00.703 S 133°14.417 E	ZMB 106685, **107238**, 127786
NT: Elsey Cr.	15°0.624 S 133°14.962 E	ZMB **127649***
NT: Elsey Cr., at Warloch ponds	15°5.083 S 133°7.439 E	ZMB 190197, 127782
NT: Roper Riv. at Roper Bar camping ground	14°42.71 S 134°30.78 E	AMS C.338661, C.338657
NT: Goyder Riv., Arnhem L.	n.d.	AMS
NT: eastern Goyder Riv. crossing, Arnhem L.	13°01.37 S 134°58.37 E	AMS C.461369
NT: Rose Riv. catchment, 150 km N of Roper Bar	13°43.40 S 135°06.2 E	NTM P8702
NT: Mumpumapu waterhole, Arnhem L.	14°22.59 S 135°19.34 E	AMS C. **461371**
NT: Wearyan Riv. crossing	16°10.05 S 136°45.25 E	VK 13.870
NT: Calvert Riv. crossing	16°56.06 S 137°21.25 E	VK 25.837, 25.846
NT: Calvert Riv., below junction with Bluey Cr.	16°56.1 S 137°21.52 E	AMS C.151987
NT: Roper Riv., Mountain Cr.	14°46.543 S 134°48.016 E	ZMB 127795
NT: Towns Riv., at crossing with Roper Hwy	15°2.57 S 135°12.718 E	ZMB 106641, **107239**, 127799
NT: Towns Riv., downstream, point 1	14°59.839 S 135°16.262 E	ZMB 127788
NT: Towns Riv., two pools on northern bank	14°59.792 S 135°17.156 E	ZMB 127796
NT: Towns Riv., backwater at junction with Cr.	14°59.999 S 135°17.03 E	ZMB 127793
NT: Cox Riv., N of causeway	15°19.394 S 135°20.669 E	ZMB 107240
NT: Wearyan Riv., at crossing	16°10.03 S 136°45.506 E	ZMB 127797
NT: Kangaroo Cr.	16°47.553 S 137°06.107 E	ZMB **107233***
QL: running Cr., 27.7 km Brookdale	18°19.25 S 139°15.45 E	VK 13.872
QL: Lawn Hill Cr., near the cascades, Lawn Hill NP	18°42.00 S 138°29.00 E	VK 26.335
QL: Gregory Riv., Riversleigh Stn	n.d.	QM 64453
QL: Gregory Riv. crossing, Riversleigh Station	19°01.15 S 138°43.25 E	VK 13.873; ZMB 107577, 127790
QL: Leichhardt Riv., east branch 2 km below dam	20°44 S 139°47 E	AMS C.300838
QL: Bynoe Riv., Burketown to Normanton Rd	17°51.53 S 140°47.58 E	AMS C.338669; VK 26.336
QL: Normanton Riv., Glenmore	15°51.199 S 141°08.048 E	ZMB **107242**, 127800; VK 26.331
QL: Walker Cr. Crossing on Normanton, Karumba Rd	17°28.22 S 141°10.42 E	VK 26.338
QL: Billabong, 1 km from Norman Riv., Kurumba Rd	17°39.64 S 141°6.1 E	AMS C.338659
QL: Norman Riv., billabong 1 km N of Normanton	17°39.712 S 141°06.154 E	ZMB **107241**, 127787
QL: Mt. Isa, Lake Moondarra	n.d.	QM 64474
QL: Soda Gorge Spring, NE of Hughenden	20°37.00 S 144°05.33 E	AMS C.145015
QL: Line Hill, Iron Range	12°45 S 143°25 E	QM 64470
QL: Lockhart Riv., S Claudie Riv. crossing	12°48 S 143°17 E	QM 21408
QL: 80 km N Cooktown, McIvor Riv.	15°0.9 S 145°06 E	AMS
QL: Endeavour Riv. Falls	15°22.270 S 145°01.770 E	ZMB 106338, **107244**
QL: McLeod Cr., tributary of Endeavour Riv.	15°25.505 S 145°06.049 E	ZMB **107243**
QL: Endeavour Riv., 12 miles WNW of Cooktown	n.d.	AMS
QL: Cooktown	n.d.	AMS C.437129
QL: Laura Riv.	15°34.680 S 144°27.410 E	ZMB **106339**
QL: Boggy Cr., W Normanby tributary	15°49.97 S 144°52.910 E	ZMB 106373
QL: Three Mile Cr./Poison Cr., tributary of Endeavour Riv.	15°25.789 S 145°07.04 E	ZMB 106356, **107245**
QL: Bloomfield Riv.	n.d.	AMS C.487
QL: Granite Cr., W of Bloomfield	15°55.99 S 145°19.54 E	ZMB 107246
QL: Bloomfield Riv. crossing, 2.9 km on N side	15°56 S 145°20 E	QM 24004
QL: Woobadda Cr.	15°57.969 S 145°22.858 E	ZMB 107247
QL: Woobadda Riv.	15°58 S 145°22.480 E	ZMB 106342
QL: Meelele Riv.	15°58.250 S 145°23.850 E	ZMB 106341, **107248**
QL: Wonga Beach, 14 km NNE of Mossman	16°20.30 S 145°25.00 E	VK 21.054
QL: Mossman Riv., Cr. at crossing	n.d.	ZMB 104150
QL: Cr. entering Mossman Riv., at Mossman Gorge	n.d.	AMS C.127104, C.426370
QL: Mossman Riv., outside Mossman Gorge NP	n.d.	AMS
QL: Mossman Riv. Gorge, small Cr. nr parking area	n.d.	AMS stn 53a
QL: Mowbray Riv., nr Port Douglas	n.d.	AMS C.324143
QL: Mowbraw Riv., near Port Douglas	16°33.812 S 145°27.877 E	ZMB **107588**
QL: W side of Daintree Riv. Valley	n.d.	AMS

Locality	Coordinates	Museum n°
QL: Martins Cr., upper Daintree Rd, Daintree Riv.	16°14.163 S 145°18.323 E	ZMB **107598**
QL: E of Dimbulah, Walsh Riv. near Mutchilba	17°7.283 S 145°16.205 E	ZMB 107600
QL: Salt Water Cr., near Lynd Brook	17°48.985 S 144°25.046 E	ZMB 107602
QL: Porcupine Cr. at Pyramid pool in gorge	20°20.752 S 144°27.676 E	ZMB 107609
QL: Kewarra Beach, N of Cairns	n.d.	AMS
QL: Barron Riv.	n.d.	BMNH 1885.3.18.3-6; AMS C.127065
QL: Cr. off Mulgrave Riv., N Cairns	17°14 S 145°46 E	AMS
QL: Mulgrave Riv., near Goldsboroug, Cairns	n.d.	QM 64451
QL: Mulgrave Riv., nr Cairns	n.d.	AMS
QL: Barron Falls	n.d.	AMS C.9283
QL: Stony Cr., nr Barron Riv.	n.d.	QM 4991
QL: Barron Riv., Hemmings Rd.	n.d.	QM 64503
QL: Barron Riv., Picnic Crossing	n.d.	QM 64478
QL: Barron Riv. Falls	n.d.	AMS C.51468
QL: Barron Riv., George Rd	16°51.632 S 145°39.791 E	ZMB **107250**
QL: Barron Riv., below 150 m Lake Placid	16°52.17 S 145°40.405 E	ZMB **107249**
QL: Mareeba, upper Barron Riv.	16°59.134 S 145°25.158 E	ZMB **107584**
QL: spring beside Barron Riv., 12 miles N of Atherton	n.d.	AMS C.324144
QL: Crystal Cascades, Redlynch, nr Cairns	n.d.	QM 64476
QL: Yarrabah Rd., Pine Cr.	17°00 S 145°50 E	QM 48184
QL: Pine Cr., NW of Malbon Thompson Rd., Cairns	n.d.	AMS stn 44
QL: Musgrave Riv., downstream from Goldsborough Bridge	17°11.40 S 145°44.13 E	VK 26.346
QL: Tributary of Musgrave Riv., near Gouldsborough Valley Camping Area	17°14.19 S 145°46.28 E	VK 26.333
QL: Dowah Cr., at Freshwater Cr. junction, W Cairns	n.d.	AMS C.324140
QL: Pelican Cr.	n.d.	BMNH 1884.12.27.1-7
QL: Near Gregory Falls	17°35.570 S 145°52.290 E	ZMB 106352
QL: Tinaroo Dam & Lake Tinaroo, Atherton Tableland	n.d.	AMS C.158125, C.158280
QL: Lake Tinaroo	n.d.	AMS C.158280
QL: Chambri Lakes, Atherton Tableland, Lake Eacham	17°17 S 145°37 E	QM 14002
QL: Atherton Tableland, Lake Tinaroo	17°10 S 145°33 E	QM 46349
QL: Tinaroo	17°08 S 145°35 E	QM 64505
QL: Johnson Riv., Innisfail	n.d.	AMS C.51806
QL: Cr. 10 miles of Innisfail, nr Johnson Riv.	n.d.	AMS C.109653
QL: Burdekin Riv.	n.d.	BMNH 1846.10.7.33-35, 1879.5.21.399-401, 406-7)
QL: Bellenden-Ker Range, nr Babinda	n.d.	AMS C.51353, C.109651
QL: Fisher's Cr., at Palmerston Hwy	17°34.167 S 145°53.876 E	ZMB **107251**
QL: Mena Cr., Innisfail	n.d.	AMS
QL: South Mission Beach	17°56.840 S 146°03.290 E	ZMB **106340**
QL: Cardwell, Rockingham Bay	n.d.	BMNH 1879.5.21.397-8, 415-6, 433-4
QL: Fisher's Cr., Palmerston Hwy	n.d.	AMS C.126522
QL: Upp Lynd Riv., Mt. Garnet to Mt. Surprise	n.d.	QM 1620
QL: Elizabeth Cr., E of Mt. Surprise	18°08.351 S 144°19.414 E	ZMB 106726
QL: Ross Riv.	19°21.73 S 146°43.93 E	AMS C.338675
QL: Saltwater Cr., Atherton	n.d.	AMS C.109132
QL: Burdekin Riv., 100 km from the coast	n.d.	VK 7780
QL: Rosetta Plains, Burdekin Riv.	n.d.	AMS C.8892
QL: Rosella Plains, near Cardwell	n.d.	BMNH
QL: Missionary Bay, Hichinbrook Island, South Cr.	n.d.	BMNH
QL: Townsville, Aplins Weir, Ross Riv.	n.d.	QM 38407
QL: Townsville	n.d.	AMS
QL: nr Almaden, Chilagoe Railway, Four Miles Cr.	n.d.	AMS C.54093
QL: off Ingham Rd, Townsville	n.d.	AMS
QL: nr Eungella, Broken Riv.	n.d.	AMS
QL: Alice Riv., at Eubenangee NP entrance	17°24.52 S 145°58.85 E	AMS C.338679
QL: Charter Towers, Tank College	n.d.	QM 1600

Locality	**Coordinates**	**Museum n°**
QL: Charters Towers, NW at Toomba Stn	19°58 S 145°34 E	QM 53803
QL: Allingham Cr., Bluff Downs Stn	19°43 S 145°36 E	QM 64450
QL: Alligator Cr., Wando Vale Rd crossing	n.d.	QM 64452
QL: Calcifer Cr., nr Chillagoe	n.d.	AMS C. 54417
QL: Euri Cr., at Bowen to Collinsville Rd	20°12.294 S, 147°57.613 E	ZMB **107955**
QL: Botanic Garden, Mackay, on Bruce Hwy	21°9.485 S, 149°9.582 E	ZMB **107963**
QL: Proserpine	n.d.	QM 4411, 35346
QL: Lethe Beach, 5 km S of Proserpine on Bruce Hwy	n.d.	AMS
QL: Conway Riv. NP, E of Prosperpine	20°16 S 148°46 E	QM 35346
QL: Myrtle Cr., crossing on Bruce Hwy	n.d.	AMS
QL: McKinley Cr., near Mackay	n.d.	VK 977
QL: Isaac and Burnett Rivers	n.d.	BMNH 1885.6.12-60
QL: Fitzroy Riv.	n.d.	BMNH 1879.5.21.468-72
QL: Fitzroy Island	n.d.	AMS C.58394
QL: Rockhampton	n.d.	BMNH 1879.5.21.420.7
QL: Rockhampton, Yeppen Lagoon	n.d.	AMS C.118148
QL: Rockhampton, western side of Frogmore Lagoon	n.d.	AMS C.109652
QL: Alligator Cr., 20 km N of Rockhampton	n.d.	AMS
QL: Addy Cr.	n.d.	BMNH
QL: Andromache Riv. crossing, via Gunyarra	20°34 S 148°29 E	QM 26376
QL: Port Curtis	n.d.	AMS
QL: Mt. Cooper, Rolleston Riv.	n.d.	AMS C.109429
QL: Hays Inlet, nr Kallangur	n.d.	QM 28789
QL: Gloucester Passage	n.d.	QM 33609
QL: Brookfield, Moggill	27°30 S 152°30 E	QM 64471
QL: North Pine Riv., Dayboro	27°15 S 152°50 E	QM 64472
QL: Kedron Brook at Brook St.	n.d.	QM 64473
QL: Fox Bridge, Mazlin Cr.	n.d.	QM 64479
QL: Beantree Bridge, Mazlin Cr.	n.d.	QM 65404
QL: South Maroochy Riv. at Yandina	26°33.626 S 152°56.629 E	ZMB 107949
QL: Kenilworth, Little Yabba Cr. junction	n.d.	QM 64495
QL: Peterson Cr.	n.d.	QM 64506
QL: Burrum Riv., Howard	n.d.	QM 64507, 64508
QL: Paluma, Mt Spec NP, Crystal Cr.	n.d.	QM 59919
QL: Walkamin, granite	n.d.	QM 64465
QL: Palms Island	n.d.	QM 64469
QL: Blackmans Cr., SW Galdstone	24°26.30 S 151°25.30 E	AMS C.324136
QL: Boyne Riv. at Rosedale, S Galdstone	24°13.40 S 151°15.30 E	AMS
QL: Brisbane, Sinnamon Pk.	n.d.	QM 64475
NSW: Clarence Riv., S Grafton	29°40 S 152°55.983 E	AMS C.170629

4.4 Discussion

Based on the data presented in this study, the suggestion is made to differentiate eleven species among the Australian Thiaridae which are mostly in accordance with the results of Glaubrecht et al. (2009) by (shell) morphology. The main differences to this previous systematic account as well as new insights in the light of the molecular results of the mitochondrial data are discussed in the following. First of all the importance of the genetical confirmation should be noted. As the shells are highly variable, many populations/individuals have been erroneously named. The collector assigned each specimen in the field, based solely on external appearance in combination with previous geographic knowledge, and not necessarily to the appropriate taxon. The extent of this source of error is only apparent in molecular results and shows that morphological classification of thiarids have to be undertaken with caution, a well-known phenomenon for other freshwater gastropods (Reid et al., 2013).

The thiarid *Thiara rudis* could be identified by molecular data and is recorded here for the first time as taxa with occurrences in Australia. The specimens were already collected in 2004 but misidentified as *"Thiara" australis* and *Sermyla venustula*. Only the molecular analyses show that they actually have typical *Thiara rudis* sequences and this assignment can be confirmed by examining the morphology.
In the genus *Sermyla*, the sequencing results show that *Sermyla riqueti* is not (or at least not often/anymore) represented in Australia and that specimens from Howard Springs (see fig. 32b), referred to as "carbonata" (Reeve, 1859), do not differ genetically from the endemic *S. venustula* which makes the latter the only species in Australia from that genus. Its occurrence in Bundara Sinkhole, far away from the normal distribution range, is quite interesting. Although alternative explanations exist, it could be a population that might have survived as relictual form from times long gone. Of course this explanation is based on the assumption that the species had a wider distribution in the past which could also explain why the type locality is outside of its present-day range.

Glaubrecht et al. (2009) conjectured that *Melanoides tuberculata* and potentially *Pseudoplotia scabra* are most likely anthropochorous introductions from Asia to Australia. The authors reasoned that the spotty occurrences on the continent with the vicinity of larger cities are indications for a possible recent introduction or invasion to the continent. New records show that they appear more often than thought and the localities are not only restricted to areas near human settlements. In any case, both species are known for being invasive in other parts of the world. Their adaptability to a wide range of environmental conditions in combination with their ability for rapid dispersal and population explosion had serious consequences, for example in case of *M. tuberculata* in Argentina (Gregoric, 2010). Actually, *M. tuberculata* has invaded the whole intertropical belt, mainly as a

result of the trade for aquarium plants (Facon et al., 2003). Concerning *P. scabra* only recently a study has been published that captures the rapid dispersal and population explosion of this invasive species in Lake Kinneret, Israel (Heller et al., 2014). The species, which is also commonly bred in aquaria, probably reached the Lake during the mid-2000s and currently comprises 95% of its snail fauna. However, it remains open whether the Australian populations of this species have been introduced recently. Further studies are necessary in order to understand the invasive behaviour and impacts of the species in Australia. The distribution of the species could rapidly spread in new water courses and it should be under supervision if new records are reported in the future.

Ripalania queenslandica was thought to be an endemic species of Australia, occurring only (as its name already reflects) on a small coastal sector along the Jardinian in Queensland. Already in 2009 it was considered that it could be more widespread in the Australasian region. Now there is evidence for this erstwhile assumption as shells from New Guinea very similar in appearance turned out to belong to the species (see chapter 5). With this range expansion it shows the same pattern as *Thiara amarula* and *Stenomelania* cf. *aspirans*: the three species are restricted to the same area in Australia, but have a more or less extensive distribution in the SE Asian and Pacific region. While all other Australian thiarids retain their eggs within the brood pouch (eu-vivipar), these three taxa, release veligers (ovo-vivipar). Anatomical studies reveal that their marsupia only contain eggs and embryos up to very early developmental stages which are then released as free swimming veligers. As they have the same potential for passive dispersal by planktonic larvae and as (not only near relatives but even) conspecifics can be found over the entire SE Asian and Pacific region, it can be assumed that these three ovo-viviparous thiarids might be recent colonizers from the north. Their marine veligers might have crossed vast areas with ocean currents coming from the north and managed to colonize in this area. The restricted appaerence in Australia (despite the capability of long-distance dispersal) seems to be connected to the climatic conditions in the Jardinian, as this region is the only area in Australia with conditions quite similar to those in other monsoonal regions of the Asia-Pacific region where these three can be found. As an alternative viewpoint to this colonization, it should be considered that these three thiarids might represent an ancient Australian freshwater faunal element. As such they might have survived as relictual forms in the Jardinian from times long gone as known about many Australian faunal and floral elements and discussed in Glaubrecht et al. (2009).

It remains a special case of interest that these three differing species *R. queenslandica*, *T. amarula* and *S.* cf. *aspirans* are easily diagnosed and distinguished from other Australian thiarids as being the biggest among the Australian thiarids with highly distinct shells (see fig.14 and tab.). The pattern observed by Glaubrecht et al. (2009) that species with a more restricted geographic range on the continent in general are also less variable, holds true not only for these three Jardinean species but in particular for *Melasma onca* as a species with

restricted occurrences and fairly constant conchology. In contrast, species with a wider distribution across Australia, like *"Thiara" australis*, *Plotiopsis balonnensis* and *Stenomelania denisoniensis*, are often extraordinarily variable even within single populations. In this context it is noteworthy to mention that *M. onca* also differs in reproductive mode as it is eu-vivipar but has a strategy distinct from other eu-vivipar Australian thiarids concerning the number of embryonic stages within the marsupium (Maaß and Glaubrecht, 2012).

Glaubrecht et al. (2009) considered the distribution of *"Thiara" australis* in comparison to *Plotiopsis balonnensis* as being "apparently completely exclusive, thus vicariant (allopatric)." The genetic results are inconsistent with this statement, confirming *"Thiara" australis* occurrences in the Coral Sea drainage (an area of *P. balonnensis*) and vice versa localities of *Plotiopsis balonnensis* near the Gulf of Carpenteria coast, i.e. Bynoe River and Gregory River, where *"Thiara" australis* clearly dominates. At Bynoe River there even is a record of sympatric and syntopic occurrences of these two thiarids in Australia (see fig.27).

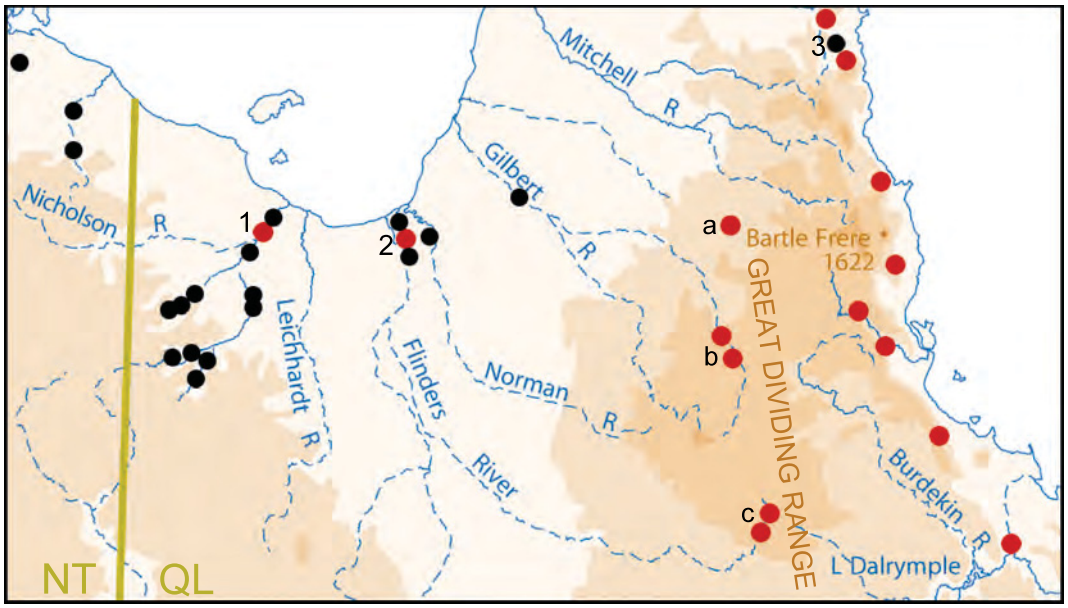

Figure 36 Region where the geographical range of *"Thiara"' australis* (black symbols) abuts against the distribution range of *P. balonnensis* (red symbols). Exceptions are marked with numbers, in which 1+2 represent cases where *P. balonnensis* sequences were found in the range of *"Thiara" australis*, and number 3 shows the contrary case. 1: Gregory River and 2: Bynoe River; 3: Three Mile (Poison) Creek (Endeavour River). a,b,c show localities in the Great Dividing range, that drain into the Gulf of Carpenteria but belong to the range of *P. balonnensis*. a: Lynd River (merges with Mitchell River); b: Gilbert-Einasleigh River; c: Porcupine Creek (Flinders River). Note that the representation of distributions is clearly influenced by collecting effort and river accessibility.

The occurrence in the Bynoe River isn't surprising that much as it is an arm of the Flinders River delta and *P. balonnensis* is known to occur in the upper river section of the Flinders River in the Great Dividing Range (see fig.36). Hence, the two species coexist in the same drainage system, with apparently with each species at opposite ends of the river, but as can be seen now with scattered exceptions. One explanation could be that often the streams are seasonally fed by rainfall and the resulting monsoonal flooding of vast areas might lead to diversion of single snails. Another possibility is passive transport for instance by humans along coastal highways which could also explain the second out-of-range occurrence in the Gregory River. However, *P. balonnensis* is notably absent in the entire Timor Sea drainage and the occurrences in the Gulf of Carpenteria are only scattered and uncommon. The best explanation for this observed disjunct distribution, is that it represents a fragmentation of a widespread ancestral species (Wiley, 1988) and with regard to the phylogenetic framework it would have been expected that the two species turn out to have a sister group relationship. Morphologically they are indeed hard to distinguish, but from the molecular point of view (speaking only of the mitochondrial data), *"Thiara" australis* is quite different from *Plotiopsis balonnensis*. In order to shed light on this paradoxon, an AFLP analyses was conducted with a special focus on these two species (see chapter 6). In addition, a bachelor thesis at the Humboldt University in Berlin, was disposed (Melanie Krause, unpublished 2014). The results are discussed in summary on page 111.

To sum up, the degree of endemisms in Australian thiarids declines from six to four species (see also tab.46), as *"Stenomelania" denisoniensis* and *Ripalania queenslandica* are not endemic to the continent. Out of the four remaining endemics, three viz. *"Thiara" australis*, *Sermyla venustula* and *Melasma onca* are restricted to only certain regions in the Leichhardtian and one i.e. *Plotiopsis balonnensis* is widespread on the continent.
Note that the aim of the present account was to incorporate the mitochondrial-data (in addition to new biogeographic data) into the existing knowledge about the Australian thiarids. For the aggregation of all results, including nuclear sequencing and AFLP data, see the corresponding and 'General discussion' chapter.

5 New insights from a lost world - Unlocking the potential of museum collections using historical specimens

5.1 Specific introduction

Museum collections contain specimens gathered over several centuries of natural history explorations and represent a fantastic and underused source of material for biological studies. In the last decade, the number of studies using natural history museum collections for genetic analyses has risen sharply and the growing demand of molecular biologists to sample museum specimens is putting an increasing pressure on the collections (Hofreiter, 2012). Exploiting museum specimens as a source of genetic data is a big challenge due to the degraded nature of the DNA which leads to low rates of amplification and high levels of contamination (Wandeler et al., 2007). The technological challenges are similar to those that occur when working with samples from the fossil record and the methodological approaches are thus similar to those used with ancient DNA (aDNA) techniques (Paijmans et al., 2012). The field of aDNA met with severe criticism when grave errors were found in some influential publications from the early 1990s. In spite of these past controversies, the study of aDNA is now a reliable research area due to recent methodological improvements (Rizzi et al., 2012). The development of next generation sequencing (NGS) has revolutionized aDNA research like almost no other field of genetics leading to reproducible and authentic results (Knapp et al., 2012).

Because of the similar methodological approaches used when working with historical museum specimens, they have often been included in the definition of ancient DNA (Pääbo et al., 2004) but beside the fact that historical samples from museums can be millennia younger than samples from the fossil record there is an important difference. In contrast to analyses of extinct organisms the results of modern degraded DNA or historical DNA (hDNA) can be confirmed by comparisons with results from high-quality DNA samples taken from extant populations. That means there is some knowledge of and control over what has been amplified (Knapp et al., 2012). For this reason, in hDNA research the state-of-the-art NGS-approach used in aDNA research is not mandatory. The most widely used technique for hDNA is whole genome extraction, PCR amplification of short, at best overlapping fragments followed by Sanger-sequencing (Rizzi et al., 2012) allowing inexpensive but confidential studies. PCR amplification is restricted to short amplicons because the high fragmentation of hDNA molecules leads to average fragment sizes of only 200 base pairs (Wandeler et al., 2007). The sequences derived from these short amplicons can be used as 'mini-barcodes' and have been shown to be effective for accurate identification in many animal groups (Hajibabaei et al., 2006; Kirchman et al., 2010; Dubey et al., 2011; Strutzenberger et al., 2012). Studies of invertebrates are surprisingly rare.

In case of molluscs, there is a variety of material stored under diverse conditions from which DNA can be extracted, such as soft bodies, operculums and shells preserved both wet or dry. However, the majority of museum holdings are represented by dry and empty shells which often constitute taxonomically or historically important lots, such as types (Köhler et al., 2008). Working with unique type material minimizing damage to the specimens is an important consideration and therefor a non-destructive extraction method should be used (Casas-Marce et al., 2010). To the best of my knowledge, there is no published protocol for non-invasive DNA extraction from entire dry mollusk shells up to now, that worked on samples older than 10 years of storage. Only few publications describe the use of dry mollusc shell material for extraction and the existing ones use shell fragments (Geist et al., 2008; Barsh and Murphy, 2007; Hawk, 2010) or they perforate the shell (Caldeira et al., 2004). The only extraction from an entire gastropod shell was published by Andree and López (2013) but the material had only been stored for ten years.

Here it is tried to extract DNA from historical material stored up to over a hundred years ago using museum specimens from the Malacological Collection at the NHM Berlin. Extractions are tested for dried entire shells, ethanol-preserved soft bodies and one dried operculum (see tab. 18). With the design of novel primers the use of short amplicons for the identification of museum specimens is explored by comparing them with fresh material using Cerithioidean gastropods as models. In five case studies concerning the Thiaridae and Paludomidae the effectiveness and relevance of this technique is shown.

5.2 Specific material and methods

DNA extraction and pre-PCR procedures of all historical samples were conducted at the Leibniz Institute for Zoo and Wildlife Research (IZW) Berlin in a separate laboratory solely dedicated to ancient DNA work to avoid contamination of historical samples with modern DNA. A list with all 34 historical samples (covering different ages, storage conditions and tissue types) used for initial extraction and PCR tests is given in the appendix (see tab. D). Samples that were chosen for further analyses representing the five case studies are provided in tab. 18.

Table 18 List of studied specimens from the Malacological Collection at the NHM Berlin. All specimen were stored at room temperature. Museum codons see appendix.

Museum n°	Species	Date	Location	Tissue	Storage	Lab n°
ZMA w/o no.	*Balanocochlis glans*	1903	New Guinea Mamapiri	foot tissue	ethanol	SC6
ZIM 3948-1	*Neoradina prasongi*	1968	Thailand Kao Tong	foot tissue	ethanol	SC10
ZIM 3948-3	*Neoradina prasongi*	1968	Thailand Kao Tong	foot tissue	ethanol	SC11
MZB 12.300-1	*Paludomus sp.*	1980	Indonesia Lombok	foot tissue		SC25
ZMB 86812-1	*Ripalania queenslandica*	1937	Papua New Guinea Manus Island	operculum	dry	SC26
ZMB 87263-1	*Ripalania queenslandica*	1937	Papua New Guinea Ramu estuary	shell	dry	SC27
ZMB 87264-1	*Ripalania queenslandica*	1937	Papua New Guinea Sattelberg	shell	dry	SC28
USNM 859456	*Simulathena papuensis*	1970	New Guinea Yule Island	foot tissue	ethanol	SC13

5.2.1 Historical DNA extractions

Extractions were done using the GENECLEAN Kit for Ancient DNA (Bio 101) according to the supplied protocol including a preincubation with Proteinase K. Dried shells, one operculum and foot tissue samples (see tab. 18) were pre-incubated overnight and removed during a centrifuge step after which only the supernatant is carried over to the next step (step 3 of alternative, supplied protocol). Extracted DNA was purified using QIAquick PCR Purification Kit (Qiagen) and stored at -20°C until further use.

5.2.2 Primerdesign & PCR

Primers were specifically designed for the group of interest based on an alignment of 16S sequences of Thiaridae targeting highly conserved regions. In order to exclude contaminations resulting primer pairs were tested with chimp DNA (as substitute for human DNA), rat DNA (Na 52 (R4864)) and bovine herpes virus from a cell growth (BHV-1 (DNA)). Two novel primer pairs S4 (16S_F_AWmod: 5‘ACAAGAAGACCCTGTCGAGC 3‘;16S_R_AS1: GATTATGCTGTTATCCCTGCGG) and S5 (16_F_Thia2: 5‘CTTYCG-CACTGATGATAGCTAG 3‘; 16S_R_FA_AWmod: 5‘CAAYTTTCGAGCTTATCCTC 3‘) did not bind to bovine, rodent or human DNA and were chosen for further analyses. Primer sets were first tested on a range of modern samples in a different laboratory

prior to being used on historical specimens. PCRs were performed in a 30,5 μl final volume containing 22,5 μl Platinum® PCR SuperMix (Invitrogen), 1 μl of each primer (10 pmol/μl), 1 μl BSA (20 mg/ml) and 5μl purified template DNA. The thermal profile of PCRs were 94°C for 4 min, then 60 cycles of 94°C for 30 seconds, 53,3°C (S4) or 50,3°C (S5) for 30 seconds and 72°C for 30 seconds. Final elongation period was 5 min at 72°C. PCR products were run on a 1.5 % agarose gel for amplification qualification and purified using QIAquick® PCR Purification Kit according to the supplied protocol. Forward and reverse DNA strands were cycle sequenced using ABI Prism BigDyeTM terminator chemistry and visualized on an ABI Prism 3130 automated sequencer (SMB Services in Molecular Biology GmbH).

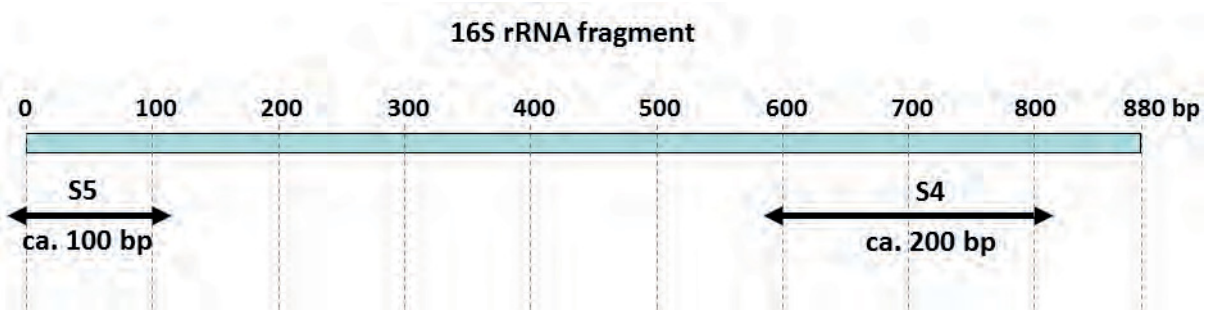

Figure 37 Position of products S4 and S5 within the 16S mtDNA sequence including primer sequences - modified from master thesis J. Ebersbach.

5.2.3 Phylogenetic analysis

Alignments of forward and reverse strands were conducted using CodonCode Aligner v. 3.7.1 (CodonCode Corporation, Dedham, MA, USA). Primer sequences were trimmed leading to hDNA sequences lengths of ∼75 (S5) and ∼150 (S4) basepairs. Sequences from fresh material (∼880bp) and hDNA sequences were aligned using MUSCLE (Edgar, 2004b,a) and corrected manually for algorithm-specific errors. As recommended by Dittmar et al. (2006) unobtainable characters were coded as missing. Bayesian inference (BI) (Huelsenbeck et al., 2001) was employed to infer phylogeny by using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) for 5,000,000 generations (samplefreq=200 burnin=35001) using the substitution model HKY+G+I, according to MODELTEST version 0.1.1 (Posada, 2008). In addition, phylogenetic trees were reconstructed by maximum parsimony (MP) using the heuristic search algorithm as implemented in PAUP* 4.0b10n (Swofford, 2002), with gaps treated as fifth base. Support for nodes was estimated by bootstrap analysis (10000 replicates). Maximum Likelihood (ML) analyses were conducted with TREEFINDER (Jobb et al., 2004) (1000 bootstrap replicates) using the model specified above. The final calibrated chronograms and node estimates were edited using FigTree version 1.3.1 (Rambaut, 2009).

5.3 Results and discussion

5.3.1 Extraction

The extractions worked in each case leading to DNA of sufficient quantity and quality for successful PCR amplification with the designed primers (see next paragraph). Amplification success was seemingly independent of used tissue type, as PCR products could be recovered from both, dry gastropod shell/operculum as well as soft tissue preserved in ethanol. The extraction procedure proved to be minimally invasive and apparently nondestructive for the entire dry shell. While the periostracum did suffer some slight damage, the shell itself appeared to be largely intact (see fig.38).

Figure 38 Shells of *Ripalania queenslandica* before (a,c) and after (b,d) extraction procedure (a, b: ZMB87264; c, d: ZMB87263). In d the upper whorls of the shell were found to be slightly damaged. Scale bar = 1 cm.

As an important application of hDNA amplicons lies in obtaining sequence information from old type specimens, any possibility of damaging this unique material must be eliminated. The method can be considered non-destructive only in the sense that the shell stays complete. However, it is semi-destructive because the periostracum suffers some damage. This organic layer is probably the source of the extracted DNA as Geist et al. (2008) found out that removal of the periostracum before the extraction resulted in decreased yield of DNA.

5.3.2 Primer design and phylogenetic analysis

Two sets of conserved primers that reliably amplified short DNA fragments (S4 and S5) from historical samples were designed and tested. The positions of these amplicons in the 16S alignment of full-lenghts sequences are shown in fig. 37.
The S5 fragment (∼75bp) was amplified in all thiarid species studied but did not work with the *Paludomus* sample which represents another family. The S4 amplicon (∼150bp)

was obtained in 7 out of 8 species. It could not be amplified from the extraction of the *Simulathena* sample (discussed in 5.3.3).

It was not possible to get a set of primers for multiple overlapping amplicons to recover the full 16S sequence, because the requirements concerning the authentication criteria could only be fulfilled by the two chosen ones. However, Meusnier et al. (2008) analysed the minimum amount of sequence information required for identifying species and found out that in 90% of the species tested in their analyses a DNA barcode of only 100bp contained nucleotide substitution(s) specific to members of a particular species. In this study the two short amplicons from old museum specimens having a total lenght of ∼225bp were also effective in identifying specimens as shown in the phylogenetic reconstruction (see fig 39). They formed monophyletic groups with sequences of freshly collected specimens of the corresponding species (discussed in the next section). The Bayesian analysis resulted in a topology very similar to those of the ML and MP analyses (see appendix fig. 55 and fig. 56), so only the BI phylogram is presented.

Figure 39 Bayesian consensus phylogram based on 16S sequences. Numbers on nodes indicate support of the shown topology by means of Bayesian posterior probabilities. Museum samples are shown in red (*Ripalania* samples) and green (see text 5.3.3). Red symbols correspond to localities and shells shown in fig. 40. Rque: *Ripalania queenslandica*; Bgla: *Balanochochlis glans*; Psia: *Paludomus siamensis*. For other abbreviations in taxa names see appendix. Four and five-digit numbers represent extraction numbers, numbers with prefix letter codes museum numbers.

5.3.3 Systematic relevance of the results

In the following five case studies the effectiveness of this technique is shown. The taxa included were chosen because their identification was needed to clarify specific biogeographical and systematical questions. As this thesis focuses on the Australian taxa, the results and the systematic relevance of non-Australian taxa are only briefly discussed.

***Ripalania queenslandica* (Smith, 1882)** is said to be endemic to Australia however the Malacozoological Collection Berlin hosts samples which closely resemble this species morphologically, but which were collected in Papua New Guinea (viz. SC27, SC28) and the Admiralty Islands (viz. SC26) in 1937. DNA from these three samples could be extracted and amplified with the two novel primer pairs. The analysis shows that two of the historic specimens (viz. SC26, SC27) from Papua New Guinea cluster with *R. queenslandica* sequences from Australia indicating that the species is, in fact, not actually endemic to Australia.

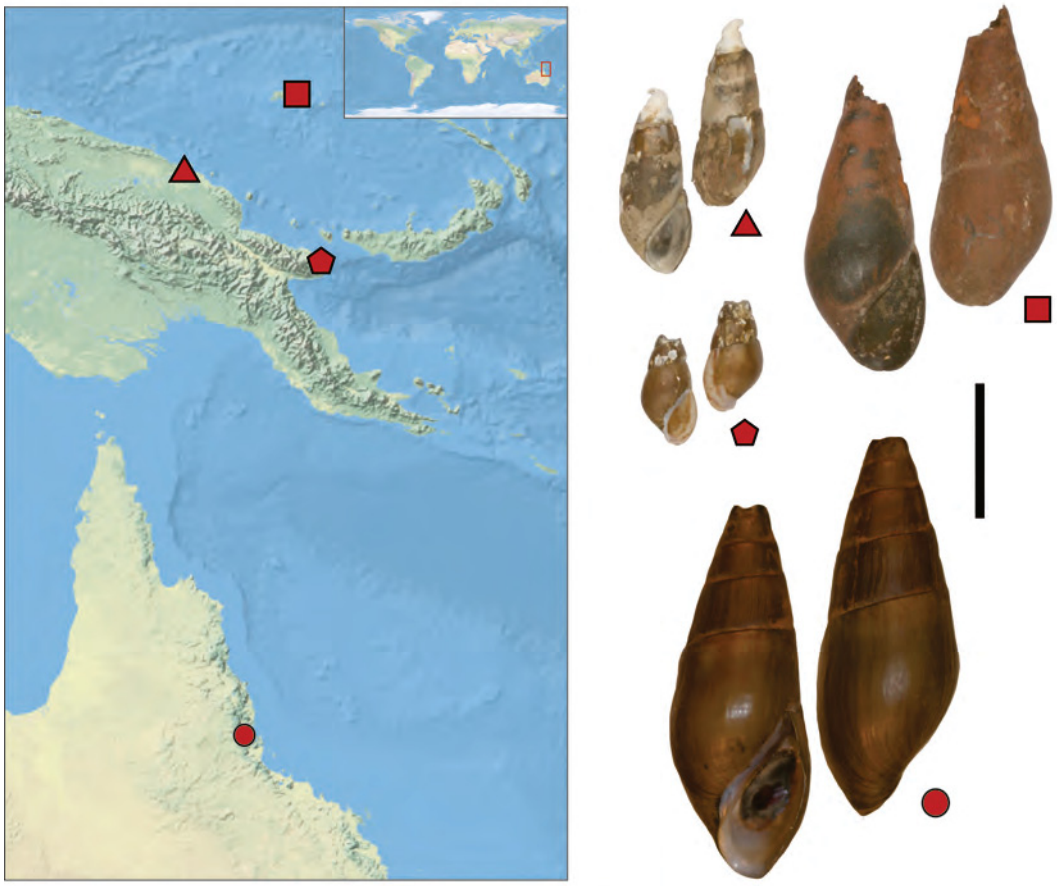

Figure 40 Formerly considered as being endemic to Australia, historic samples from the Bismarck Archipelago, suggest a wider distribution of *R. queenslandica* outside of Australia. Scale bar: 1 cm. Symbols: quad - SC26; triangle - SC27; pentagon - SC28; circle - fresh material from Australia represented by one typical shell.

One sample (viz. SC28) that was thought to be *R. queenslandica* does not cluster within the *R. queenslandica* cluster. The shell is much smaller than the others and the parietal wall of the aperture is slightly different in shape (see fig 40) indicating that this shell might belong to another species.

***Neoradina prasongi* (Brandt, 1974)** is the type species of the genus and only known from Thailand where it has been exclusively reported from the type locality near Kao Tong (Brandt, 1974). Up to now, the genus has been neglected by phylogenetic scrutiny because species identification is difficult due to its close resemblance to other thiarid genera (*Melanoides* and *Stenomelania*). The phylogenetic reconstruction indicates that the cluster of the two historic specimens of *N. prasongi*, verified here with types (viz. SC10 and 11), and fresh topotypical material from southern Thailand, represents an independent thiarid lineage, distinct both from *Melanoides* and *Stenomelania*.

***Balanocochlis glans* (Busch, 1842)** (viz. SC6) clusters with the appropriate sequences from fresh material of the species. This taxa is discussed separately in Glaubrecht et al. 2013: The two clades of *Balanocochlis* constitute strong evidence for an interesting geographical break across the island of New Guinea, clearly separating the western clade from an eastern clade.

***Simulathena papuensis* (Houbrick, 1992)** was originally described as a member of the Planaxidae (Mollusca: Cerithioidea) (Houbrick, 1992). Morphologically, particularly regarding shell, radula and reproductive biology, it might rather represent a member of the limnic Thiaridae (Glaubrecht, personal communication), rendering its interest for genetic analysis. Only the product of primer pair S5 could be amplified from the historical sample. The short amplicon (73bp) clusters with a sequence of *Pseudoplotia scabra* from Laos differing in only 3bp. In a separate analyses including outgroups of Pachychilidae, Planaxidae, Potamididae, Cerithiidae and Pleuroceridae, the *Simulathena papuensis* sequence (viz SC13) appears at the same position within the Thiaridae (see fig. 54 in appendix). This provides the final proof that it is a representative of this family.

***Paludomus sp.* (Swainson, 1840)** The genus is widespread in the Indo-West Pacific from the Seychelles and India to Indonesia and the Philippines. Sample SC25 was collected in Lombok, Indonesia, and is one of the only *Paludomus* samples ever to be found beyond the Wallace Line. Only the product of primer pair S4 could be amplified. The 150 bp amplicon from the old museum specimen (viz SC25) formed a monophyletic group with freshly collected specimens of the corresponding genus. The result reveals that the taxon is apparently not strictly Oriental in its distribution.

5.3.4 Summary and conclusion

Two sets of novel primers for targeting regions within the 16S gene to produce ∼75bp (S5) and ∼150bp (S4) amplicons from hDNA were designed. Comparison of sequence information obtained from these hDNA fragments with recently collected samples provided excellent corroboration. The mini-barcodes enabled the identification of species by phylogeny reconstructions as illustrated by five thiarid case studies.
Concerning the extraction from entire shells, shell DNA provides a useful additional source for DNA-based analyses but damage of the periostracum makes the procedure not safe enough when using type material. However, the extraction method was only minimally invasive and even dried tissue of soft body proved to be suitable material for amplifying DNA.
It should be noted that if samples were collected and handled without DNA studies in mind, as is common for most museum specimens, the extracts can contain significant amounts of contaminating human DNA (Knapp et al., 2012). Thus, this approach should only be used if enough data for comparison is available and if dedicated laboratory facilities exist. Fulfilling these conditions, the shown procedure enables to contextualize old museum material within biosystematics research in an inexpensive but confidential way. Since it was feasible to amplify mtDNA fragments from a historical sample stored over a hundred years ago (viz. SC6), it is possible to think about amplifying material even older.

6 AFLP fingerprints reveal contrasting patterns of genetic structure between nuclear and mitochondrial data

6.1 Specific introduction

In the preceding sections it was shown that the nuclear data does not comprehend enough information to clear the relationships among closely related species of thiarid taxa. In contrast to the mitochondrial based inferences, the relationships among the genera and taxa within the thiarids are poorly resolved in the nuclear gene trees providing little phylogenetic signal.

The lack of population structure in nuclear genealogies within highly differentiated mtDNA lineages is not uncommon. But the sole use of mitochondrial data to infer a family's evolutionary history can be greatly misleading, as the genealogy does not necessarily match the true history of the species (Ballard and Whitlock, 2004). Hence more nuclear data is needed to uncover the genetic differences between species and to build a more robust phylogeny of the family. When working with molluscs, the absence of suitable sequencable loci is a phenomenon one is often faced with (Greve et al., 2012; Haase et al., 2014). With regard to the extensive process of developing sequence-based nuclear markers useful for phylogenetic reconstructions, a number of recent studies have emphasized the utility of amplified fragment length polymorphism (AFLP) markers for the analysis of species where other markers have yet to be developed (Dasmahapatra et al., 2009). The AFLP technique has become an attractive tool in phylogenetics as it allows molecular genetic analyses without any prior DNA sequence information of the organism under study. Because AFLP markers are sampled throughout the genome, they are likely to uncover rare genetic differences in groups with low sequence variation and they have proved to be valuable characters to resolve phylogenetic relationships among closely related taxa, but also at the family-level (Dasmahapatra et al., 2009; Meudt and Clarke, 2007).

In addition, the AFLP technique is an attractive tool in population genetics and might help to assess genetic variation and population structuring of the thiarids with regard to the different river drainage systems. This could lead to a better understanding of the distribution patterns in Australia and their connection with different dispersal potential as dicsussed in chapter 4.

However, due to the nature of the AFLP technique its reliability has been an issue since the introduction of this method. Unlike DNA sequencing, where each nucleotide can be determined with high degree of confidence, AFLPs can contain amplification failures and there is a lack of a reliable control. For this reason a new automated scoring approach, called AMARE, is used in this study that works in an objective and perfectly reproducible way (Kück et al., 2012). In order to be comparable with the preliminary sequencing

results a similar data set (see chapter 3) was aimed for with an additional focus on the two endemic species *"Thiara" australis* and *Plotiopsis balonnensis*. As mentioned in the introduction and discussed in the biogeographical revision (chapter 4), the simplest explanation for their observed disjunct distribution and morphological similarity, is that they represents a fragmentation of a widespread ancestral species. If so, the two species are sister groups, but the mitochondrial data reject this relationship even showing that *"Thiara" australis* is genetically quite different from *Plotiopsis balonnensis* (see fig. 5). In order to shed light on the evolution of the thiarid taxa the phylogenetic signal in AFLP data is compared to the efficacy of mtDNA sequences.

6.2 Specific material and methods

6.2.1 Sample choice

To have comparable results with the dataset of the sequence-based trees from the first chapter, it would, of course, have been best to take exactly the same samples as in these anaylses. However, electrophoresis tests showed that in most cases the DNA quality was insufficient for the AFLP technique. Especially older samples or older extractions showed a high degree of degradation. As low DNA quantity and/or quality are known to promote genotyping errors (Pompanon et al., 2005) these samples had to be excluded.
To get results that are still comparable with the sequence data, an effort was made to replace samples that did not work by congeners from the same locality. Over 200 samples from older collections were checked (see section DNA concentration adjustment) in order to make the dataset as similar as possible. For taxa from Australia fresh material from a special expedition in 2011 was taken. The list of material is given in the appendix (see page 169).

6.2.2 Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a fingerprinting technology based on the polymerase chain reaction (PCR) and was first described by Vos and Hogers (1995). AFLPs are generated by complete restriction endonuclease digestion of total genomic DNA, followed by selective PCR amplification and electrophoresis of a subset of fragments, resulting in a unique, reproducible fingerprint (or profile) for each individual. The markers that make up the fingerprint are widely distributed throughout the genome, allowing an assessment of genome wide variation (Meudt and Clarke, 2007).

DNA concentration adjustment To yield comparable and homogeneous fingerprints, quality and quantity of DNA was checked on a 1.5% agarose gel and the DNA concentration was standardized among samples. Concentrations were measured using the Thermo Scientific NanoDrop^{TM}ND1000 spectrophotometer. TE buffer in which the DNA was dis-

solved after extraction was used as reference material (blank). When a measurement of a sample is taken, the intensity of light that is transmitted through the sample is recorded. The sample intensities along with the blank intensities are used to calculate the sample absorbance at a given wavelength (260 nm). The analyte concentration is correlated with the calculated absorbance. For each sample, two independent measurements were taken and averaged afterwards. After measurement, the concentration of DNA samples was adjusted to 50 ng/μl. Samples with higher concentration were diluted with TE buffer.

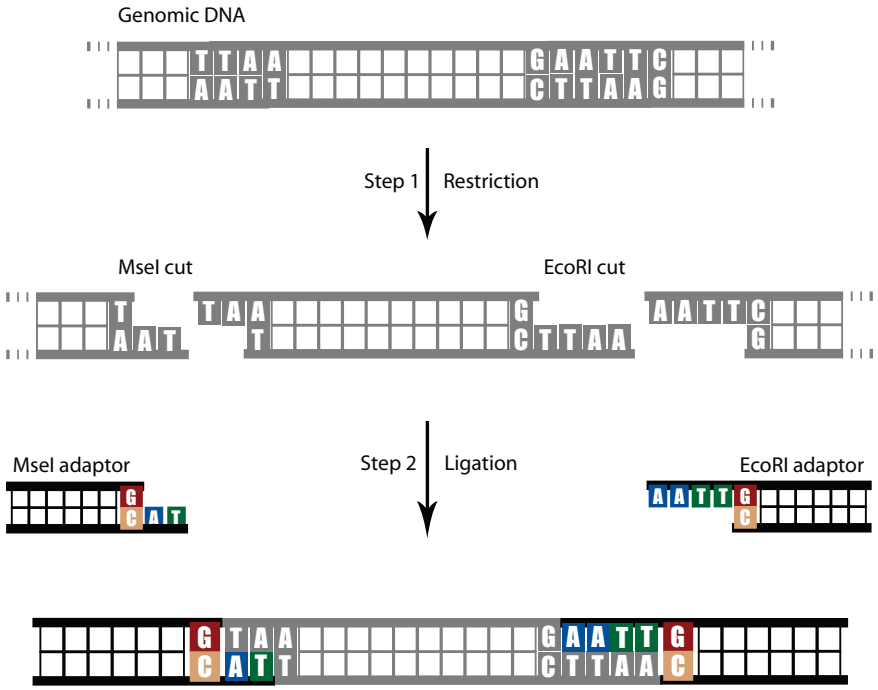

Figure 41 Restriction and ligation. In step 1, genomic DNA is digested with a pair of restriction endonucleases (EcoRI and MseI), producing three categories of DNA fragments (see text). In step 2, double-stranded EcoRI and MseI adaptors with complementary sticky ends are ligated to the restriction fragments.

Restriction and ligation (RL) For the AFLP procedure, restriction and ligation (see fig. 41) were carried out in a single step. Genomic DNA was digested with two restriction enzymes, a frequent cutter MseI (4bp restriction site) and a rare cutter EcoRI (6bp restriction site). After digestion, three categories of fragments exist in the mixture: fragments with EcoRI cuts at both ends (longer ones on average), fragments with MseI cuts at both ends (smaller ones on average), and fragments with an EcoRI cut at one end and a MseI cut at the other end. The AFLP protocol is designed to amplify and preferentially detect this last kind of fragments. Each obtained fragment possesses sticky ends on both sides, which consist of a few bases to which adapters are ligated using DNA ligase (Bonin et al., 2005). The adaptors (see fig. 42) contain core sequences, which are complementary to primers used in the following amplification steps (Vos and Hogers,

1995). In a 0.2 ml Eppendorf tube, 2.75 μl DNA sample and 8.25 μl mastermix (see appendix tab.26) were merged. After short centrifugation at 8000 rpm, samples were incubated over night in a thermocycler (2h at 37°C, 8h at 16°C). After incubation, the reaction product was diluted in 39 μl of TE buffer.

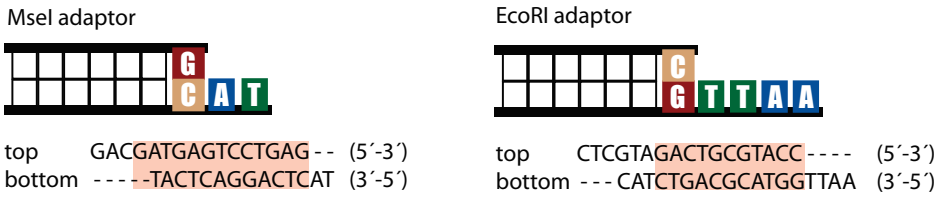

Figure 42 AFLP adaptors: short DNA fragments with sticky ends corresponding to the cuttings of the enzymes. The highlighted part shows sequences complementary to amplification primers.

Preselective amplification (PA) Preselective amplification aims to decrease the complexity of the initial fragment mixture by amplifying only a subset of fragments (Bonin et al., 2005). It is conducted as normal PCR with two primers (see tab. 19) whose structure consists of a core sequence, an enzyme specific sequence, and a selective single-base extension at the 3‘ end of the primer (Zabeau and Vos, 1993). Only fragments exhibiting the chosen bases inside the fragments will be amplified resulting in a reduction of fragment numbers by 1\16 of the initial amount (Meudt and Clarke, 2007).

Table 19 Primer used for preselective amplification. enz.= enzyme specific sequence, ext.= selective extension.

primer	core sequence	enz.	ext.
preselective EcoRI	GACTGCGTACC -	AATTC	- **A**
preselective Mse I	GATGAGTCCTGAG -	TAA	- **C**

For PCR reaction, 4 μl diluted RL product and 16 μl mastermix were mixed in a 2 ml Eppendorf tube and shortly centrifuged at 8000 rpm. The used mastermix and PCR profile is shown in the appendix (fig.58 and tab.27).
To ensure complete digestion and to prevent later amplification of uncut fragments, an important quality control consists of running a portion of the preamplification product on an agarose gel (Mueller and Wolfenbarger, 1999). Successful samples should show a smear and the intensity of this smear should be similar across samples (Bensch and Akesson, 2005). Of the resulting PA product 9 μl were analyzed via agarose gel electrophoresis for

one hour with 55 V and 400 mA. The remaining PCR products were diluted in 50 μl of TE buffer.

Selective amplification The selective amplification is based on the same principle as the preselective one. A small aliquot of preamplified fragments is used in a second PCR with two primers that additionally extend two bases inwards. This further reduces the number of fragments by 1\256. The EcoRI primer is labeled with a fluorescent dye (a fluorophore), so that all strands synthesized from this primer are fluorescently labeled (Meudt and Clarke, 2007). Because the restriction/ligation step results in three types of fragments (i.e. EcoRI-EcoRI, EcoRI-MseI and MseI-MseI), labeling of the EcoRI primer has the advantage that fragments amplified only by the MseI primer will not be visualized (Bensch and Akesson, 2005).
For selective amplification, 4 μl diluted product from the PA reaction was taken and mixed with 18 μl mastermix. Samples were centrifuged (1 min, 8000 rpm) and placed in the thermocycler. EcoRI selective primers are specially designed to have a higher annealing temperature than MseI selective primers. As a result, a touchdown PCR allows a preferential amplification of EcoRI/MseI versus MseI/MseI fragments (Bonin et al., 2005). The first cycle of the PCR had an annealing-temperature of 65°C and was then gradually reduced (1°C per cycle). The used mastermix and complete touchdown PCR profile is given in the appendix (fig.59 and tab.28).

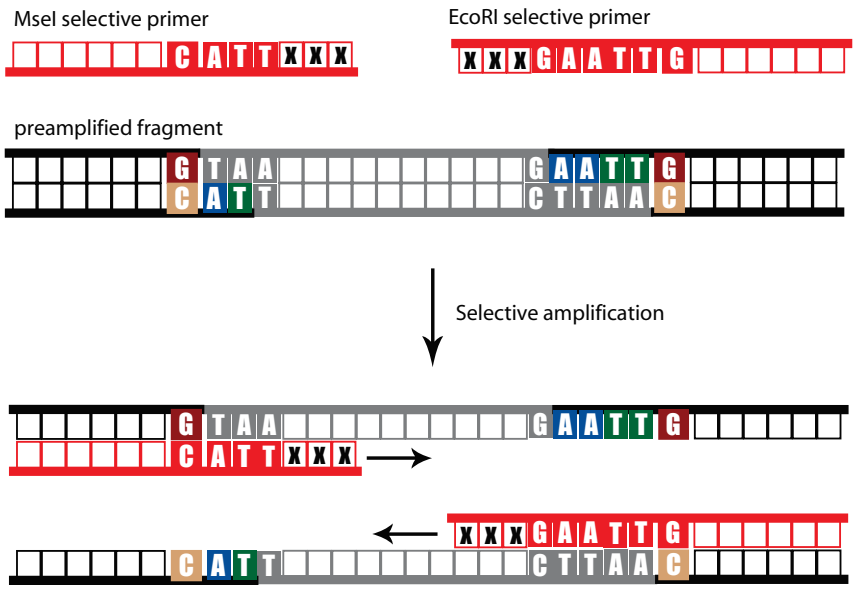

Figure 43 The selective amplification further reduces the amount of fragments, due to the extension of the primers by two selective bases (”X”).

Selection of primers Before embarking on the full project, different primer combinations were tested on a small number of samples. Initial tests of primers for AFLP experiments are necessary to ensure that they produce an appropriate number of fragments (Fink et al., 2010). High quality profiles show well separated peaks, a lack of shoulder or stutter peaks, fragments distributed throughout the available size range, and clear polymorphisms (Meudt and Clarke, 2007). Using these criteria, we found five primer combinations out of 17 that produced profiles suitable for high throughput genotyping. The EcoRI selective primers were labeled with different fluorophores, enabling the products from different primer combinations to be pooled for capillary electrophoresis (see tab. 20).

Table 20 Primer combinations used for selective amplification. Selective bases are shown in bold type. The EcoRI primers are labeled with two kinds of fluorescent dye (blue and green) in order to enable poolplexing of differently labeled products.

selective primer	core sequence	extension	dye	color
EcoRI ACA	GACTGCGTACCAATTC	**ACA**	HEX	green
MseI CTT	GATGAGTCCTGAGTAA	**CTT**	-	-
EcoRI AGA	GACTGCGTACCAATTC	**AGA**	FAM	blue
MseI CGG	GATGAGTCCTGAGTAA	**CGG**	-	-
EcoRI ACC	GACTGCGTACCAATTC	**ACC**	HEX	green
MseI CTG	GATGAGTCCTGAGTAA	**CTG**	-	-
EcoRI AGC	GACTGCGTACCAATTC	**AGC**	FAM	blue
MseI CGG	GATGAGTCCTGAGTAA	**CGG**	-	-
EcoRI AGG	GACTGCGTACCAATTC	**AGG**	HEX	green
MseI CGA	GATGAGTCCTGAGTAA	**CGA**	-	-

Capillary electrophoresis of fluorescently labeled AFLPs In order to separate the different fragments after selective amplification and to estimate their size, samples were loaded on an ABI Genetic Analyser from Applied Biosystems by SMB Services in Molecular Biology GmbH. Capillary electrophoresis follows the same principle as agarose gel electrophoresis and occurs when an electric field is applied to an electrolyte solution within a capillary, causing ions to migrate. The labeled DNA fragments are automatically denatured, separated and then detected by laser-induced fluorescence. The capillary instrument detects fragments present in the spectrum of each fluorophore, producing an electronic profile of relative fluorescence units (RFU) versus fragment size (Meudt and Clarke, 2007). The program determines the size of the amplified restriction fragments with the help of a size standard, then it classifies them according to their size with single-base resolution (Bonin et al., 2005). Blue labeled products and green labeled products were

pooled for capillary electrophoresis. 1 μl blue labeled PCR product and 1 μl green labeled PCR product was added to 11μl HiDi with formamide GS 500(-250) and denaturated at 90°C in a thermal cycler (2720 Applied Biosystems) for two minutes before capillary electrophoresis.

Preparation of samples Since replicated pairs are the only objective measure of quality of AFLPs (Meudt and Clarke, 2007) replicates were produced for more than 40% of the 115 samples for each primer combination. To pick up handling errors at any stage of the analysis the 52 replicates were of different kinds representing different treatments. In 26 cases the extraction product was used twice for restriction/ligation and 26 times the same PA product from the same individual was used twice for selective amplification. These replicates were analyzed independently partly on different plates to detect differences between the electrophoresis runs and to account for potential position effects on the 96-well plate of the sequencer.

Analysis of raw data The software program GeneMapper version 4.0 (Applied Biosystems) was used to analyze the raw fluorescent AFLP data and to convert it into binary matrices. The peak height threshold (PHT) determines if a peak is called present (1) or absent (0). That is why it should not be too low because also backround noise could be scored. If, on the other hand, it is set too high, a peak would not be scored although it is present (Holland and Clarke, 2008). In this study the PHT was set to 50 rfu (relative fluorecent units). The minimum fragment length (MFL) determines the size of the characters in base pairs that are scored and included in the profile. Short fragments show a higher risk of homoplasy (Vekemans et al., 2002) so scoring was conducted between 50 and 500 bases (MFL value of 50 rfu). As recommended by Holland (2008), bin width was reduced from the default 1.0 bp setting and set to 0.85 bp, because this helps to distinguish between nonidentical fragments that differ in mobility by less than 1 bp. All profiles were checked concerning correct fit of the size standard and distribution of fragments throughout the available size range. Low quality profiles were discarded. After automated scoring, binary 0-1 matrices were exported as text files having a format compatible with the AMARE software, see next section.

6.2.3 Automatic masking - marker selection

In the AFLP fingerprint, a marker is an amplified locus that is identified as peaks of equal fragment size across multiple samples (Meudt and Clarke, 2007). The AFLP scoring software produces marker matrices by converting fluorescence data to binary data by first binning the data (grouping the peaks of equal fragment size from different accessions into a single marker) and then scoring the peaks as 1 (present) or 0 (absent) (Holland and Clarke, 2008). After this procedure it can happen that markers are grouped multiply

(multiple markers with identical fragment size) or that two peaks are present in one marker. As the number of markers is around several hundreds per primer combination manual correction is time-consuming. In addition the remaining markers after correction differ in reliability and with such an amount it is hard to get a correct impression of the quality of the dataset. Furthermore, manual selection of markers is subjective and not repeatable.
For these reasons a procedure was designed by the author and colleagues (Kück et al., 2012) that can be used to optimize the selection of markers in an automated way. A PERL script was written and applied to clean up the data and to detect unreliable markers (markers that are unstable or difficult to score). The procedure is shortly described in the following.

AMARE - AFLP MAtrix REduction (Kück et al., 2012)
AMARE serves as a second filter for marker selection after using commercial software packages for bin width definition and peak height detection (as described above). The approach is based on replicates and makes marker selection dependent on marker reproducibility to control for scoring errors. Strength and accuracy of the approach depend on the number of replicates and whether they are representative for the whole data set. The starting point is the assumption that these replicates can help to detect unreliable bins. Unreliable bins are defined as bins which show a high number of incongruent scorings among replicates. AMARE uses three criteria (tresholds) to mask the matrix: A treshold of bin reliability (BR) sets the acceptance value of the minimal number of reproducible (0,0) and (1,1) bin states. If a bin has a BR below this treshold it is considered unreliable and will be masked in the matrix. The BR treshhold is automatically incremented by 0.01 starting from the user defined treshold until BR=0.95. Hence, after the execution of AMARE different output matrices (for each individual threshold set) are recorded. The user might most likely choose the largest (n' x m')-character matrix where n' is the number of replicates and m' is the number of remainig bins.
AMARE tries to keep as many characters as possible by inspecting the quality of replicates of individuals. Low quality replicates are discarded from the data set dependent on a replicate reliability threshold (RR). Further, the user can indicate a minimum bin distance threshold (BD) of allowed distances between differently sized bins corresponding to the standard deviation of the sequencer's sizing precision. For more details please read the detailed description in Kück et al. 2012. The approach of bin masking among replicates is outlined in figure 44. In the present study, AMARE matrices were obtained by setting the minimum BR threshold to 0.7 and the BD treshold to 0.15 according to the sizing precision of the ABI sequencer.

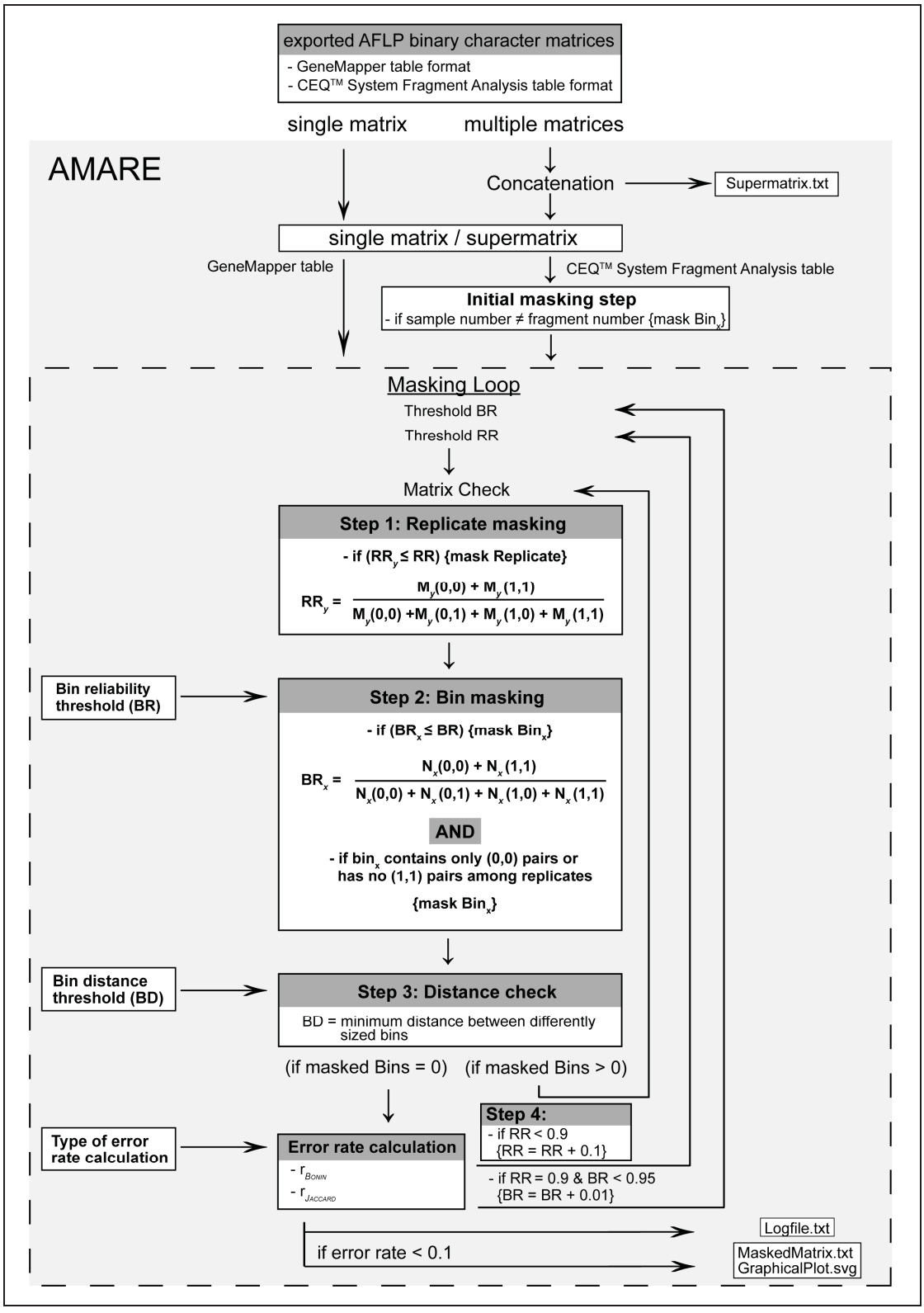

Figure 44 Flowchart of AMARE (Kück et al. 2012). The approach of bin masking among replicates can be separated into four steps.

6.2.4 Analyses

Phylogenetic analysis of the AFLP data was carried out in PAUP* v4.0b10 (Swofford, 2002) using the neighbour-joining algorithm (Saitou and Nei, 1987) with 1000 bootstrap replicates. The principle of the neighbour-joining method is to find pairs of operational taxonomic units (neighbours) that minimize the total branch length at each stage of clustering of neighbours starting with a starlike tree (Saitou and Nei, 1987). The process of testing all pairs of neighbours is repeated until no more joining can be done (Hartl and Clark, 1997).

6.3 Results

All samples turned out to have poor-quality electropherograms with weak signal peaks. The weakness of the signal led to peaks that weren't high enough to be clearly detectable, measurable and delimitable. Due to this, even the fingerprints from replicate DNA extractions showed an unexpected high degree of differences. There was no correlation to the age of the samples, so degradation of DNA as a cause could be ruled out. Despite extensive attempts including variations of primer combinations, recipes, PCR programs, chemicals and concentrations the profiles stayed unclear. Also the separation of restriction and ligation didn't improve the results neither did the separate analyses of the different dyes. However, having treated all samples in the same manner, the electropherograms were analysed showing at least moderate signal.

6.3.1 AMARE output

Starting with a BR treshhold of 0.7 260 matrices were generated. A summary of all threshold sets and corresponding error rates is shown in the appendix (see p.166). Note that after a first run the replicate sample of *Neoradina* was excluded. As this sample is of high importance, it was not treated as replicate again but still included in the analyses. Doing so the largest (n' x m')-character matrix (n': number of replicates = 51; m': number of remaining bins = 704) was BR: 84 % RR: 80 % and selected for further analyses. The number of selected markers decreased from 1998 to 704 markers in the AMARE masked matrix (see tab. 21).

Table 21 Number of bins per primer combination before and after masking with AMARE (BR 84% RR 80%). The numbers of initial bins and those that were excluded are in the same range for each primer combination.

primer combination	no. of bins before AMARE	no. of bins after AMARE
EcoRI ACA & MseI CTT	436	144
EcoRI AGA & MseI CGG	352	116
EcoRI ACC & MseI CTG	437	116
EcoRI AGC & MseI CGG	401	178
EcoRI AGG & MseI CGA	372	150
	in total 1998	704

6.3.2 Neighbour-joining tree

The neighbour-joining tree of the AMARE reduced character matrix is partly unresolved containing 78 polytomies and all in all moderate bootstrap support (BS) (see fig. 45). The included replicates group as sister taxa with bootstrap support $\geq 50\%$ in 33 cases (out of 52). Out of those a bootstrap support of 100% is present in only 9 cases. 16 replicated samples do not even group as sister taxa.

Despite the general pattern of weak signal peaks in the electropherograms, the neighbour joining tree shows phylogenetic signal, as most of the predefined species are well separated from each other. From the Australian taxa the phylogenetic tree clearly resolves *Thiara rudis*, *Ripalania queenslandica*, *Thiara amarula*, *Pseudoplotia scabra* and *Melasma onca*. The single Australian *Stenomelania aspirans* sequence clusters with congeners from South Bali and *Stenomelania sp.* from Seram (BS: 98,8%).

Discrepancies exist in five species, that did not form a monophyletic cluster: In case of *Sermyla venustula* two individuals from Howard springs are not included in the main cluster. Furthermore *Melanoides tuberculata* and *"Stenomelania" denisoniensis* are polyphyletic in the tree as they were in the sequence based topologies (see fig. 5 and 6).

The main discrepancy to the mitochondrial data and the most suprising result is that all individuals of *"Thiara" australis* and *Plotiopsis balonnensis* build a monophyletic group (BS: 87,7%). The high genetic divergence between the mitochondrial data of these two species are not corroborated at all by AFLP data.

Looking at the phylogenetic relationships between the species, the AFLP tree does not support the majority of the mtDNA lineages. The position of the *M. onca* sequences that are closely related to a clade comprising individuals of *Tarebia lineata* from Indonesia is in concordance. These two branches cluster with *Tarebia granifera* from Indonesia and Timor as they do in the sequence based mt-topology (see fig. 5). The close relationship between *Sermyla venustula*, *Sermyla riqueti* and sequences of *Melanoides tuberculata* is another congruence between AFLP tree and mitochondrial data.

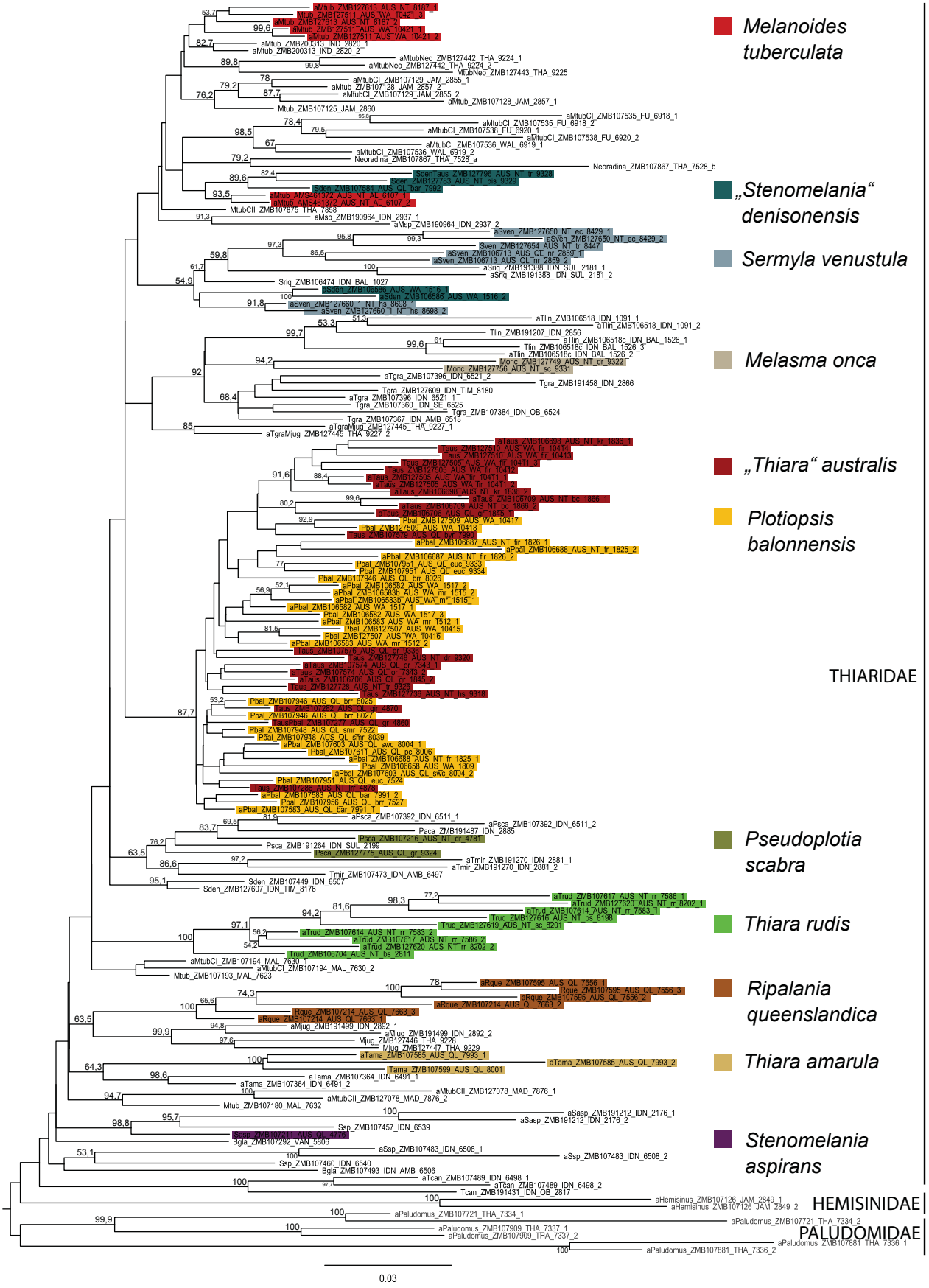

Figure 45 Neighbour-joining tree of the AMARE reduced character matrix (BR: 84 % RR: 80 %). Replicated individuals are indicated by _1 and _2 at the end of the name. Bootstrap values are shown at nodes if higher than 50. Samples from Australia are highlighted in colour, corresponding to species assignment (see legend on right side of tree).

6.4 Discussion

In case of thiarids for which the AFLP technique had not been previously developed, the establishment and modification of the AFLP protocol was extremely time-consuming and the result still underperforming. Probably due to the weak signal, scoring was often misleading, a problem with which also the program AMARE cannot help, as it concerns the whole dataset and not specific unreliable bins. The bad quality of the profiles can be seen in the high number of incongruent scorings among the included replicates: 30% do not even group as sister taxa. Only 60% of the replicated samples group as sister taxa with bootstrap support $\geq 50\%$. It would have been expected that all or at least a great majority of replicates have identical AFLP profiles resulting in a sister group relationship with 100% bootstrap support, as was the case in only 17% of the samples.
The difficulty to obtain good quality profiles should be further analysed as there might be a connection to the problems in DNA extraction in this taxonomic group. In general, snails have enormous quantities of mucopolysaccharides which make standard DNA extraction problematic (Skujienė and Soroka, 2003). Common ready-made extraction kits (e.g. Qiagen DNeasy) cannot be successfully applied with thiarids, here only extraction techniques including chloroform/phenol steps lead to purified DNA. Although the inserted DNA should be freed from mucopolysaccharides through the purification steps, the problem might be linked to the underperforming AFLP analyses.

Because of the low confidential degree, a population based approach with regard to the genetic differentation within species between different river drainage systems was abandoned. Based on this data also confidential inferences about the evolutionary relationships among the species are difficult to be made. Nevertheless, there is quite interesting phylogenetic signal in the neighbour-joining tree that can't be ignored: The AFLP data indicates a very close relationship between the two endemic species *"Thiara" australis* and *Plotiopsis balonnensis*. This is in striking conflict with the results from the mitochondrial sequences, but consistent with morphological expectations. As mentioned, the two cannot be distinguished by external appearance and their geographical distribution looks like a result of vicariance as they usually do not occur together (see chapter 4). Assuming that they share a most recent common ancestor as indicated by the AFLP data and in the absence of clear morphological differences, these two species could be treated as species (or even populations) which have diverged only very recently. However, robust phylogenies are necessary to gain insight into the evolutionary histories of these taxa. The results concerning *"Thiara" australis* and *Plotiopsis balonnensis* are discussed in more detail on page 111 and the AFLP results in a broader context are debated in the following 'General discussion' chapter.

7 General discussion

The subject of this thesis was to reconstruct the evolutionary history of the freshwater snail family Thiaridae with particular focus on phylogeography and the biogeographic origin of the Australian fauna. The results presented here reject the long-held view of the thiarid fauna being an appendage to the southeast Asian biota. Instead the revealed analyses are largely congruent with a vicariance scenario within the framework of Gondwanian fragmentation and an ancestral thiarid lineage that originated in Australia. In addition, emphasis in this work was laid on the extensive inventory of the current species diversification and distributional areas on the continent. In summary, a total of eleven distinct clades were confirmed by the molecular data, contextualizing even historical museum material within biosystematic research. With over 1000 records and recent distribution maps on a drainage based scale, the extensive data on extant species gives new insights into the spatial patterns and the degree of endemism. In the following, a summary of the molecular results with focus on the detected mito-nuclear discord is provided and discussed in the light of the proposed Gondwanian origin.

The fact that estimating the phylogenetic history of species by gene trees can be misleading, is a well known phenomenon and the mechanisms underlying it are understood (Pamilo and Nei, 1988; Maddison and Maddison, 1992; Funk and Omland, 2003; Edwards, 2009). This work gives another example of the potential pitfalls of inferring relationships from only a small portion of the genome and elucidates the importance and necessity to include a broad spectrum of approaches, combining data from morphology, genetics and other sources to delimit species. The comprehensive molecular study presented here does not have sufficient conclusiveness to satisfactorily resolve the relationships among the species in the Thiaridae. The different genes and techniques paint contradictory pictures of evolutionary relationships, which is a known phenomenon especially in freshwater taxa (Puslednik et al., 2009). The classification of limnic gastropods can present difficulties as a result of fine-scale geographical differentiation, isolation in separate drainage systems, introgressive hybridization and ancestral polymorphism, resulting in a poor match between morphological and genetic delineated species (Lee et al., 2007; Köhler and Deein, 2010; Miura et al., 2013; Reid et al., 2013).

This thesis presents the first molecular study of the freshwater snail family Thiaridae based on four DNA sequence markers (COI, 16S, H3 and 28S) and amplified fragment length polymorphisms (AFLPs). Taken together, the comparison of the phylogeographic patterns inferred from these different molecular marker types support the suggestion that in case of thiarids the mitochondrial DNA might not reflect the phylogeographic structure of the species correctly, although it shows high structuring and clearly separates different thiarid species phylogenetically. On this level, however, the investigations based on the nuclear sequences have been unsuccessful in this case. Overall, the nDNA phylogeny of-

fers little power for resolving relationships among younger evolutionary lineages, but the tree shows consistent and well-supported deeper nodes: The Paludomidae are at the base of a clade uniting the Hemisinidae and Thiaridae as sister taxa. The topology of these three freshwater families builds the foundation for a Gondwanian vicariant scenario and it is supported by both nDNA and mtDNA. Although the resolution is generally poor in the nDNA phylogeny, at least the tendencies reveal congruence with the (mostly nuclear) AFLP data, which in turn is more congruent with relationships inferred from morphological data than the mitochondrial splits are. AFLP markers provide a nuclear, multilocus, genome-wide picture of genetic divergence and as they sample noncoding variation, they have relatively rapid rates of evolution. In contrast, the mitochondrion bears the evolutionary history of only a small fragment of a single gene and genetic clustering revealed by multiple AFLP markers is more likely to discover 'real species' than mtDNA data (Dasmahapatra et al., 2010). The most notable point where the nuclear and mitochondrial analyses disagree is the relation between *"Thiara" australis* and *Plotiopsis balonnensis* as discussed in chapter 6 and summarised later on. Another indication for the misleading information in the mtDNA data comes from non-Australian thiarid taxa or rather sequences. The species *Thiara rudis* and *Sermyla riqueti* appear as non-monophyletic in the mtDNA tree but each comprise two distinct mt-clusters in each case (see fig. 5). Indeed, in the nDNA tree these individuals build monophyletic clusters according to the morphologically delimited species (see fig. 6).

From the congruence between the AFLP, the nuclear sequencing data and the morphological results in combination with the non-monophyly of morphologically delimited species in the mt phylogeny, it is concluded that the mitochondrial DNA (COI and 16S) is misleading in case of Thiaridae and does not reflect the phylogeographic structure of the species. Of course, this conclusion calls into question the practice of species identification based on mtDNA sequences alone (as done in the biogeographical revision, chapter 4) and launches the same discussion as in the barcoding debate. In general, there has been a striking discord about the suitability of mtDNA in phylogenetics and taxonomy since Hebert et al. (2003) argued that mtDNA barcodes can be used as a universal barcode for all life. Although a lively debate about what it can and should be used for is continuing (Moritz and Cicero, 2004; Ebach and Holdrege, 2005; DeSalle et al., 2005; Hajibabaei et al., 2007; Waugh, 2007; Valentini et al., 2009; Casiraghi et al., 2010; Goldstein and DeSalle, 2011; Kekkonen and Hebert, 2014), DNA barcoding is a well-established research field attracting large amounts of funding today (Taylor and Harris, 2012). However, species identification via tree-based methods gives the impression of inferring phylogenies and relationships from single gene trees which is widely recognized as a problem by phylogeneticists (DeSalle et al., 2005; Valentini et al., 2009). In this work it is shown that the mitochondrial COI (the standard barcoding gene) and 16S gene provide useful markers for thiarid species identification but not for the inference of phylogenetic rela-

tionships between them. Although the signal embedded diverges from the true phylogeny and must therefore be interpreted with caution, it is still a powerful signal that has its causes. Well differentiated monophyletic clades of COI and 16S haplotypes were detected and each of the morphologically delimited species is characterized by fixed mutational differences. With such a substructure and high genetic divergence between mitochondrial lineages it is suprising that these are not corroborated by the nuclear data. There are many ways in which the biology of the mitochondria differs from the nuclear genome, and these affect the pattern and process of its evolution substantively (Ballard and Whitlock, 2004; Toews and Brelsford, 2012). The resulting conflicting patterns between mtDNA and nDNA can be ascribed to different reasons namely incomplete lineage sorting of ancestral polymorphisms (Avise and Wollenberg, 1997; Pollard et al., 2006; Rato et al., 2010; McKay and Zink, 2010), introgression resulting from interspecific gene flow in the early stages of speciation (Gompert et al., 2008; Rheindt and Edwards, 2011) or mitochondrial gene rearrangements (Inoue et al., 2003; Rawlings et al., 2010; Lin et al., 2014). An alternative explanation is that the conflicting patterns obtained could be due to a gender-biased gene flow, with recurrent dispersion of males and rare dispersion of females or vice versa (Pardini et al., 2001). Of course this would assume the existence of males, which is not secured in case of most thiarids. Selection could also cause increased mtDNA divergence relative to weak nuclear differentiation and the revealed deep mitochondrial splits may correspond to ecologically distinct groups or geographical areas (Cheviron and Brumfield, 2009; Nosil et al., 2009).

The latter in particular could be the case for *"Thiara" australis* and *Plotiopsis balonnensis*. An interesting outcome of this thesis is that these two species, although morphologically similar, exhibit extensive differences among mtDNA haplotypes (chapter 4). However, the AFLP data indicate that these two species share a most recent common ancestor which is congruent with the morphological overlap (chapter 6). The suggestion of Glaubrecht et al. (2009) to distinguish a lineage *Plotiopsis* and to accommodate *balonnensis* as taxon quite distinct from the very similar *australis*, was based on preliminary results of molecular genetic analyses using mtDNA fragments in connection with the distinctive parapatric distribution of both. Prior to this finding B. J. Smith (1992, 1996) considered *Plotiopsis* as subgenus of *Thiara* and Iredale (1943), in addition to *balonnensis*, included four other species among them also *Plotiopsis australis*. However, both purely nomenclatorial-taxonomic treatments were not substantiated by any detailed study or data of any nature. Nevertheless, the close affinity of these two entities is confirmed by their morphology while the new interpretation is based on apparently misleading molecular data. The two species are obviously closely related based on the AFLP data and in the absence of clear morphological differences, they can be treated as para-species (or even populations within a superspecies) which diverged only very recently. The questions arise if they are indeed reproductively isolated, biological species or if they may have consisted of two formerly

allopatric taxa that may be in the process of remerging and fusion. Thus, the situation here is probably best explained by introgression of mitochondrial genes due to secondary contact of previously geographically isolated populations or species. It is known, that in other cases of freshwater Cerithioidea, such as e.g. the Asian pachychilids geographical separation is the main factor that drives speciation and that secondary contact between originally allopatric populations frequently leads to the introgression of neutral markers (Köhler and Deein, 2010). If we are dealing with recently diverged sister species whereby no isolation mechanisms have evolved that prevent species from cross-breeding, there is the possibility of hybridization in the overlap of the geographic ranges. The dynamics of hybrid zones are of considerable interest from an evolutionary point of view because such regions often play important roles in models of speciation (Barton and Hewitt, 1985; Hewitt, 2001). Hybridization with introgression of alleles is discussed as potentially enhancing speciation, thus with potentially important consequences in evolutionary biology and speciation theory (Dowling and Secor, 1997; Barton, 2001; Mallet, 2007; Twyford and Ennos, 2012; Abbott et al., 2013). Its evolutionary role has recently been revisited in the literature now that larger nuclear datasets are increasingly available but we still lack textbook studies of particular molluscs for hybridisation (Glaubrecht, 2011). In the present case, a potential hybrid zone is found in the northern Dividing Range between the Jardinian and the Leichhardtian province, where one documented case of a *"Thiara" australis* mtDNA haplotype was found in the *P. balonnensis* region and vice versa two cases of *P. balonnensis* haplotypes in the *"Thiara" australis* range. Even by looking at the shells of two of these outlier individuals, it is directly apparent that they are much smaller than the shells of their congeners (see fig.17d and fig.26). Such a discrepancy in shell size wasn't found in other individuals and might be a hint to preexisting reproductive isolation in the form of reduced hybrid fitness. An alternative explanation is that these individuals are translocated specimens that do not find their favourable living conditions in the new habitat which would be consistent with the relative stability of the parapatric distribution not allowing the further fusion of both species' ranges. Future genetic and field research is needed to determine whether there is current gene flow between the two clades and whether the two clades are ecologically distinct. If hybridization plays a role in the evolution of these two species the geographical distribution and phylogenetic patterns make them an excellent group for studying the process of speciation, or possibly the processes that allow separate populations to merge and not differentiate as species.

One disadvantage in the case of thiarids is that the application of a particular species concept is not made easy given the prediction that these gastropods reproduce largely via, at least partial, parthenogenesis. For the discussion of the applicability of currently used species concepts see Glaubrecht et al. (2009) and Glaubrecht (2011) . The delimitation of species using genetic data is based on the criterion that species are groups of organisms with similar genotypes as suggested in the genotypic cluster definition of

species given by Mallet (1995), a concept that focuses only on the identification of species and not on their origin (Coyne and Orr, 2004). In this study the delimitation of provisional species is, in the first instance, based on morphological characters as conducted by Glaubrecht et al. (2009). Congruence between these morphologically delimited groups, clades in the mitochondrial gene tree and/or in the nj tree based on the AFLP data corroborates that such groups are evolutionary units that can be considered provisionally as (bio-)species. Two morphologically delimited species from Australia could neither be distinguished by mtDNA data nor by the AFLP data, i.e. *Melanoides tuberculata* and *Stenomelania denisoniensis*. Given the polyphyletic nature revealed by molecular analyses, these taxa deserve further investigation. In the present situation, defining the taxonomic status of the various clades is an extremely difficult task. The phylogenetic investigation has called former taxonomic assignments into question. Especially a new concept for the widely delineated genus *Thiara*, which was found to be polyphyletic in each analysis is needed. In order to solve the obvious discrepancies between molecular and conchological data, additional morphological and anatomical studies are needed to shed light on the taxonomic status and evolutionary history of each particular taxon. The present study fortifies the finding of Glaubrecht et al. (2009) that characters of shell plus radula that are classically utilized in limnic malacology (e.g. Martens 1883; Thiele 1928; Rensch 1934; Starmühlner 1969) are only of very limited use in genus or even species level taxonomy in freshwater Cerithioidea which all exhibit large phenotypic plasticity.

A resolved phylogeny and knowledge of geographical range are prerequisites for the inference of biogeographic processes and evolutionary history in general. Unfortunately a robust molecular phylogeny of Thiaridae could not be established, so that suggestions about speciation patterns remain highly speculative, as discussed above for one case study. Although many details of the historical processes and their evolutionary consequences remain to be studied, some of the relevant aspects for the Thiaridae will be discussed in the following in the light of a Gondwanian origin. The synthesis of the findings concerning the species occurrences in Australia is summarized and visualized in Figure 46. One striking biogeographical pattern in the Australian thiarids that is immediately obvious is the widespread occurrence of *P. balonnensis* over vast areas of the continent. As the only thiarid *P. balonnensis* is recorded to be extant in highly isolated water-bodies in desert springs of central Australia. During the Miocene the climate of Australia was much more humid, sustaining large rainforest areas that were drained by systems of rivers and lakes. Changing climatic events during the Pliocene and Pleistocene resulted in the progression of aridity in the central deserts and, accordingly, in the isolation of water bodies and the erasure of limnic faunal elements (Unmack, 2001). The isolated occurrences of *P. balonnensis* represent most likely relictual populations of the previously widespread species that were trapped in these desert refugia.

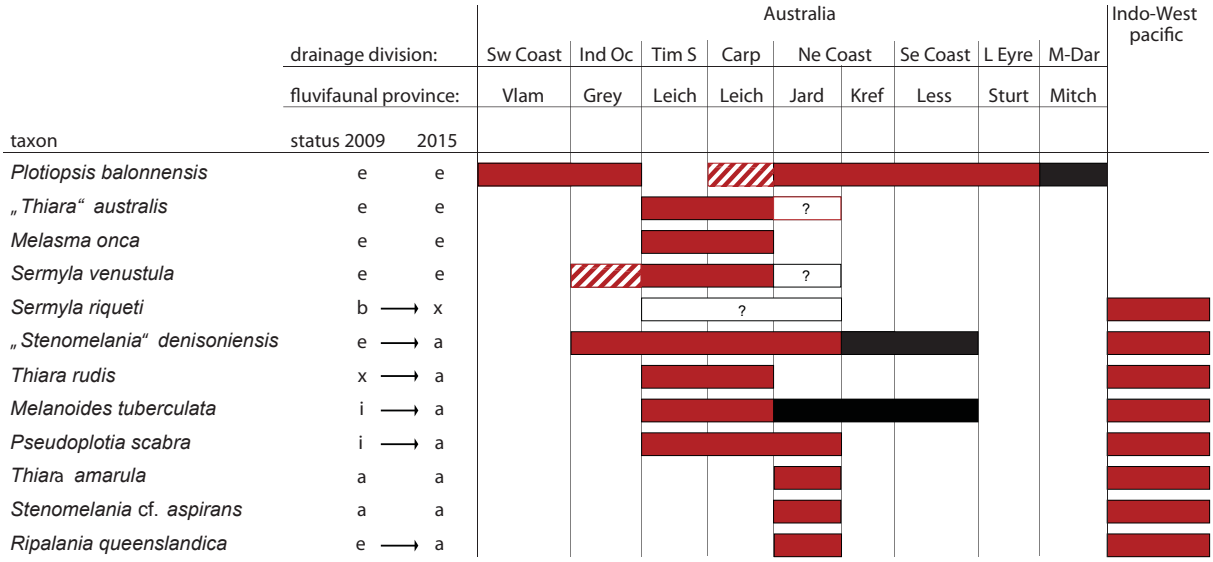

Figure 46 Summary of the geographic ranges of thiarid species in Australia according to their occurrences in major drainage systems and fluvifaunal provinces. Red bars represent genetically confirmed localities, black ones dry material, diagonally striped bars represent cases with scattered and deviant occurrences and white bars with questionmarks symbolise doubtful records as discussed in chapter 4. The following abbreviations are used for status description as determined by Glaubrecht et al. (2009): e - endemic to Australia; a - autochthonous (indigenous) in Australia, but widely distributed elsewhere; b - possibly occassional brackish water invasion; i - recently introduced; x - not occurring in Australia. For abbreviations of fluvifaunal provinces and major drainage systems see appendix.

As mentioned the rainforests are known to have been much more abundant and widespread during the Miocene and the remaining rainforest habitats in the Jardinian are generally considered ancient and providing places of refuge for many Miocene faunal elements of Australia (Webb and Tracey, 1981; Sanderson, 2008; Rossetto, 2015). These formerly continous rainforest habitats could have provided suitable environments also for *Thiara amarula*, *Stenomelania* cf. *aspirans* and *Ripalania queenslandica*. Despite their capability of long-distance dispersal these three species occur only in few streams along the coast of Queensland. Their restricted appearence seems to be connected to the climatic conditions in the Jardinian, as this region is the only area in Australia with conditions quite similar to those in other monsoonal regions of the Asia-Pacific region where these three can be found. In the light of the presented scenario, it should be considered that these three thiarids might rather represent an ancient Australian freshwater faunal element than being recent invaders from the north through passive dispersal by planktonic larvae.

Thiarids in Australia occur in the northern-central region of the Leichhardtian zoogeographic region in particular which is approximately represented by the Timor Sea and Gulf of Carpenteria drainage systems (see fig.46). The thiarids might have had a wider distribution in the past and with the increasing aridity that had occurred earlier in the southern parts of the continent, the southern populations went extinct. This scenario also

helps to understand the occurrence of *S. venustula* which is distributed highly disjunctly in the Greyian region of West Australia, far away from the otherwise northern range of the species. As mentioned in chapter 4, the highly isolated location of Bundara Sinkhole could harbour a population that might have survived as relictual form from times long gone.

Taken together the continent "down under" possesses some unique freshwater lineages with peculiar elements that are endemic on a continental scale, but also some species that are restricted to only certain regions - in particular to the northern coastal wet-dry region of the Leichhardtian province - and even to certain river drainage systems. The peculiar and, possibly highly specialised and adapted, thiarid fauna of Australia has apparently managed to persist for a long time. Their current distribution and past diversification on the continent is the result of a complex history most likely promoted by the interplay between the hydrogeomorphological impacts leading to phenomena like river captures or drying-up of waterbodies provoking spreading or extinctions of populations under a fusion-fission scenario.

Prospects Although there are still ambiguities to be resolved concerning phylogenetical relationships among the thiarid species, the comparison of the resultant phylogenies offers considerable insight into this enigmatic group. Australian thiarids represent a challenging but also extremely interesting case for studying biogeography due to their now assumed long history on the continent. In the light of the upcoming global warming, understanding the influence of past climate change on the biogeography, evolution and extinction of faunas is critical for the development of conservation strategies. This is of particular importance in Australia, where intensified aridity has shaped large portions of the continent. In fact, no other continent of its size underwent such radical shift to intensive aridity as Australia. The potential use of the phylogeographic framework to predict the possible future responses of species to climate change scenarios is noteworthy. The Australian thiarids represent an important and realistic model system in speciation research which provides detailed insight into the dynamics of the underlying mechanisms of speciation under the influence of climate change.

It is of further concern that Thiaridae serve as first intermediate snail host for several trematode species, among them the human lung and intestinal fluke (Chaniotis et al., 1980; Krailas et al., 2011). Regarding their known potential for being invasive they might become established and spread elsewhere and of course mollusc-transmitted diseases need recognition and emphasis due to their importance for the veterinary and public health. A focus of future work should be on the mitigation of the effect of invasive species and on preventing future invasions. The diversity of life on Earth is rapidly declining under

the current biodiversity crisis (Olson et al., 2002) and invasive species are one of the most commonly cited causes of this biodiversity loss (Ricciardi, 2004; Didham et al., 2007; Hermoso et al., 2011). This situation is especially worrying in freshwater environments worldwide (Dudgeon et al., 2006; Abell et al., 2008; Stow et al., 2014). Of the global terrestrial fauna, freshwater molluscs are among the most diverse and threatened groups, so that their conservation is a matter of concern (Lydeard et al., 2004; Lysne et al., 2008; Lopes-Lima et al., 2014).

The results of the present study offer a solid basis for further profound investigation on the study of biodiversity and evolution in freshwater snails. New technological advances facilitate the generation of huge amounts of genomic sequence data which may allow for more informed decisions on phylogenetic relationships and taxonomic assignments. In combination with the untapped genetic data within archived museum specimens that is now available due to the presented method developed within the scope of this thesis. Broad application of this approach to other taxa will further enhance our ability to accurately estimate the true number of species on Earth. In the light of the increasing biodiversity crisis, the study of this biological diversity on all levels as well as the underlying evolutionary forces is becoming even more urgent.

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APPENDIX

A List of sampling localities sorted by sampling year

Table 22 Sampling localities of Australia sorted by sampling year.

Exp. & Loc n°		Taxa	ZMBn°	Locality	Coordinates
AUS 2002	14	*S. denisoniensis*	106340	QL: South Mission Beach	17°56,84'S, 146°3,29'E
AUS 2002	15	*P. balonnensis*	106345	QL: nr. Gregory Falls	17°35,57'S, 145°52,29'E
AUS 2002	15	*S. denisoniensis*	106352	QL: nr. Gregory Falls	17°35,57'S, 145°52,29'E
AUS 2002	16	*R. queenslandica*	106355	QL: North Johnston River	17°30,34'S, 145°59,55'E
AUS 2002	16	*R. queenslandica*	192474	QL: North Johnston River	17°30,34'S, 145°59,55'E
AUS 2002	16	*Stenomelania* sp.	106343	QL: North Johnston River	17°30,34'S, 145°59,55'E
AUS 2002	16	*T. amarula*	106349	QL: North Johnston River	17°30,34'S, 145°59,55'E
AUS 2002	16	*T. amarula*	106354	QL: North Johnston River	17°30,34'S, 145°59,55'E
AUS 2002	21	*T. amarula*	193470	QL: Mowbray River	16°33,87'S, 145°27,83'E
AUS 2002	24	*S. denisoniensis*	106341	QL: Meelele River	15°58,25'S, 145°23,85'E
AUS 2002	25	*S. denisoniensis*	106342	QL: Woobadda River	15°58'S, 145°22,48'E
AUS 2002	26	*T. amarula*	106348	QL: Woobadda River, Tributary of Bloomfield River	15°57,35'S, 145°21,11'E
AUS 2002	28	*P. scabra*	106351	QL: Three Mile - Poison Creek	15°25,81'S, 145°7,05'E
AUS 2002	28	*P. balonnensis*	106347	QL: Three Mile - Poison Creek	15°25,81'S, 145°7,05'E
AUS 2002	28	*S. denisoniensis*	106356	QL: Three Mile - Poison Creek	15°25,81'S, 145°7,05'E
AUS 2002	30	*P. balonnensis*	106346	QL: Endeavour River Falls	15°22,27'S, 145°1,77'E
AUS 2002	30	*S. denisoniensis*	106338	QL: Endeavour River Falls	15°22,27'S, 145°1,77'E
AUS 2002	31	*S. denisoniensis*	106339	QL: Laura River	15°34,68'S, 144°27,41'E
AUS 2002	32	*S. denisoniensis*	106373	QL: Boggy Creek, W Normanby tributary	15°49,97'S, 144°52,91'E
AUS 2002	35	*S. aspirans*	106344	QL: Mowbray River	16°33,87'S, 145°27,83'E
AUS 2004	76	*M. tuberculata*	106592	NT: Darwin: George Brown Botanic Garden, pool	12°26,739'S, 130°50,179'E
AUS 2004	76	*M. tuberculata*	106592	NT: Darwin: George Brown Botanic Garden, pool	12°26,739'S, 130°50,179'E
AUS 2004	79	*S. carbonata*	106593	NT: Howard Springs	12°27,345'S, 131°3,146'E
AUS 2004	79	*S. carbonata*	106593	NT: Howard Springs	12°27,345'S, 131°3,146'E
AUS 2004	80	*S. carbonata*	106595	NT: Howard Springs Creek, N of Howard Springs	12°27,268'S, 131°3,108'E
AUS 2004	80	*S. carbonata*	106595	NT: Howard Springs Creek, N of Howard Springs	12°27,268'S, 131°3,108'E
AUS 2004	80	*T. australis*	106594	NT: Howard Springs Creek, N of Howard Springs	12°27,268'S, 131°3,108'E
AUS 2004	80	*T. australis*	106594	NT: Howard Springs Creek, N of Howard Springs	12°27,268'S, 131°3,108'E
AUS 2004	81	*S. denisoniensis*	106597	NT: Howard River, crossing	12°27,752'S, 131°5,008'E
AUS 2004	81	*S. denisoniensis*	106597	NT: Howard River, crossing	12°27,752'S, 131°5,008'E
AUS 2004	81	*T. australis*	106596	NT: Howard River, crossing	12°27,752'S, 131°5,008'E
AUS 2004	81	*T. australis*	106596	NT: Howard River, crossing	12°27,752'S, 131°5,008'E
AUS 2004	81	*T. australis*	106598	NT: Howard River, crossing	12°27,752'S, 131°5,008'E
AUS 2004	81	*T. australis*	106598	NT: Howard River, crossing	12°27,752'S, 131°5,008'E
AUS 2004	82	*S. denisoniensis*	106600	NT: Berry Springs	12°42,153'S, 130°59,875'E
AUS 2004	82	*S. denisoniensis*	106600	NT: Berry Springs	12°42,153'S, 130°59,875'E
AUS 2004	82	*T. australis*	106599	NT: Berry Springs	12°42,153'S, 130°59,875'E
AUS 2004	82	*T. australis*	106599	NT: Berry Springs	12°42,153'S, 130°59,875'E
AUS 2004	84	*M. tuberculata*	106603	NT: Manton River	12°50,282'S, 131°7,998'E
AUS 2004	84	*S. denisoniensis*	106603	NT: Manton River	12°50,282'S, 131°7,998'E
AUS 2004	87	*T. australis*	106610	NT: Adelaide River, North, c. 18km downstream from highway crossing	13°8,742'S, 131°13,14'E
AUS 2004	87	*T. australis*	106610	NT: Adelaide River, North, c. 18km downstream from highway crossing	13°8,742'S, 131°13,14'E
AUS 2004	88	*T. australis*	106611	NT: Adelaide River, South, at crossing	13°28,975'S, 131°5,853'E
AUS 2004	88	*T. australis*	106611	NT: Adelaide River, South, at crossing	13°28,975'S, 131°5,853'E
AUS 2004	89	*M. onca*	106614	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E

Exp. & Loc	n°	Taxa	ZMBn°	Locality	Coordinates
AUS 2004	89	*M. onca*	106614	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E
AUS 2004	89	*T. australis*	106612	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E
AUS 2004	90	*T. australis*	106615	NT: Douglas River crossing, Bond bridge	13°47,36'S, 131°21,185'E
AUS 2004	90	*T. australis*	106615	NT: Douglas River crossing, Bond bridge	13°47,36'S, 131°21,185'E
AUS 2004	91	*M. onca*	106617	NT: Katherine River, at Katherine, Low Level crossing, downstream from bridge	14°29,441'S, 132°14,991'E
AUS 2004	91	*M. onca*	106617	NT: Katherine River, at Katherine, Low Level crossing, downstream from bridge	14°29,441'S, 132°14,991'E
AUS 2004	92	*T. australis*	106618	NT: Katherine Falls, at Flora River N.P.	14°45,412'S, 131°35,791'E
AUS 2004	93	*T. australis*	106619	NT: Victoria River, Old Victoria River Crossing	15°34,862'S, 131°6,142'E
AUS 2004	93	*T. australis*	106619	NT: Victoria River, Old Victoria River Crossing	15°34,862'S, 131°6,142'E
AUS 2004	94	*T. australis*	106621	NT: Victoria River, at Victoria River Gorge	15°37,79'S, 131°8,099'E
AUS 2004	94	*T. australis*	106621	NT: Victoria River, at Victoria River Gorge	15°37,79'S, 131°8,099'E
AUS 2004	95	*M. onca*	106622	NT: Roper River, at Mataranka	14°56,771'S, 133°12,609'E
AUS 2004	95	*S. venustula*	106623	NT: Roper River, at Mataranka	14°56,771'S, 133°12,609'E
AUS 2004	95	*T. australis*	106624	NT: Roper River, at Mataranka	14°56,771'S, 133°12,609'E
AUS 2004	95	*T. australis*	106624	NT: Roper River, at Mataranka	14°56,771'S, 133°12,609'E
AUS 2004	96	*M. onca*	106626	NT: Roper River, at 4 Mile Point	14°56,137'S, 133°10,033'E
AUS 2004	96	*M. onca*	106626	NT: Roper River, at 4 Mile Point	14°56,137'S, 133°10,033'E
AUS 2004	96	*T. australis*	106625	NT: Roper River, at 4 Mile Point	14°56,137'S, 133°10,033'E
AUS 2004	96	*T. australis*	106625	NT: Roper River, at 4 Mile Point	14°56,137'S, 133°10,033'E
AUS 2004	97	*M. onca*	106628	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2004	97	*S. venustula*	106629	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2004	97	*S. venustula*	106629	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2004	97	*T. australis*	106627	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2004	97	*T. australis*	106627	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2004	97	*T. australis*	106630	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2004	97	*T. australis*	106630	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2004	98	*S. venustula*	106632	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2004	98	*S. venustula*	106632	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2004	98	*T. australis*	106631	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2004	98	*T. australis*	106633	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2004	98	*T. australis*	106633	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2004	98	*T. australis*	106634	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2004	98	*T. australis*	106634	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2004	99	*M. onca*	106636	NT: Roper River, at Roper Bar	14°42,802'S, 134°30,474'E
AUS 2004	99	*M. onca*	106636	NT: Roper River, at Roper Bar	14°42,802'S, 134°30,474'E
AUS 2004	99	*T. australis*	106635	NT: Roper River, at Roper Bar	14°42,802'S, 134°30,474'E
AUS 2004	99	*T. australis*	106635	NT: Roper River, at Roper Bar	14°42,802'S, 134°30,474'E
AUS 2004	100	*S. denisoniensis*	106641	NT: Towns River, at crossing	15°2,57'S, 135°12,718'E
AUS 2004	100	*T. australis*	106640	NT: Towns River, at crossing	15°2,57'S, 135°12,718'E
AUS 2004	100	*T. australis*	106640	NT: Towns River, at crossing	15°2,57'S, 135°12,718'E
AUS 2004	101	*S. venustula*	106654	NT: Elsey River, at Elsey Cemetery	
AUS 2004	101	*S. denisoniensis*	106651	NT: Elsey River, at Elsey Cemetery	
AUS 2004	101	*T. australis*	106652	NT: Elsey River, at Elsey Cemetery	
AUS 2004	102	*T. australis*	106642	NT: East Alligator River, Ubir, at Cahills Crossing to Arnhemland	12°25,542'S, 132°57,882'E
AUS 2004	102	*T. australis*	106642	NT: East Alligator River, Ubir, at Cahills Crossing to Arnhemland	12°25,542'S, 132°57,882'E
AUS 2004	103	*S. denisoniensis*	106644	NT: Black Jungle Springs, Kakadu N.P.	13°2,898'S, 132°9,889'E
AUS 2004	103	*S. denisoniensis*	106644	NT: Black Jungle Springs, Kakadu N.P.	13°2,898'S, 132°9,889'E
AUS 2004	104	*S. denisoniensis*	106645	NT: Coomalie Creek, at crossing of road to W	13°0,602'S, 131°6,85'E
AUS 2004	105	*T. australis*	106648	NT: Finnis River, NW of Batchelor	13°1,316'S, 130°57,093'E
AUS 2004	105	*T. australis*	106648	NT: Finnis River, NW of Batchelor	13°1,316'S, 130°57,093'E

Exp. & Loc n°		Taxa	ZMBn°	Locality	Coordinates
AUS 2004	106	*S. venustula*	106650	NT: Daly River Crossing	13°46,026'S, 130°42,688'E
AUS 2004	109	*P. balonnensis*	106582	West SE of Geraldton, Ellendale Pool at Greenough River	28°51,63'S, 114°58,43'E
AUS 2004	109	*P. balonnensis*	106582	West SE of Geraldton, Ellendale Pool at Greenough River	28°51,63'S, 114°58,43'E
AUS 2004	109	*S. denisoniensis*	106586	West SE of Geraldton, Ellendale Pool at Greenough River	28°51,63'S, 114°58,43'E
AUS 2004	109	*S. denisoniensis*	106586	West SE of Geraldton, Ellendale Pool at Greenough River	28°51,63'S, 114°58,43'E
AUS 2004	112	*P. balonnensis*	106583	West Murchinson River, Kalbarri N.P., at Ross Graham Lookout	27°48,77'S, 114°28,54'E
AUS 2004	112	*P. balonnensis*	106583	West Murchinson River, Kalbarri N.P., at Ross Graham Lookout	27°48,77'S, 114°28,54'E
AUS 2004	112	*P. balonnensis*	106583	West Murchinson River, Kalbarri N.P., at Ross Graham Lookout	27°48,77'S, 114°28,54'E
AUS 2005	1	*S. denisoniensis*	106660	NT: Crater Lake, S Bachelor, NE of Adelaide River	13°2,76'S, 131°5,445'E
AUS 2005	1	*S. denisoniensis*	106660	NT: Crater Lake, S Bachelor, NE of Adelaide River	13°2,76'S, 131°5,445'E
AUS 2005	1	*T. australis*	106659	NT: Crater Lake, S Bachelor, NE of Adelaide River	13°2,76'S, 131°5,445'E
AUS 2005	1	*T. australis*	106659	NT: Crater Lake, S Bachelor, NE of Adelaide River	13°2,76'S, 131°5,445'E
AUS 2005	2	*S. denisoniensis*	106662	NT: Rum Jungle at Litchfield Road, 2nd lake	13°2,604'S, 130°59,862'E
AUS 2005	2	*T. australis*	106661	NT: Rum Jungle at Litchfield Road, 2nd lake	13°2,604'S, 130°59,862'E
AUS 2005	2	*T. australis*	106661	NT: Rum Jungle at Litchfield Road, 2nd lake	13°2,604'S, 130°59,862'E
AUS 2005	3	*T. australis*	106664	NT: Finnis River, NW of Batchelor	13°1,316'S, 130°57,093'E
AUS 2005	3	*T. australis*	106664	NT: Finnis River, NW of Batchelor	13°1,316'S, 130°57,093'E
AUS 2005	4	*M. onca*	106667	NT: Oolloo Crossing, Daly River	14°4,24'S, 131°15,056'E
AUS 2005	4	*M. onca*	106667	NT: Oolloo Crossing, Daly River	14°4,24'S, 131°15,056'E
AUS 2005	4	*P. scabra*	106668	NT: Oolloo Crossing, Daly River	14°4,24'S, 131°15,056'E
AUS 2005	4	*T. australis*	106666	NT: Oolloo Crossing, Daly River	14°4,24'S, 131°15,056'E
AUS 2005	4	*T. australis*	106666	NT: Oolloo Crossing, Daly River	14°4,24'S, 131°15,056'E
AUS 2005	5	*M. onca*	106671	NT: Daly River Crossing	13°46,026'S, 130°42,688'E
AUS 2005	5	*M. onca*	106671	NT: Daly River Crossing	13°46,026'S, 130°42,688'E
AUS 2005	5	*T. australis*	106670	NT: Daly River Crossing	13°46,026'S, 130°42,688'E
AUS 2005	6	*M. onca*	106673	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E
AUS 2005	6	*M. onca*	106673	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E
AUS 2005	6	*T. australis*	106672	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E
AUS 2005	6	*T. australis*	106674	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E
AUS 2005	6	*T. australis*	106674	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E
AUS 2005	7	*S. venustula*	106722	NT: Bitter Springs at Mataranka	14°54,669'S, 133°5,319'E
AUS 2005	7	*Stenomelania* sp.	106721	NT: Bitter Springs at Mataranka	14°54,669'S, 133°5,319'E
AUS 2005	8	*S. venustula*	106676	NT: Roper River, at Jalmurark Camp Ground	14°57,158'S, 133°13,29'E
AUS 2005	8	*S. venustula*	106723	NT: Roper River, at Jalmurark Camp Ground	14°57,158'S, 133°13,29'E
AUS 2005	8	*Stenomelania* sp.	106724	NT: Roper River, at Jalmurark Camp Ground	14°57,158'S, 133°13,29'E
AUS 2005	8	*T. australis*	106675	NT: Roper River, at Jalmurark Camp Ground	14°57,158'S, 133°13,29'E
AUS 2005	9	*P. scabra*	106679	NT: Little Roper River, South bank at old crossing	14°55,589'S, 133°7,137'E
AUS 2005	9	*S. venustula*	106678	NT: Little Roper River, South bank at old crossing	14°55,589'S, 133°7,137'E
AUS 2005	9	*S. venustula*	106678	NT: Little Roper River, South bank at old crossing	14°55,589'S, 133°7,137'E
AUS 2005	9	*T. australis*	106677	NT: Little Roper River, South bank at old crossing	14°55,589'S, 133°7,137'E
AUS 2005	9	*T. australis*	106677	NT: Little Roper River, South bank at old crossing	14°55,589'S, 133°7,137'E
AUS 2005	10	*M. onca*	106681	NT: Stevie's Hole at Waterhouse River, Elsey N.P.	14°55,782'S, 133°8,732'E
AUS 2005	10	*M. onca*	106681	NT: Stevie's Hole at Waterhouse River, Elsey N.P.	14°55,782'S, 133°8,732'E
AUS 2005	10	*S. venustula*	106682	NT: Stevie's Hole at Waterhouse River, Elsey N.P.	14°55,782'S, 133°8,732'E
AUS 2005	10	*T. australis*	106680	NT: Stevie's Hole at Waterhouse River, Elsey N.P.	14°55,782'S, 133°8,732'E
AUS 2005	11	*S. riqueti*	106684	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2005	11	*S. riqueti*	106684	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E

Exp. & Loc	n°	Taxa	ZMBn°	Locality	Coordinates
AUS 2005	11	*S. denisoniensis*	106685	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2005	11	*T. australis*	106683	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2005	12	*P. balonnensis*	106686	NT: Red Centre: Ormiston Gorge, outlet, waterhole with vegetation	23°37,704'S, 132°43,375'E
AUS 2005	12	*P. balonnensis*	106717	NT: Red Centre: Ormiston Gorge, outlet, waterhole with vegetation	23°37,704'S, 132°43,375'E
AUS 2005	13	*P. balonnensis*	106687	NT: Red Centre: Finke River, at Glen Helen Gorge nr. resort	23°41,322'S, 132°40,606'E
AUS 2005	13	*P. balonnensis*	106716	NT: Red Centre: Finke River, at Glen Helen Gorge nr. resort	23°41,322'S, 132°40,606'E
AUS 2005	14	*P. balonnensis*	106718	NT: Red Centre: Palm Valley, 2 km into the valley	24°3,036'S, 132°41,68'E
AUS 2005	15	*P. balonnensis*	106719	NT: Red Centre: Finke River, shallow waterhole in dry river bed, nr. Boggy Hole	24°8,113'S, 132°50,233'E
AUS 2005	16	*P. balonnensis*	106688	NT: Red Centre: Boggy Hole, Campground, Finke River	24°8,174'S, 132°51,768'E
AUS 2005	16	*P. balonnensis*	106688	NT: Red Centre: Boggy Hole, Campground, Finke River	24°8,174'S, 132°51,768'E
AUS 2005	17	*P. balonnensis*	106689	NT: Red Centre: Three Mile Point, Finke River at crossing of Stuart HWY	24°33,182'S, 133°14,355'E
AUS 2005	17	*P. balonnensis*	106689	NT: Red Centre: Three Mile Point, Finke River at crossing of Stuart HWY	24°33,182'S, 133°14,355'E
AUS 2005	18	*M. tuberculata*	106690	West Kimberley Region: Lilly Lagoon, nr. Lake Kununurra, above Diversion Dam	15°46,825'S, 128°44,477'E
AUS 2005	18	*M. tuberculata*	106690	West Kimberley Region: Lilly Lagoon, nr. Lake Kununurra, above Diversion Dam	15°46,825'S, 128°44,477'E
AUS 2005	21	*T. australis*	106692	West Kimberley Region: Kununurra, 250 m along canal at pump station, Lake Kununurra	15°47,34'S, 128°43,005'E
AUS 2005	21	*T. australis*	106692	West Kimberley Region: Kununurra, 250 m along canal at pump station, Lake Kununurra	15°47,34'S, 128°43,005'E
AUS 2005	22	*T. australis*	106693	West Kimberley Region: Fitzroy Crossing, Fitzroy River	18°12,653'S, 125°34,74'E
AUS 2005	22	*T. australis*	106693	West Kimberley Region: Fitzroy Crossing, Fitzroy River	18°12,653'S, 125°34,74'E
AUS 2005	23	*T. australis*	106696	West Kimberley Region: Geiki Gorge, Fitzroy River	18°6,521'S, 125°41,891'E
AUS 2005	24	*T. australis*	106697	NT: East Baines River, crossing at Victoria HWY	15°45,737'S, 130°1,75'E
AUS 2005	24	*T. australis*	106697	NT: East Baines River, crossing at Victoria HWY	15°45,737'S, 130°1,75'E
AUS 2005	25	*M. onca*	106699	NT: Katherine River, 500 m downstream from Lower Land Bridge at Springvale Homestead detour	14°29,49'S, 132°14,73'E
AUS 2005	25	*M. onca*	106699	NT: Katherine River, 500 m downstream from Lower Land Bridge at Springvale Homestead detour	14°29,49'S, 132°14,73'E
AUS 2005	25	*T. australis*	106698	NT: Katherine River, 500 m downstream from Lower Land Bridge at Springvale Homestead detour	14°29,49'S, 132°14,73'E
AUS 2005	26	*S. carbonata*	1000	NT: Howard Springs	12°27,345'S, 131°3,146'E
AUS 2005	26	*S. carbonata*	106700	NT: Howard Springs	12°27,345'S, 131°3,146'E
AUS 2005	27	*T. australis*	106701	NT: Howard River, crossing	12°27,752'S, 131°5,008'E
AUS 2005	27	*T. australis*	106701	NT: Howard River, crossing	12°27,752'S, 131°5,008'E
AUS 2005	27	*T. australis*	106702	NT: Howard River, crossing	12°27,752'S, 131°5,008'E
AUS 2005	28	*M. tuberculata*	106726	NT: Darwin River, at crossing with HWY to Cox Peninsula	12°44,527'S, 130°57,93'E
AUS 2005	28	*Stenomelania* sp.	106726	NT: Darwin River, at crossing with HWY to Cox Peninsula	12°44,527'S, 130°57,93'E
AUS 2005	29	*T. australis*	106704	NT: Berry Springs	12°42,153'S, 130°59,875'E
AUS 2007	67	*S. venustula*	107228	NT: Howard Springs Creek, N of Howard Springs	12°27,268'S, 131°3,108'E

Exp. & Loc	n°	Taxa	ZMBn°	Locality	Coordinates
AUS 2007	68	*P. scabra*	107215	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E
AUS 2007	68	*T. australis*	107263	NT: Bamboo Creek, c. 3-10m from Daly River	13°40,118'S, 130°39,501'E
AUS 2007	69	*M. onca*	107221	NT: Daly River Crossing	13°46,026'S, 130°42,688'E
AUS 2007	69	*T. australis*	107264	NT: Daly River Crossing	13°46,026'S, 130°42,688'E
AUS 2007	70	*M. onca*	107222	NT: Roper River, at Jalmurark Camp Ground	14°57,158'S, 133°13,29'E
AUS 2007	70	*S. venustula*	107229	NT: Roper River, at Jalmurark Camp Ground	14°57,158'S, 133°13,29'E
AUS 2007	70	*T. australis*	107265	NT: Roper River, at Jalmurark Camp Ground	14°57,158'S, 133°13,29'E
AUS 2007	71	*S. venustula*	107230	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2007	71	*S. denisoniensis*	107238	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2007	71	*T. australis*	107266	NT: Salt Creek nr Elsey Creek, at crossing of Roper Highway	15°0,703'S, 133°14,417'E
AUS 2007	72	*S. venustula*	107231	NT: Elsey Creek on Roper Highway (Roper River Catchment)	15°0,627'S, 133°15,096'E
AUS 2007	72	*T. australis*	107267	NT: Elsey Creek on Roper Highway (Roper River Catchment)	15°0,627'S, 133°15,096'E
AUS 2007	73	*M. onca*	107223	NT: Roper River at Roper Bar (Roper River Catchment)	14°42,795'S, 134°30,575'E
AUS 2007	73	*T. australis*	107268	NT: Roper River at Roper Bar (Roper River Catchment)	14°42,795'S, 134°30,575'E
AUS 2007	74	*S. denisoniensis*	107239	NT: Towns River, at crossing	15°2,57'S, 135°12,718'E
AUS 2007	74	*T. australis*	107269	NT: Towns River, at crossing	15°2,57'S, 135°12,718'E
AUS 2007	75	*S. denisoniensis*	107240	Cox River, in last along stagnant waterhole N of couseway	15°19,394'S, 135°20,699'E
AUS 2007	75	*T. australis*	107270	Cox River, in last along stagnant waterhole N of couseway	15°19,394'S, 135°20,699'E
AUS 2007	76	*T. australis*	107271	NT: Limmen Bight River	15°28,865'S, 135°24,054'E
AUS 2007	77	*T. australis*	107272	NT: McArthur River at Boorolooa	16°4,866'S, 136°19,026'E
AUS 2007	78	*T. australis*	107273	NT: Wearyan River, along beach at crossing	16°10,02'S, 136°45,481'E
AUS 2007	79	*S. venustula*	107232	NT: Foelsche River	16°12,628'S, 136°53,034'E
AUS 2007	79	*T. australis*	107274	NT: Foelsche River	16°12,628'S, 136°53,034'E
AUS 2007	80	*T. australis*	107275	NT: Robinson River	16°28,27'S, 137°2,932'E
AUS 2007	81	*S. venustula*	107233	NT: Kangaroo Creek	16°47,553'S, 137°6,107'E
AUS 2007	82	*T. australis*	107276	NT: Blueys Creek on The Savannah Way (Clavert River Catchment)	16°56,066'S, 137°21,578'E
AUS 2007	83	*T. australis*	107277	QL: Gregory River, SE of Burketown at Savannah Highway crossing	17°53,517'S, 139°17,209'E
AUS 2007	84	*T. australis*	107278	QL: Gregory River at Gregory Downs	18°38,695'S, 139°14,875'E
AUS 2007	85	*S. venustula*	107234	QL: Gregory River at Riversleigh (Gregory River Catchment)	19°1,116'S, 138°43,529'E
AUS 2007	85	*T. australis*	107279	QL: Gregory River at Riversleigh (Gregory River Catchment)	19°1,116'S, 138°43,529'E
AUS 2007	86	*T. australis*	107280	QL: O'Shanassy River	19°1,354'S, 138°45,741'E
AUS 2007	87	*T. australis*	107281	QL: Lawn Hill, Boodjamulla Creek, downstream of Indarri Falls	18°42,051'S, 138°29,196'E
AUS 2007	89	*S. venustula*	107209	QL: Norman River, Billabong 1 km N of Normanton	17°39,712'S, 141°6,154'E
AUS 2007	89	*S. venustula*	107235	QL: Norman River, Billabong 1 km N of Normanton	17°39,712'S, 141°6,154'E
AUS 2007	89	*S. denisoniensis*	107241	QL: Norman River, Billabong 1 km N of Normanton	17°39,712'S, 141°6,154'E
AUS 2007	90	*S. denisoniensis*	107242	QL: Normanton River, Glenmore, SE of Normanton River	15°51,199'S, 141°8,048'E
AUS 2007	91	*S. denisoniensis*	107243	QL: McLeod Creek, at crossing, tributary to Endeavour River	15°25,503'S, 145°6,049'E
AUS 2007	92	*P. balonnensis*	107256	QL: Endeavour River Falls	15°22,27'S, 145°1,77'E
AUS 2007	92	*S. denisoniensis*	107244	QL: Endeavour River Falls	15°22,27'S, 145°1,77'E

Exp. & Loc	n°	Taxa	ZMBn°	Locality	Coordinates
AUS 2007	93	*S. denisoniensis*	107245	QL: Three Mile Creek/ Poisson Creek (Tributary to Endeavour River)	15°25,789'S, 145°7,04'E
AUS 2007	94	*S. aspirans*	107211	QL: Granite Creek, W of Bloomsfield	15°55,99'S, 145°19,54'E
AUS 2007	94	*S. denisoniensis*	107246	QL: Granite Creek, W of Bloomsfield	15°55,99'S, 145°19,54'E
AUS 2007	94	*T. amarula*	107217	QL: Granite Creek, W of Bloomsfield	15°55,99'S, 145°19,54'E
AUS 2007	95	*S. denisoniensis*	107247	QL: Woobadda Creek	15°57,969'S, 145°22,858'E
AUS 2007	96	*S. denisoniensis*	107248	QL: Meelele River	15°58,25'S, 145°23,85'E
AUS 2007	97	*R. queenslandica*	107213	QL: Douglas Creek, near Daintree at crossing	16°16,194'S, 145°58,6'E
AUS 2007	97	*T. amarula*	107218	QL: Douglas Creek, near Daintree at crossing	16°16,194'S, 145°58,6'E
AUS 2007	98	*S. aspirans*	107212	QL: Mowbray River	16°33,87'S, 145°27,83'E
AUS 2007	98	*T. amarula*	107219	QL: Mowbray River	16°33,87'S, 145°27,83'E
AUS 2007	98	*T. amarula*	107219	QL: Mowbray River	16°33,87'S, 145°27,83'E
AUS 2007	99	*P. balonnensis*	107257	QL: Barron River, below 150 m Lake Placid	16°52,17'S, 145°40,405'E
AUS 2007	99	*S. denisoniensis*	107249	QL: Barron River, below 150 m Lake Placid	16°52,17'S, 145°40,405'E
AUS 2007	99	*T. amarula*	107220	QL: Barron River, below 150 m Lake Placid	16°52,17'S, 145°40,405'E
AUS 2007	100	*P. balonnensis*	107258	QL: Barron River Gorge Road, half way to hydro station at River Access	16°51,632'S, 145°39,791'E
AUS 2007	100	*S. denisoniensis*	107250	QL: Barron River Gorge Road, half way to hydro station at River Access	16°51,632'S, 145°39,791'E
AUS 2007	101	*R. queenslandica*	107214	QL: North Johnston River	17°30,34'S, 145°59,55'E
AUS 2007	102	*P. balonnensis*	107259	QL: Fisher's Creek, Palmeston Rock, at Palmerston Hwy	17°34,167'S, 145°53,876'E
AUS 2007	102	*S. denisoniensis*	107251	QL: Fisher's Creek, Palmeston Rock, at Palmerston Hwy	17°34,167'S, 145°53,876'E
AUS 2007	103	*P. balonnensis*	107260	QL: Einasleigh River, 4 km E of Einasleigh	18°30,938'S, 144°6,682'E
AUS 2007	104	*T. australis*	107282	QL: Gilbert River, downstream from crossing Burke Dev. Road, 10 km NE of Normanton	17°10,117'S, 141°45,999'E
AUS 2007	104	*T. australis*	107282	QL: Gilbert River, downstream from crossing Burke Dev. Road, 10 km NE of Normanton	17°10,117'S, 141°45,999'E
AUS 2007	105	*M. onca*	107224	NT: Roper River, Roper Falls, 4 km E of Jarmurak	14°57,401'S, 133°15,018'E
AUS 2007	105	*S. denisoniensis*	107252	NT: Roper River, Roper Falls, 4 km E of Jarmurak	14°57,401'S, 133°15,018'E
AUS 2007	105	*T. australis*	107283	NT: Roper River, Roper Falls, 4 km E of Jarmurak	14°57,401'S, 133°15,018'E
AUS 2007	106	*M. onca*	107225	NT: Roper River, at 4 Mile Point	14°56,137'S, 133°10,033'E
AUS 2007	106	*T. australis*	107284	NT: Roper River, at 4 Mile Point	14°56,137'S, 133°10,033'E
AUS 2007	107	*S. venustula*	107236	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2007	107	*T. australis*	107285	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2007	108	*S. venustula*	107237	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2007	108	*S. denisoniensis*	107253	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2007	108	*T. australis*	107286	NT: Little Roper River, at crossing	14°55,581'S, 133°7,176'E
AUS 2007	109	*M. onca*	107226	NT: Stevie's Hole at Waterhouse River, Elsey N.P.	14°55,782'S, 133°8,732'E
AUS 2007	109	*S. denisoniensis*	107254	NT: Stevie's Hole at Waterhouse River, Elsey N.P.	14°55,782'S, 133°8,732'E
AUS 2007	109	*T. australis*	107287	NT: Stevie's Hole at Waterhouse River, Elsey N.P.	14°55,782'S, 133°8,732'E
AUS 2007	110	*T. australis*	107288	NT: Katherine River, at Katherine, Low Level crossing, downstream from bridge	14°29,441'S, 132°14,991'E
AUS 2007	111	*M. onca*	107227	NT: Oolloo Crossing, Daly River	14°4,24'S, 131°15,056'E
AUS 2007	111	*P. scabra*	107216	NT: Oolloo Crossing, Daly River	14°4,24'S, 131°15,056'E
AUS 2007	111	*T. australis*	107289	NT: Oolloo Crossing, Daly River	14°4,24'S, 131°15,056'E
AUS 2007	112	*S. venustula*	107210	NT: Berry Springs, S of Darwin	12°42,111'S, 130°59,854'E
AUS 2007	112	*S. denisoniensis*	107255	NT: Berry Springs, S of Darwin	12°42,111'S, 130°59,854'E
AUS 2007	112	*T. australis*	107290	NT: Berry Springs, S of Darwin	12°42,111'S, 130°59,854'E
AUS 2009	1	*M. tuberculata*	107545	NT: Berry Springs on Stuart Highway	12°42,309'S, 131°0,401'E
AUS 2009	1	*S. venustula*	107544	NT: Berry Springs on Stuart Highway	12°42,309'S, 131°0,401'E
AUS 2009	1	*T. australis*	107592	NT: Berry Springs on Stuart Highway	12°42,309'S, 131°0,401'E
AUS 2009	2	*M. onca*	107264	NT: Daly River Crossing	13°46,142'S, 130°42,874'E
AUS 2009	2	*P. scabra*	107546	NT: Daly River Crossing	13°46,142'S, 130°42,874'E
AUS 2009	2	*T. australis*	107548	NT: Daly River Crossing	13°46,142'S, 130°42,874'E
AUS 2009	3	*M. onca*	107550	NT: Bamboo Creek at Daly River	13°40,083'S, 130°39,542'E
AUS 2009	3	*P. scabra*	107551	NT: Bamboo Creek at Daly River	13°40,083'S, 130°39,542'E

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AUS 2009	4	*T. australis*	107554	NT: Adelaide River	13°10,353'S, 131°11,501'E
AUS 2009	5	*M. onca*	107555	NT: Elsey Falls at junction of Salt Creek, 50 m above confluence with Roper River, below Mataranka Falls	14°57,412'S, 133°15,103'E
AUS 2009	5	*T. australis*	107556	NT: Elsey Falls at junction of Salt Creek, 50 m above confluence with Roper River, below Mataranka Falls	14°57,412'S, 133°15,103'E
AUS 2009	6	*S. venustula*	107557	NT: Roper River at Jalmurark camp	14°57,1'S, 133°13,42'E
AUS 2009	6	*S. venustula*	107558	NT: Roper River at Jalmurark camp	14°57,1'S, 133°13,42'E
AUS 2009	6	*T. australis*	107559	NT: Roper River at Jalmurark camp	14°57,1'S, 133°13,42'E
AUS 2009	7	*P. scabra*	107564	NT: Little Roper River at crossing	14°55,63'S, 133°7,105'E
AUS 2009	7	*S. venustula*	107561	NT: Little Roper River at crossing	14°55,63'S, 133°7,105'E
AUS 2009	7	*S. venustula*	107562	NT: Little Roper River at crossing	14°55,63'S, 133°7,105'E
AUS 2009	7	*S. denisoniensis*	107563	NT: Little Roper River at crossing	14°55,63'S, 133°7,105'E
AUS 2009	7	*T. australis*	107560	NT: Little Roper River at crossing	14°55,63'S, 133°7,105'E
AUS 2009	8	*M. onca*	107565	NT: Roper River at 4 Mile Point	14°56,151'S, 133°10,034'E
AUS 2009	8	*T. australis*	107567	NT: Roper River at 4 Mile Point	14°56,151'S, 133°10,034'E
AUS 2009	9	*T. australis*	107569	NT: MacArthur River, Borroloola	16°4,889'S, 136°19,148'E
AUS 2009	10	*T. australis*	107573	NT: Robinson River, West of border to Queensland	16°28,27'S, 137°2,995'E
AUS 2009	11	*T. australis*	107574	QL: O'Shanassy River, nr Riversleigh	19°1,322'S, 138°45,697'E
AUS 2009	12	*P. scabra*	107575	QL: Gregory River at Riversleigh	19°1,101'S, 138°43,5'E
AUS 2009	12	*S. denisoniensis*	107577	QL: Gregory River at Riversleigh	19°1,101'S, 138°43,5'E
AUS 2009	12	*T. australis*	107576	QL: Gregory River at Riversleigh	19°1,101'S, 138°43,5'E
AUS 2009	13	*T. australis*	107578	QL: Beame Brook, lower part of Lawn Hill Creek System, above Albert River junction	17°52,77'S, 139°20,445'E
AUS 2009	14	*T. australis*	107579	QL: Bynoe River, west of Normanton	17°51,685'S, 140°48,231'E
AUS 2009	17	*P. balonnensis*	107583	QL: Mareeba, upper Barron River	16°59,134'S, 145°25,158'E
AUS 2009	17	*S. denisoniensis*	107584	QL: Mareeba, upper Barron River	16°59,134'S, 145°25,158'E
AUS 2009	18	*S. aspirans*	107586	QL: Mowbraw River, near Port Douglas	16°33,812'S, 145°27,877'E
AUS 2009	18	*S. denisoniensis*	107588	QL: Mowbraw River, near Port Douglas	16°33,812'S, 145°27,877'E
AUS 2009	18	*T. amarula*	107585	QL: Mowbraw River, near Port Douglas	16°33,812'S, 145°27,877'E
AUS 2009	18	*T. amarula*	107590	QL: Mowbraw River, near Port Douglas	16°33,812'S, 145°27,877'E
AUS 2009	20	*T. amarula*	107594	QL: Douglas Creek, Daintree River	16°16,19'S, 145°18,579'E
AUS 2009	21	*P. scabra*	107596	QL: Martins Creek, upper Daintree Road, Daintree River	16°14,163'S, 145°18,323'E
AUS 2009	21	*R. queenslandica*	107595	QL: Martins Creek, upper Daintree Road, Daintree River	16°14,163'S, 145°18,323'E
AUS 2009	21	*S. denisoniensis*	107598	QL: Martins Creek, upper Daintree Road, Daintree River	16°14,163'S, 145°18,323'E
AUS 2009	21	*T. amarula*	107599	QL: Martins Creek, upper Daintree Road, Daintree River	16°14,163'S, 145°18,323'E
AUS 2009	22	*S. denisoniensis*	107600	QL: E of Dimbulah, Walsh River near Mutchilba	17°7,283'S, 145°16,205'E
AUS 2009	23	*P. balonnensis*	107603	QL: Salt Water Creek, S of Lynd River crossing, near Lynd Brook	17°48,985'S, 144°25,046'E
AUS 2009	23	*S. denisoniensis*	107602	QL: Salt Water Creek, S of Lynd River crossing, near Lynd Brook	17°48,985'S, 144°25,046'E
AUS 2009	26	*P. balonnensis*	107611	QL: Porcupine Creek at Pyramid pool in gorge, trib. to Flinders River	20°20,752'S, 144°27,676'E
AUS 2009	26	*S. denisoniensis*	107609	QL: Porcupine Creek at Pyramid pool in gorge, trib. to Flinders River	20°20,752'S, 144°27,676'E
AUS 2009	27	*M. onca*	107613	NT: Stevie's Hole at Waterhouse River, junction to Roper River	14°55,734'S, 133°8,712'E
AUS 2009	27	*T. australis*	107612	NT: Stevie's Hole at Waterhouse River, junction to Roper River	14°55,734'S, 133°8,712'E
AUS 2009	28	*M. onca*	107587	NT: Wabalarr, Roper River, E of 4 Mile Point on way to Jalmurark	14°56,028'S, 133°10,44'E

Exp. & Loc	n°	Taxa	ZMBn°	Locality	Coordinates
AUS 2009	28	*M. onca*	107610	NT: Wabalarr, Roper River, E of 4 Mile Point on way to Jalmurark	14°56,028'S, 133°10,44'E
AUS 2009	28	*M. onca*	107619	NT: Wabalarr, Roper River, E of 4 Mile Point on way to Jalmurark	14°56,028'S, 133°10,44'E
AUS 2009	28	*S. venustula*	107614	NT: Wabalarr, Roper River, E of 4 Mile Point on way to Jalmurark	14°56,028'S, 133°10,44'E
AUS 2009	28	*S. venustula*	107615	NT: Wabalarr, Roper River, E of 4 Mile Point on way to Jalmurark	14°56,028'S, 133°10,44'E
AUS 2009	28	*S. venustula*	107616	NT: Wabalarr, Roper River, E of 4 Mile Point on way to Jalmurark	14°56,028'S, 133°10,44'E
AUS 2009	28	*S. venustula*	107617	NT: Wabalarr, Roper River, E of 4 Mile Point on way to Jalmurark	14°56,028'S, 133°10,44'E
AUS 2009	28	*S. venustula*	107618	NT: Wabalarr, Roper River, E of 4 Mile Point on way to Jalmurark	14°56,028'S, 133°10,44'E
AUS 2009	28	*T. australis*	107620	NT: Wabalarr, Roper River, E of 4 Mile Point on way to Jalmurark	14°56,028'S, 133°10,44'E
AUS 2009	29	*M. onca*	107625	NT: Mulurark, Roper River, 2km W of Jalmurark, 6km E of Wabalarr	14°56,789'S, 133°12,626'E
AUS 2009	29	*S. venustula*	107621	NT: Mulurark, Roper River, 2km W of Jalmurark, 6km E of Wabalarr	14°56,789'S, 133°12,626'E
AUS 2009	29	*T. australis*	107626	NT: Mulurark, Roper River, 2km W of Jalmurark, 6km E of Wabalarr	14°56,789'S, 133°12,626'E
AUS 2009	30	*S. venustula*	107627	NT: Howard Springs Creek	12°27,268'S, 131°3,108'E
AUS 2009	30	*S. venustula*	107630	NT: Howard Springs Creek	12°27,268'S, 131°3,108'E
AUS 2009	30	*S. denisoniensis*	107629	NT: Howard Springs Creek	12°27,268'S, 131°3,108'E
AUS 2009	30	*T. australis*	107628	NT: Howard Springs Creek	12°27,268'S, 131°3,108'E
AUS 2010	1	*P. balonnensis*	107946	QL: Upper Brisbane River, at Fernvale, W Brisbane, at old bridge crossing	27°26,226'S, 152°38,056'E
AUS 2010	1	*P. balonnensis*	107946	QL: Upper Brisbane River, at Fernvale, W Brisbane, at old bridge crossing	27°26,226'S, 152°38,056'E
AUS 2010	2	*P. balonnensis*	107957	QL: South Pine River at Samford, NW Brisbane	27°21,319'S, 152°52,912'E
AUS 2010	4	*P. balonnensis*	107948	QL: South Maroochy River at Yandina, Coleman's Road crossing	26°33,626'S, 152°56,629'E
AUS 2010	4	*P. balonnensis*	107948	QL: South Maroochy River at Yandina, Coleman's Road crossing	26°33,626'S, 152°56,629'E
AUS 2010	4	*S. denisoniensis*	107949	QL: South Maroochy River at Yandina, Coleman's Road crossing	26°33,626'S, 152°56,629'E
AUS 2010	5	*P. balonnensis*	107950	QL: Litte Widgee Creek, trib. to Mary River, W Gympie	26°12,31'S, 152°27,193'E
AUS 2010	6	*P. balonnensis*	107951	QL: Euri Creek, at Bowen to Collinsville road	20°12,294'S, 147°57,613'E
AUS 2010	6	*S. denisoniensis*	107955	QL: Euri Creek, at Bowen to Collinsville road	20°12,294'S, 147°57,613'E
AUS 2010	8	*S. denisoniensis*	107963	QL: Botanic Garden, Mackay, on Bruce Hwy	21°9,485'S, 149°9,582'E
AUS 2010	9	*P. balonnensis*	107956	QL: Broken River, trib. to Bowen River, at Eungella	21°10,13'S, 148°30,129'E
AUS 2011	1	*S. venustula*	127634	NT: Berry Springs, SE of Darwin	12°42,111'S, 130°59,875'E
AUS 2011	1	*S. denisoniensis*	127789	NT: Berry Springs, SE of Darwin	12°42,111'S, 130°59,875'E
AUS 2011	1	*T. australis*	127720	NT: Berry Springs, SE of Darwin	12°42,111'S, 130°59,875'E
AUS 2011	1	*T. australis*	127734	NT: Berry Springs, SE of Darwin	12°42,111'S, 130°59,875'E
AUS 2011	1	*T.rudis*	127616	NT: Berry Springs, SE of Darwin	12°42,111'S, 130°59,875'E
AUS 2011	1	*T.rudis*	127617	NT: Berry Springs, SE of Darwin	12°42,111'S, 130°59,875'E
AUS 2011	2	*M. onca*	127750	NT: Daly River, at crossing	13°46,001'S, 130°42,638'E
AUS 2011	3	*M. onca*	127752	NT: Kathrine River, downstream lower level, water channel next to main river	14°29,5'S, 132°14,847'E
AUS 2011	3	*S. denisoniensis*	127785	NT: Kathrine River, downstream lower level, water channel next to main river	14°29,5'S, 132°14,847'E
AUS 2011	3	*T. australis*	127747	NT: Kathrine River, downstream lower level, water channel next to main river	14°29,5'S, 132°14,847'E

Exp. & Loc	n°	Taxa	ZMBn°	Locality	Coordinates
AUS 2011	4	*M. onca*	127753	NT: Flora River, at Flora Falls, Djarrung campground	14°45,456'S, 131°35,705'E
AUS 2011	4	*T. australis*	127731	NT: Flora River, at Flora Falls, Djarrung campground	14°45,456'S, 131°35,705'E
AUS 2011	5	*M. onca*	127754	NT: Flora River, below Kathleen Falls, 3km down, at boat ramp	14°43,992'S, 131°36,487'E
AUS 2011	5	*T. australis*	127746	NT: Flora River, below Kathleen Falls, 3km down, at boat ramp	14°43,992'S, 131°36,487'E
AUS 2011	6	*M. onca*	127755	NT: Flora River, near junction, c. 18km from Djarrung campground	14°40,092'S, 131°40,963'E
AUS 2011	6	*T. australis*	127745	NT: Flora River, near junction, c. 18km from Djarrung campground	14°40,092'S, 131°40,963'E
AUS 2011	7	*T. australis*	127725	NT: Victoria River, at old crossing	15°34,866'S, 131°6,137'E
AUS 2011	8	*T. australis*	127744	NT: Timber Creek, above junction of Victoria River	15°38,203'S, 130°28,529'E
AUS 2011	8	*T.rudis*	127618	NT: Timber Creek, above junction of Victoria River	15°38,203'S, 130°28,529'E
AUS 2011	9	*P. scabra*	127763	NT: Big Horse Creek, at Victoria River	15°36,878'S, 130°23,7'E
AUS 2011	9	*T. australis*	127621	NT: Big Horse Creek, at Victoria River	15°36,878'S, 130°23,7'E
AUS 2011	10	*M. onca*	127756	NT: Salt Creek at junction to Roper River	14°57,453'S, 133°15,095'E
AUS 2011	10	*P. scabra*	127779	NT: Salt Creek at junction to Roper River	14°57,453'S, 133°15,095'E
AUS 2011	10	*P. balonnensis*	117733	NT: Salt Creek at junction to Roper River	14°57,453'S, 133°15,095'E
AUS 2011	10	*S. venustula*	127635	NT: Salt Creek at junction to Roper River	14°57,453'S, 133°15,095'E
AUS 2011	10	*S. venustula*	127636	NT: Salt Creek at junction to Roper River	14°57,453'S, 133°15,095'E
AUS 2011	10	*S. venustula*	127637	NT: Salt Creek at junction to Roper River	14°57,453'S, 133°15,095'E
AUS 2011	10	*S. denisoniensis*	127784	NT: Salt Creek at junction to Roper River	14°57,453'S, 133°15,095'E
AUS 2011	10	*T. australis*	127723	NT: Salt Creek at junction to Roper River	14°57,453'S, 133°15,095'E
AUS 2011	10	*T.rudis*	127619	NT: Salt Creek at junction to Roper River	14°57,453'S, 133°15,095'E
AUS 2011	11	*M. onca*	127757	NT: Roper River, between Mataranka Falls and Jalmurark campground, 2km below Jalmurark (downstream)	14°57,515'S, 133°14,275'E
AUS 2011	11	*S. venustula*	127638	NT: Roper River, between Mataranka Falls and Jalmurark campground, 2km below Jalmurark (downstream)	14°57,515'S, 133°14,275'E
AUS 2011	11	*T. australis*	127732	NT: Roper River, between Mataranka Falls and Jalmurark campground, 2km below Jalmurark (downstream)	14°57,515'S, 133°14,275'E
AUS 2011	12	*M. tuberculata*	127613	NT: Bitter Springs, at Mataranka	14°54,642'S, 133°5,362'E
AUS 2011	12	*S. denisoniensis*	127783	NT: Bitter Springs, at Mataranka	14°54,642'S, 133°5,362'E
AUS 2011	13	*M. onca*	127760	NT: Little Roper River at crossing, south bank, lilly pond	14°55,589'S, 133°7,137'E
AUS 2011	13	*P. scabra*	127514	NT: Little Roper River at crossing, south bank, lilly pond	14°55,589'S, 133°7,137'E
AUS 2011	13	*S. venustula*	127515	NT: Little Roper River at crossing, south bank, lilly pond	14°55,589'S, 133°7,137'E
AUS 2011	13	*S. venustula*	127639	NT: Little Roper River at crossing, south bank, lilly pond	14°55,589'S, 133°7,137'E
AUS 2011	13	*S. denisoniensis*	127794	NT: Little Roper River at crossing, south bank, lilly pond	14°55,589'S, 133°7,137'E
AUS 2011	13	*T. australis*	127516	NT: Little Roper River at crossing, south bank, lilly pond	14°55,589'S, 133°7,137'E
AUS 2011	13	*T. australis*	127719	NT: Little Roper River at crossing, south bank, lilly pond	14°55,589'S, 133°7,137'E
AUS 2011	13	*T. australis*	127721	NT: Little Roper River at crossing, south bank, lilly pond	14°55,589'S, 133°7,137'E
AUS 2011	13	*T. australis*	127735	NT: Little Roper River at crossing, south bank, lilly pond	14°55,589'S, 133°7,137'E

Exp. & Loc n°		Taxa	ZMBn°	Locality	Coordinates
AUS 2011	14	*P. scabra*	127778	NT: Little Roper River at crossing, north bank, among palms	14°55,63'S, 133°7,105'E
AUS 2011	14	*S. venustula*	127640	NT: Little Roper River at crossing, north bank, among palms	14°55,63'S, 133°7,105'E
AUS 2011	14	*S. venustula*	127641	NT: Little Roper River at crossing, north bank, among palms	14°55,63'S, 133°7,105'E
AUS 2011	14	*S. denisoniensis*	127792	NT: Little Roper River at crossing, north bank, among palms	14°55,63'S, 133°7,105'E
AUS 2011	14	*T. australis*	127726	NT: Little Roper River at crossing, north bank, among palms	14°55,63'S, 133°7,105'E
AUS 2011	14	*T. australis*	127730	NT: Little Roper River at crossing, north bank, among palms	14°55,63'S, 133°7,105'E
AUS 2011	15	*P. scabra*	127780	NT: Roper River, at Botanic Walk	14°56,126'S, 133°8,532'E
AUS 2011	15	*S. venustula*	127642	NT: Roper River, at Botanic Walk	14°56,126'S, 133°8,532'E
AUS 2011	15	*S. venustula*	127643	NT: Roper River, at Botanic Walk	14°56,126'S, 133°8,532'E
AUS 2011	15	*S. denisoniensis*	127791	NT: Roper River, at Botanic Walk	14°56,126'S, 133°8,532'E
AUS 2011	15	*T. australis*	127727	NT: Roper River, at Botanic Walk	14°56,126'S, 133°8,532'E
AUS 2011	16	*M. onca*	127751	NT: Roper River, at 4Mile Point	14°56,12'S, 133°10,069'E
AUS 2011	16	*P. scabra*	127770	NT: Roper River, at 4Mile Point	14°56,12'S, 133°10,069'E
AUS 2011	16	*S. venustula*	127644	NT: Roper River, at 4Mile Point	14°56,12'S, 133°10,069'E
AUS 2011	16	*T. australis*	127722	NT: Roper River, at 4Mile Point	14°56,12'S, 133°10,069'E
AUS 2011	16	*T. australis*	127742	NT: Roper River, at 4Mile Point	14°56,12'S, 133°10,069'E
AUS 2011	17	*P. scabra*	127772	NT: Wabalarr, at Roper River	14°56,028'S, 133°10,444'E
AUS 2011	17	*S. venustula*	127645	NT: Wabalarr, at Roper River	14°56,028'S, 133°10,444'E
AUS 2011	17	*T. australis*	127724	NT: Wabalarr, at Roper River	14°56,028'S, 133°10,444'E
AUS 2011	17	*T. australis*	127743	NT: Wabalarr, at Roper River	14°56,028'S, 133°10,444'E
AUS 2011	18	*M. onca*	127761	NT: Mulurark, at Roper River	14°56,763'S, 133°12,614'E
AUS 2011	18	*P. scabra*	127769	NT: Mulurark, at Roper River	14°56,763'S, 133°12,614'E
AUS 2011	18	*S. venustula*	127646	NT: Mulurark, at Roper River	14°56,763'S, 133°12,614'E
AUS 2011	18	*T. australis*	127729	NT: Mulurark, at Roper River	14°56,763'S, 133°12,614'E
AUS 2011	18	*T. australis*	127741	NT: Mulurark, at Roper River	14°56,763'S, 133°12,614'E
AUS 2011	19	*P. scabra*	127766	NT: Salt Creek, at crossing of Roper Hwy	15°0,703'S, 133°14,417'E
AUS 2011	19	*S. venustula*	127647	NT: Salt Creek, at crossing of Roper Hwy	15°0,703'S, 133°14,417'E
AUS 2011	19	*S. venustula*	127648	NT: Salt Creek, at crossing of Roper Hwy	15°0,703'S, 133°14,417'E
AUS 2011	19	*S. denisoniensis*	127786	NT: Salt Creek, at crossing of Roper Hwy	15°0,703'S, 133°14,417'E
AUS 2011	19	*T. australis*	127740	NT: Salt Creek, at crossing of Roper Hwy	15°0,703'S, 133°14,417'E
AUS 2011	20	*S. venustula*	127649	NT: Elsey Creek, at crossing of Roper Hwy	15°0,624'S, 133°14,962'E
AUS 2011	20	*S. venustula*	127650	NT: Elsey Creek, at crossing of Roper Hwy	15°0,624'S, 133°14,962'E
AUS 2011	20	*T. australis*	127717	NT: Elsey Creek, at crossing of Roper Hwy	15°0,624'S, 133°14,962'E
AUS 2011	21	*M. onca*	127762	NT: Roper River, at Roper Bar	14°42,802'S, 134°30,474'E
AUS 2011	21	*P. scabra*	127768	NT: Roper River, at Roper Bar	14°42,802'S, 134°30,474'E
AUS 2011	21	*T. australis*	127733	NT: Roper River, at Roper Bar	14°42,802'S, 134°30,474'E
AUS 2011	22	*P. scabra*	127771	NT: Roper River, Mountain Creek, 500m below crossing of Roper Hwy	14°46,543'S, 134°48,016'E
AUS 2011	22	*S. denisoniensis*	127795	NT: Roper River, Mountain Creek, 500m below crossing of Roper Hwy	14°46,543'S, 134°48,016'E
AUS 2011	22	*T. australis*	127739	NT: Roper River, Mountain Creek, 500m below crossing of Roper Hwy	14°46,543'S, 134°48,016'E
AUS 2011	22	*T.rudis*	127620	NT: Roper River, Mountain Creek, 500m below crossing of Roper Hwy	14°46,543'S, 134°48,016'E
AUS 2011	23	*S. venustula*	127651	NT: Towns River, at crossing with Roper Hwy	15°2,57'S, 135°12,718'E
AUS 2011	23	*S. denisoniensis*	127799	NT: Towns River, at crossing with Roper Hwy	15°2,57'S, 135°12,718'E
AUS 2011	23	*T. australis*	127623	NT: Towns River, at crossing with Roper Hwy	15°2,57'S, 135°12,718'E
AUS 2011	23	*P. scabra*	127776	NT: Towns River, at crossing with Roper Hwy	15°2,57'S, 135°12,718'E
AUS 2011	24	*T. australis*	127718	NT: Towns River, at boat ramp	15°2,09'S, 135°13,161'E
AUS 2011	25	*S. venustula*	127652	NT: Limmen Bight River, delta	15°15,19'S, 135°31,7'E
AUS 2011	26	*S. venustula*	127653	NT: Towns River, downstream, point 1	14°59,839'S, 135°16,262'E
AUS 2011	26	*S. denisoniensis*	127788	NT: Towns River, downstream, point 1	14°59,839'S, 135°16,262'E

Exp. & Loc	n°	Taxa	ZMBn°	Locality	Coordinates
AUS 2011	26	*T. australis*	127737	NT: Towns River, downstream, point 1	14°59,839'S, 135°16,262'E
AUS 2011	27	*S. denisoniensis*	127796	NT: Towns River, two pools on northern bank	14°59,792'S, 135°17,156'E
AUS 2011	27	*T. australis*	127738	NT: Towns River, two pools on northern bank	14°59,792'S, 135°17,156'E
AUS 2011	28	*S. venustula*	127654	NT: Towns River, backwater at junction with creek, "Sermyla point"	14°59,999'S, 135°17,03'E
AUS 2011	28	*S. venustula*	127655	NT: Towns River, backwater at junction with creek, "Sermyla point"	14°59,999'S, 135°17,03'E
AUS 2011	28	*S. denisoniensis*	127793	NT: Towns River, backwater at junction with creek, "Sermyla point"	14°59,999'S, 135°17,03'E
AUS 2011	28	*T. australis*	127728	NT: Towns River, backwater at junction with creek, "Sermyla point"	14°59,999'S, 135°17,03'E
AUS 2011	29	*S. denisoniensis*	127797	NT: Wearyan River, at crossing	16°10,03'S, 136°45,506'E
AUS 2011	31	*T. australis*	127622	QL: Lawn Hill Creek, at Adels Grove	18°41,383'S, 138°31,655'E
AUS 2011	32	*P. scabra*	127775	QL: Gregory River, at 2nd crossing, Riversleight	19°1,195'S, 138°43,567'E
AUS 2011	32	*S. denisoniensis*	127790	QL: Gregory River, at 2nd crossing, Riversleight	19°1,195'S, 138°43,567'E
AUS 2011	32	*T. australis*	127624	QL: Gregory River, at 2nd crossing, Riversleight	19°1,195'S, 138°43,567'E
AUS 2011	32	*T. australis*	127625	QL: Gregory River, at 2nd crossing, Riversleight	19°1,195'S, 138°43,567'E
AUS 2011	32	*T. australis*	127626	QL: Gregory River, at 2nd crossing, Riversleight	19°1,195'S, 138°43,567'E
AUS 2011	33	*P. scabra*	127764	QL: O'Shanassy River, at crossing	19°1,378'S, 138°45,73'E
AUS 2011	34	*T. australis*	127627	QL: Lawn Hill Creek, at Boudjamulla camp	18°42,056'S, 138°29,211'E
AUS 2011	35	*P. scabra*	127765	QL: Gregory River, at crossing, Gregory Downs	18°38,829'S, 139°14,912'E
AUS 2011	35	*P. scabra*	127773	QL: Gregory River, at crossing, Gregory Downs	18°38,829'S, 139°14,912'E
AUS 2011	35	*T. australis*	127628	QL: Gregory River, at crossing, Gregory Downs	18°38,829'S, 139°14,912'E
AUS 2011	36	*P. scabra*	127777	QL: Gregory River, Beame Brook, at crossing	17°52,708'S, 139°20,576'E
AUS 2011	36	*T. australis*	127629	QL: Gregory River, Beame Brook, at crossing	17°52,708'S, 139°20,576'E
AUS 2011	38	*S. venustula*	127656	QL: Norman River, 1km N of Normanton	17°39,712'S, 141°6,154'E
AUS 2011	38	*S. denisoniensis*	127787	QL: Norman River, 1km N of Normanton	17°39,712'S, 141°6,154'E
AUS 2011	39	*T. australis*	127630	QL: Bynoe River, at crossing	17°51,719'S, 140°48,067'E
AUS 2011	40	*S. denisoniensis*	127800	QL: Norman River, at Glenmore, crossing at old bridge	17°51,228'S, 141°8,047'E
AUS 2011	40	*T. australis*	127631	QL: Norman River, at Glenmore, crossing at old bridge	17°51,228'S, 141°8,047'E
AUS 2011	41	*P. balonnensis*	127614	QL: Einasleigh River, 4km E of Einasleigh	18°30,915'S, 144°6,654'E
AUS 2011	42	*P. balonnensis*	127615	QL: Porcupine Creek at Porcupine Gorge	20°21,197'S, 144°28,01'E
AUS 2011	43	*P. scabra*	127767	NT: Elsey Creek, at Warloch ponds	15°5,083'S, 133°7,439'E
AUS 2011	43	*S. venustula*	127657	NT: Elsey Creek, at Warloch ponds	15°5,083'S, 133°7,439'E
AUS 2011	43	*S. venustula*	127658	NT: Elsey Creek, at Warloch ponds	15°5,083'S, 133°7,439'E
AUS 2011	43	*S. denisoniensis*	127782	NT: Elsey Creek, at Warloch ponds	15°5,083'S, 133°7,439'E
AUS 2011	43	*T. australis*	127632	NT: Elsey Creek, at Warloch ponds	15°5,083'S, 133°7,439'E
AUS 2011	44	*M. onca*	127749	NT: Daly River, at Oolloo crossing	14°4,24'S, 131°15,084'E
AUS 2011	44	*P. scabra*	127774	NT: Daly River, at Oolloo crossing	14°4,24'S, 131°15,084'E
AUS 2011	44	*T. australis*	127633	NT: Daly River, at Oolloo crossing	14°4,24'S, 131°15,084'E
AUS 2011	44	*T. australis*	127748	NT: Daly River, at Oolloo crossing	14°4,24'S, 131°15,084'E
AUS 2011	45	*S. venustula*	127659	NT: Spring creek at Howard Springs	12°27,553'S, 131°3,069'E
AUS 2011	45	*S. denisoniensis*	127798	NT: Spring creek at Howard Springs	12°27,553'S, 131°3,069'E
AUS 2011	46	*S. venustula*	127660	NT: Howard Springs, below pond	12°27,366'S, 131°3,19'E
AUS 2011	46	*S. denisoniensis*	127781	NT: Howard Springs, below pond	12°27,366'S, 131°3,19'E
AUS 2011	46	*T. australis*	127736	NT: Howard Springs, below pond	12°27,366'S, 131°3,19'E
AUS 2012	4	*T. australis*	127505	Fitzroy River, at Fitzroy Crossing, 500m S of bridge	18°12,659'S, 125°34,801'E
AUS 2012	5	*T. australis*	127506	Lennard River, near Windjana Gorge	17°25'S, 124°50'E
AUS 2012	7	*P. balonnensis*	127507	De Grey River, E of Port Hedland	20°18,665'S, 119°15,383'E
AUS 2012	8	*P. balonnensis*	117731	Millstream National Park, Fortescue River, at Millstream Creek, below pond, among stones, at Millstream old Homestead	21°35,355'S, 117°4,33'E
AUS 2012	8	*P. balonnensis*	127508	Millstream National Park, Fortescue River, at Millstream Creek, below pond, among stones, at Millstream old Homestead	21°35,355'S, 117°4,33'E

Exp. & Loc	n°	Taxa	ZMBn°	Locality	Coordinates
AUS 2012	8	*P. balonnensis*	127509	Millstream National Park, Fortescue River, at Millstream Creek, below pond, among stones, at Millstream old Homestead	21°35,355'S, 117°4,33'E
AUS 2012	9	*T. australis*	127510	Geikie Gorge, at Fitzroy River	18°6,394'S, 125°42,026'E
AUS 2012	10	*M. tuberculata*	127511	Lake Ord, Ord River, at Kununurra	15°47,203'S, 128°44,163'E
AUS 2012	11	*T. australis*	127512	Ord River at Lake Ord, along canal, downstream pump station	15°47,339'S, 128°43,01'E
AUS 2012	12	*T. australis*	127513	East Bearns River, at cossing, 50 km W of Timber Creek	15°45,67'S, 130°1,653'E

B Mastermix and PCR profiles

Table 23 Mastermix used for PCR of COI, 16S, H3 and 28S

reagent	**volume for 1 reaction**
ddH_2O	18.8 μl
primer I	1.0 μl
primer II	1.0 μl
dNTPs	0.5 μl
buffer	2.5 μl
Taq	0.2 μl
DNA	1.0 μl
sum	25.0 μl

Table 24 Multiplex Mastermix used for PCR of COI

reagent	**volume for 1 reaction**
H_2O	4.3 μl
primer I	1.6 μl
primer II	1.6 μl
Q-solution	2.0 μl
Multiplex	9.5 μl
DNA	1.0 μl
sum	20.0 μl

Thermal cycling conditions for COI multiplex approach were as follows: 95 °C for 15 min, 35 cycles of touchdown PCR (94 °C for 0:30 min, 55-40 °C annealing for 1:30 min and 72 °C extension for 1:30 min) followed by 5 cycles (94 °C for 0:30 min, 40 °C annealing for 1:30 min and 72 °C extension for 1:30 min) and a final extension step at 72 °C for 10 min.

Table 25 Multiplex Mastermix used for PCR of 16S.

reagent	**volume for 1 reaction**
H_2O	4.3 μl
primer I	1.6 μl
primer II	1.6 μl
BSA	2.0 μl
Multiplex	9.5 μl
DNA	1.0 μl
sum	20.0 μl

Thermal cycling conditions for 16S multiplex PCR were as follows: 95 °C for 15 min, 35 cycles of touchdown PCR (94 °C for 0:30 min, 67.5-50 °C annealing for 1:30 min and 72 °C extension for 1:30 min) followed by 5 cycles (94 °C for 0:30 min, 50 °C annealing for 1:30 min and 72 °C extension for 1:30 min) and a final extension step at 72 °C for 10 min.

C Additional analyses of phylogenetic trees

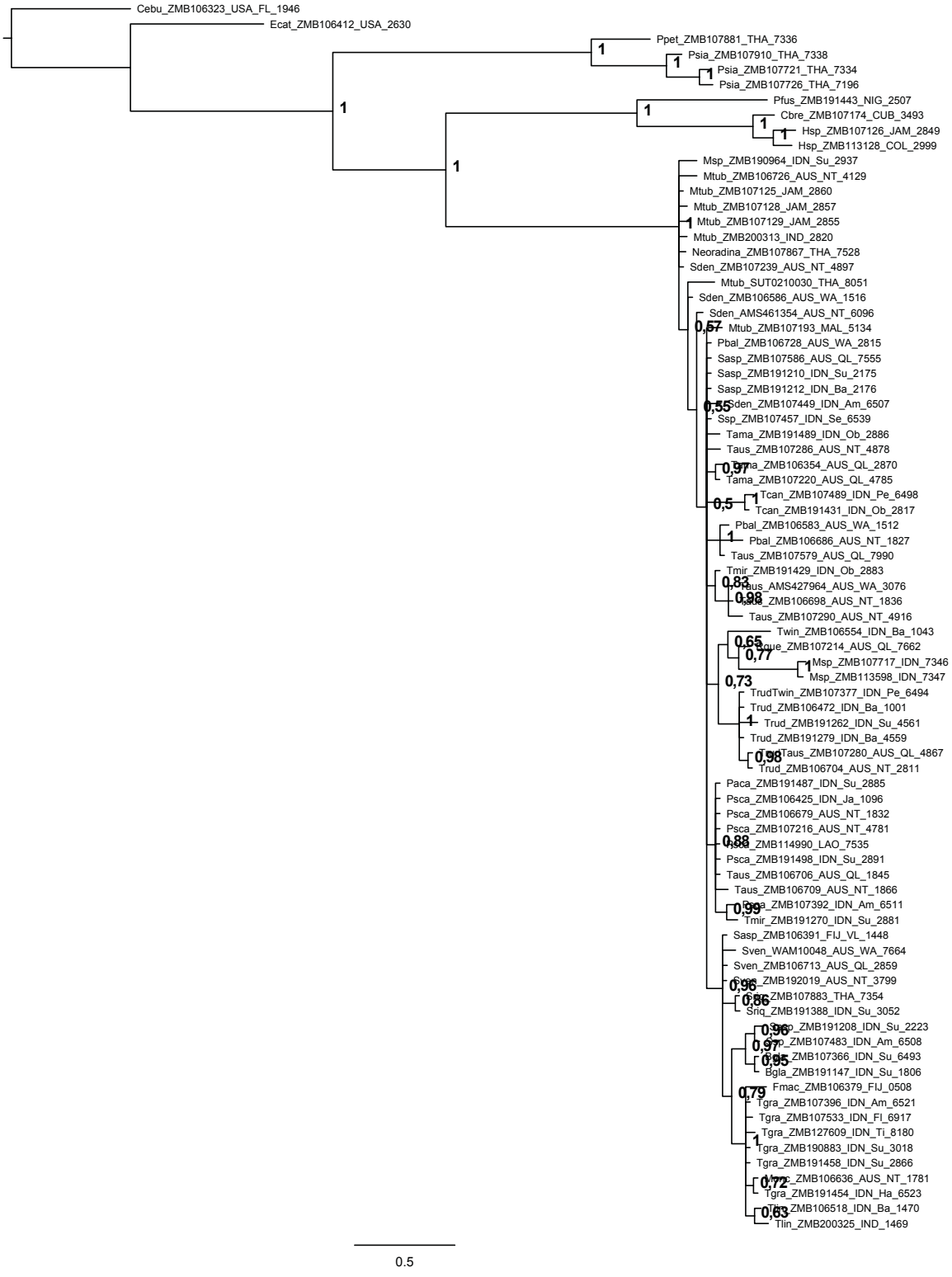


Figure 47 Bayesian inference phylogram based on combined 28S and H3 sequences (81 taxa). Analyses was conducted by using MrBayes (ngen: 5000000; samplefreq: 100; burnin: 35001).

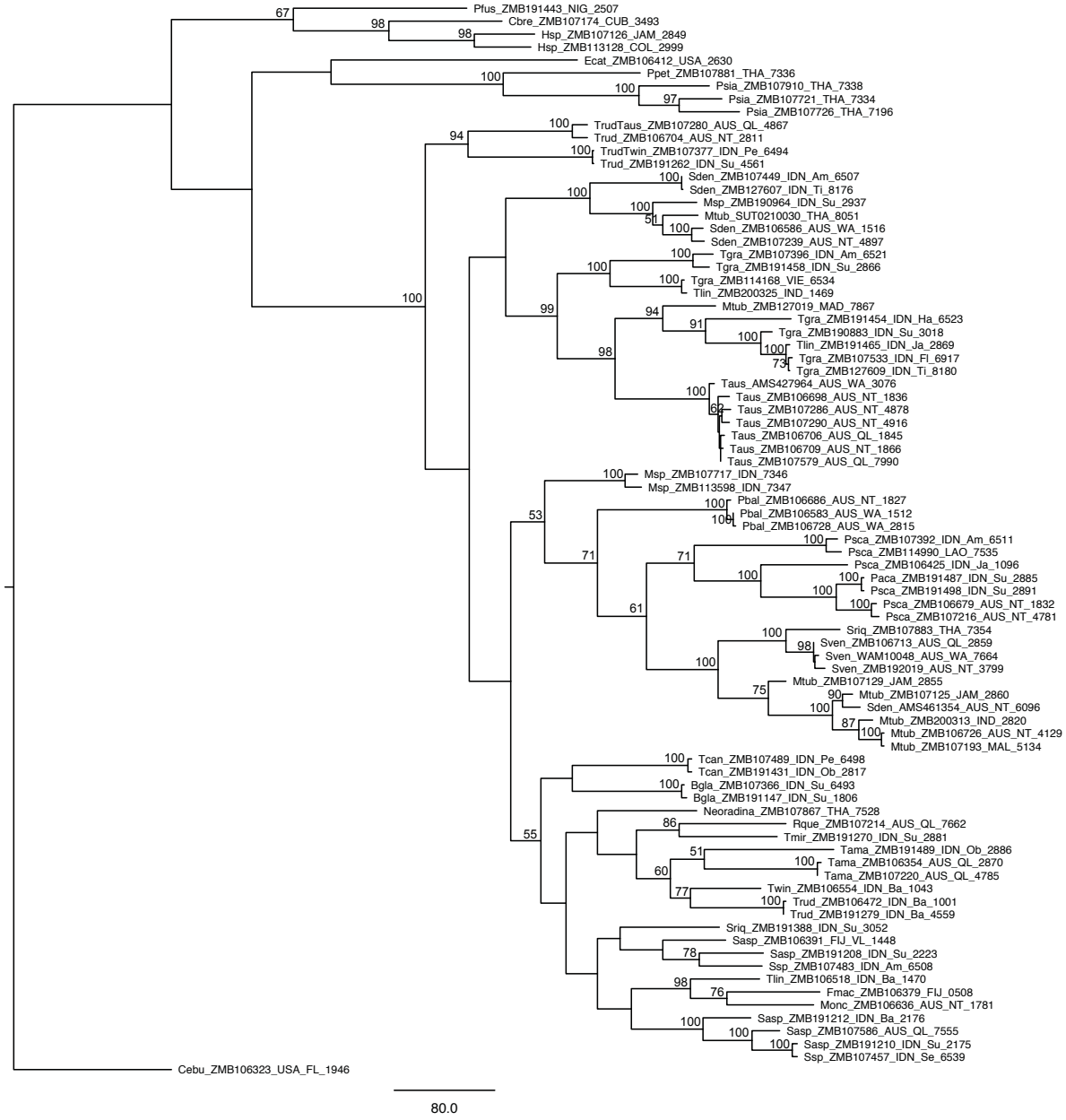


Figure 48 Maximum Parsimony topology based on concatenated mtDNA estimated by PAUP 4.0. Numbers on branches denote bootstrap values which are only shown when higher than 50%.

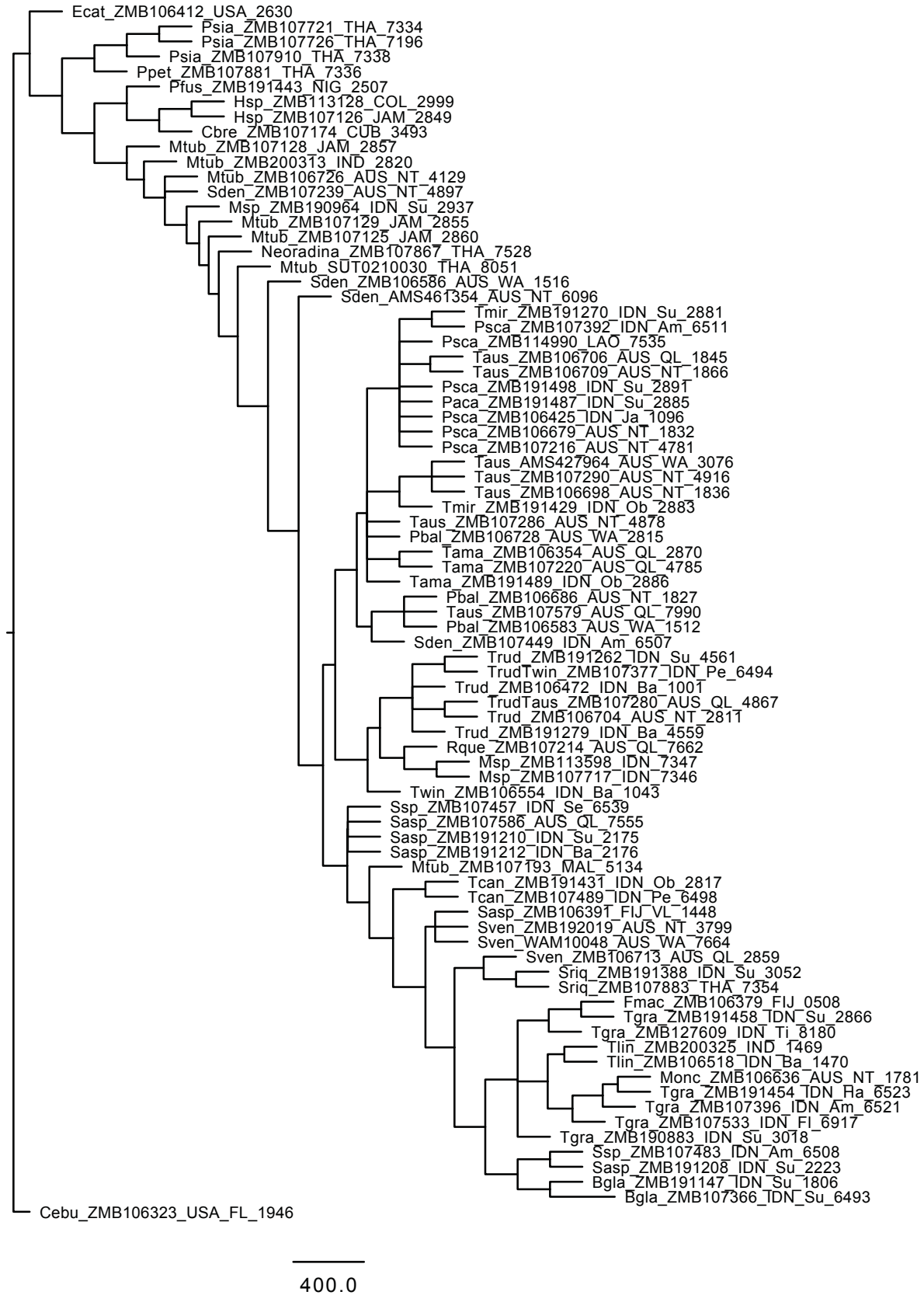


Figure 49 Majority rule (extended) consensus tree based on combined 28S and H3 sequences and obtained by using PAUP 4.0.

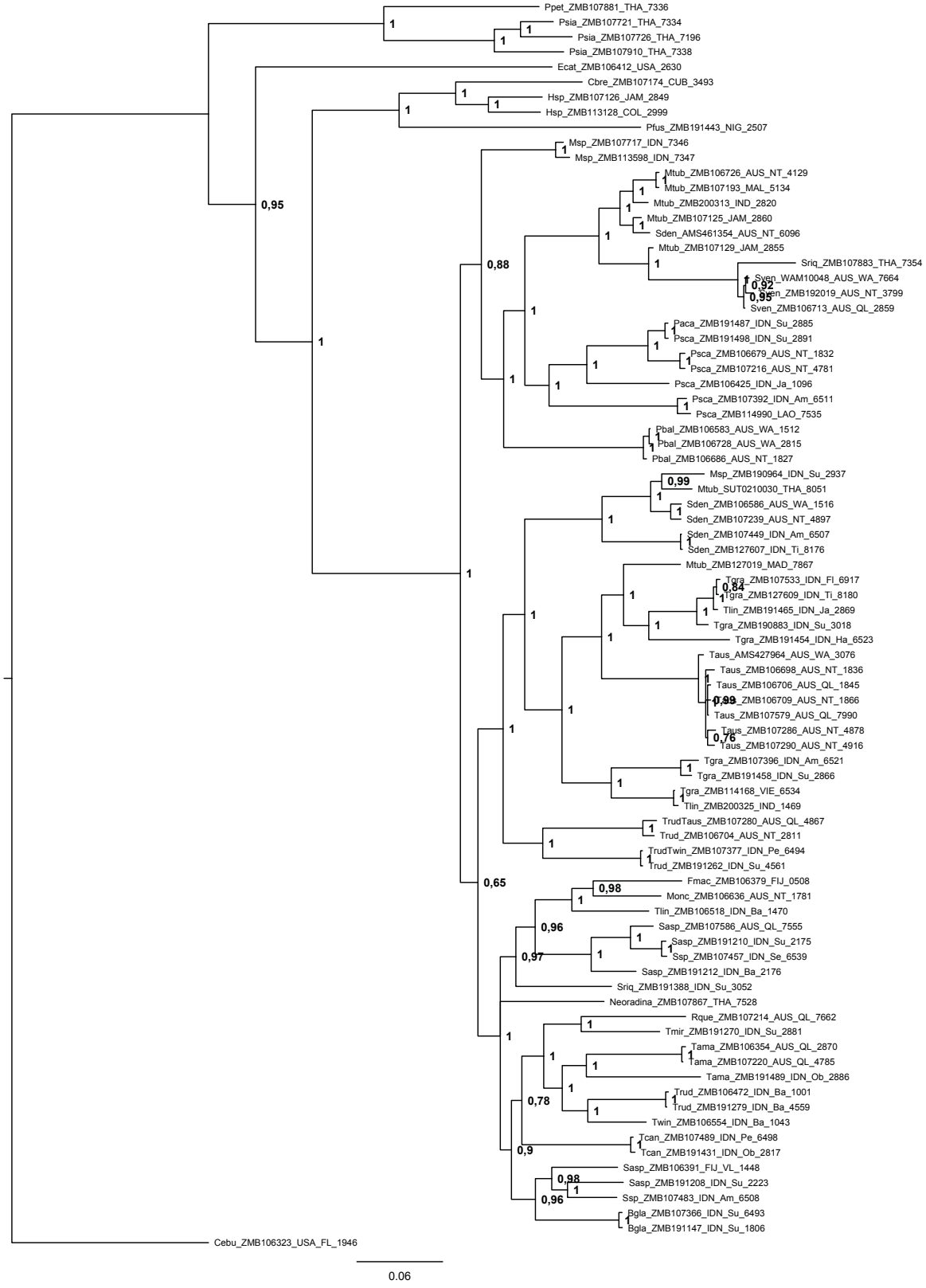


Figure 50 Bayesian inference based on combined mtDNA dataset (COI and 16S rRNA). Analyses was conducted by using MrBayes (ngen: 5000000; samplefreq: 100; burnin: 35001).

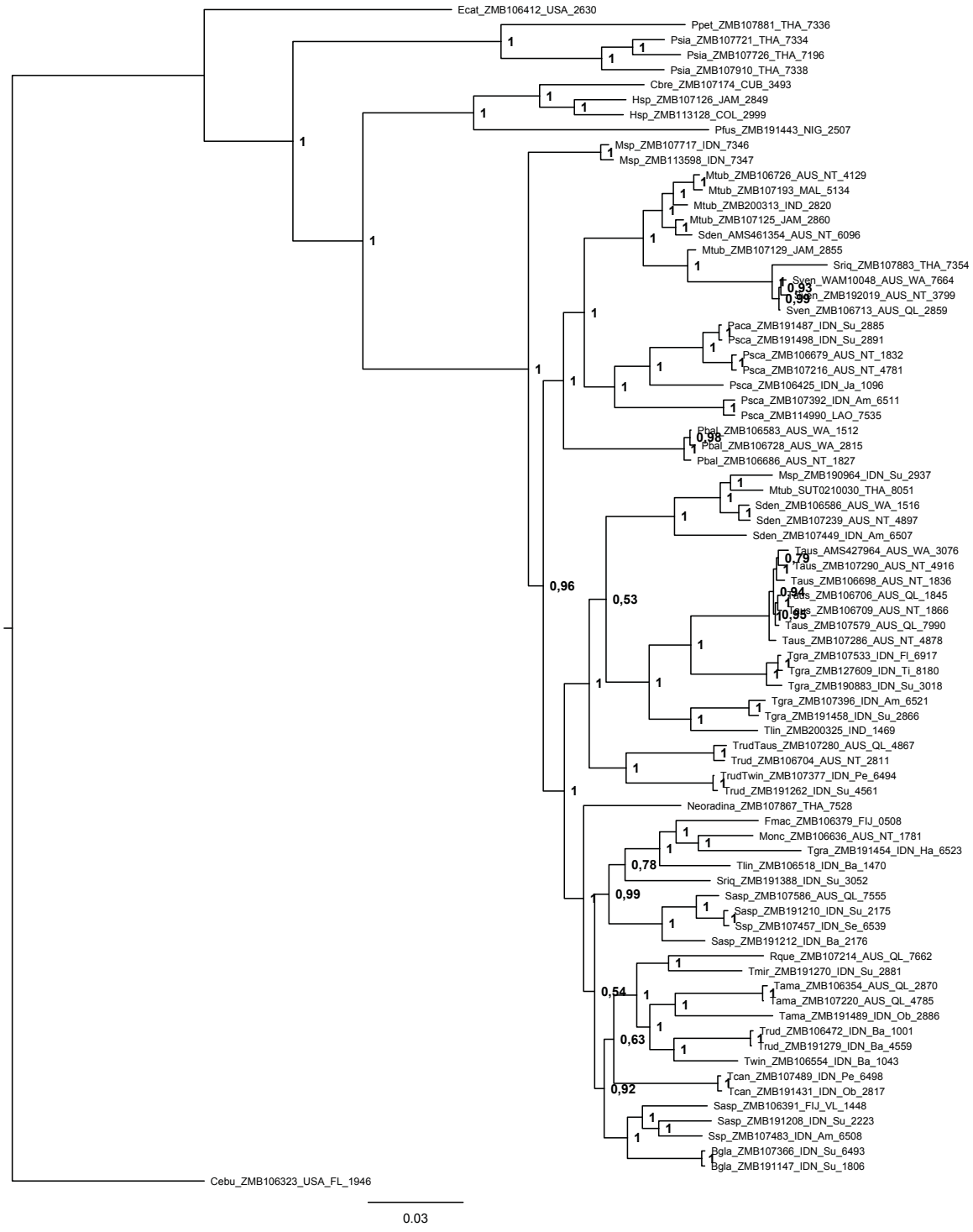


Figure 51 Bayesian inference phylogram based on the concatenated data including 79 taxa (combined mitochondrial and nuclear DNA). Analyses was conducted by using MrBayes (ngen: 5000000; samplefreq: 100; burnin: 35001).

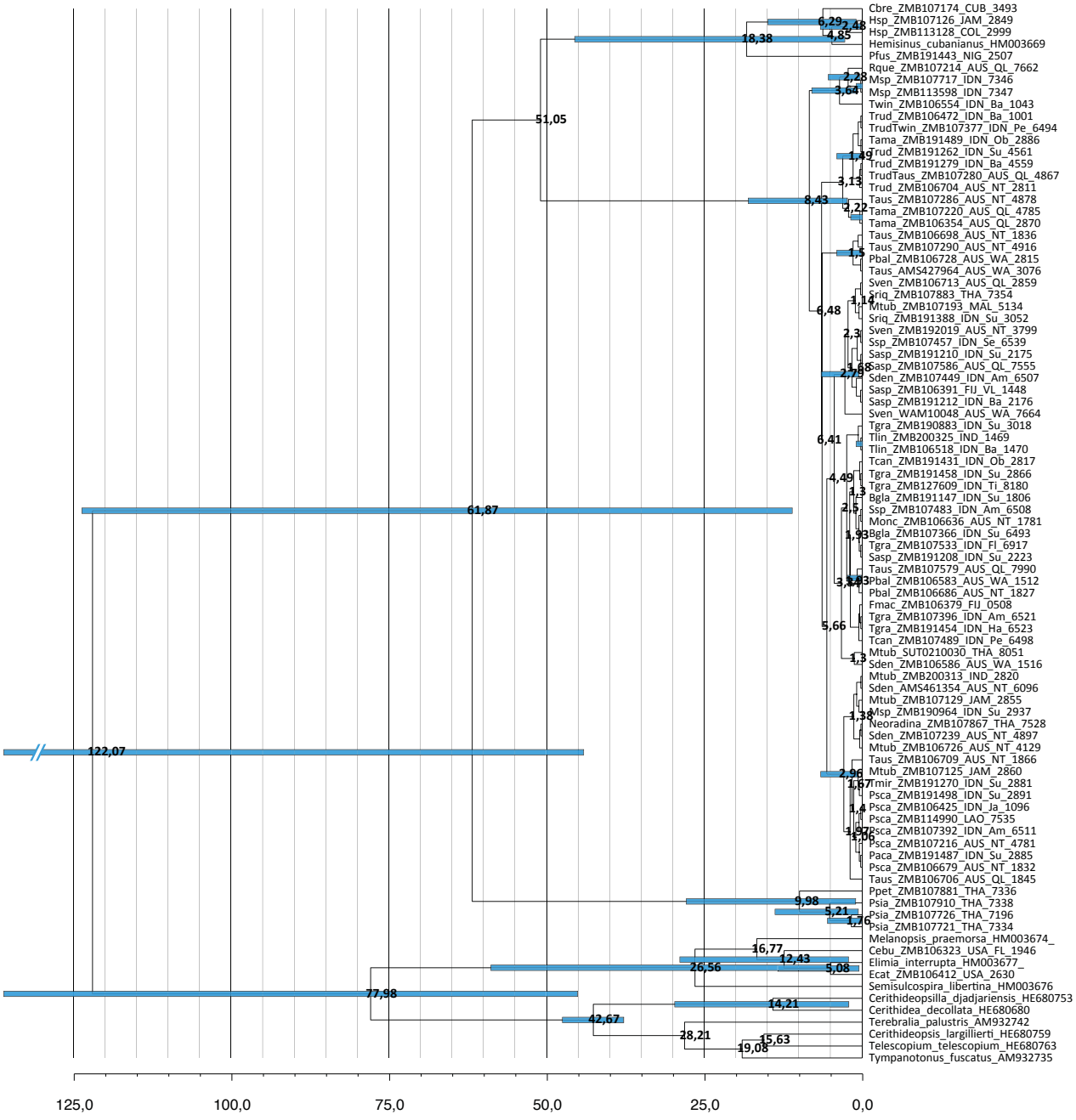


Figure 52 BEAST chronogram based on 28S sequences. First approach with only one calibration point: A normally distributed calibration prior with mean 42.9 and standard deviation 2.5 (95% range: 38.8-47.8 ma) was set for the node age of the Potamididae. Numbers at nodes represent divergence dates. Blue bars represent 95% highest posterior density intervals. Scale is given in millions of years before present.

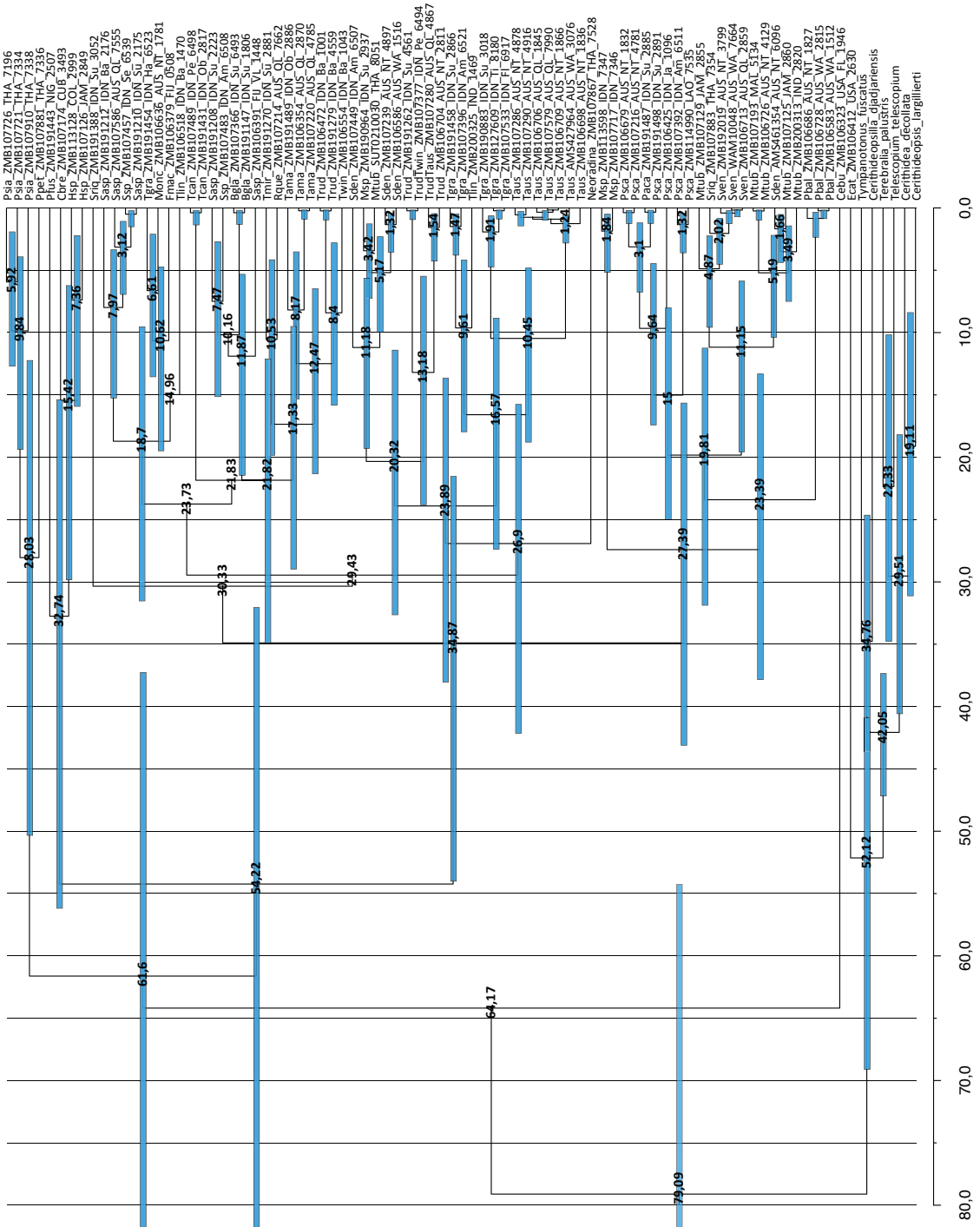


Figure 53 BEAST chronogram based on combined COI and 16S sequences. First approach with only one calibration point: A normally distributed calibration prior with mean 42.9 and standard deviation 2.5 (95% range: 38.8-47.8 ma) was set for the node age of the Potamididae. Numbers at nodes represent divergence dates. Blue bars represent 95% highest posterior density intervals. Scale is given in millions of years before present.

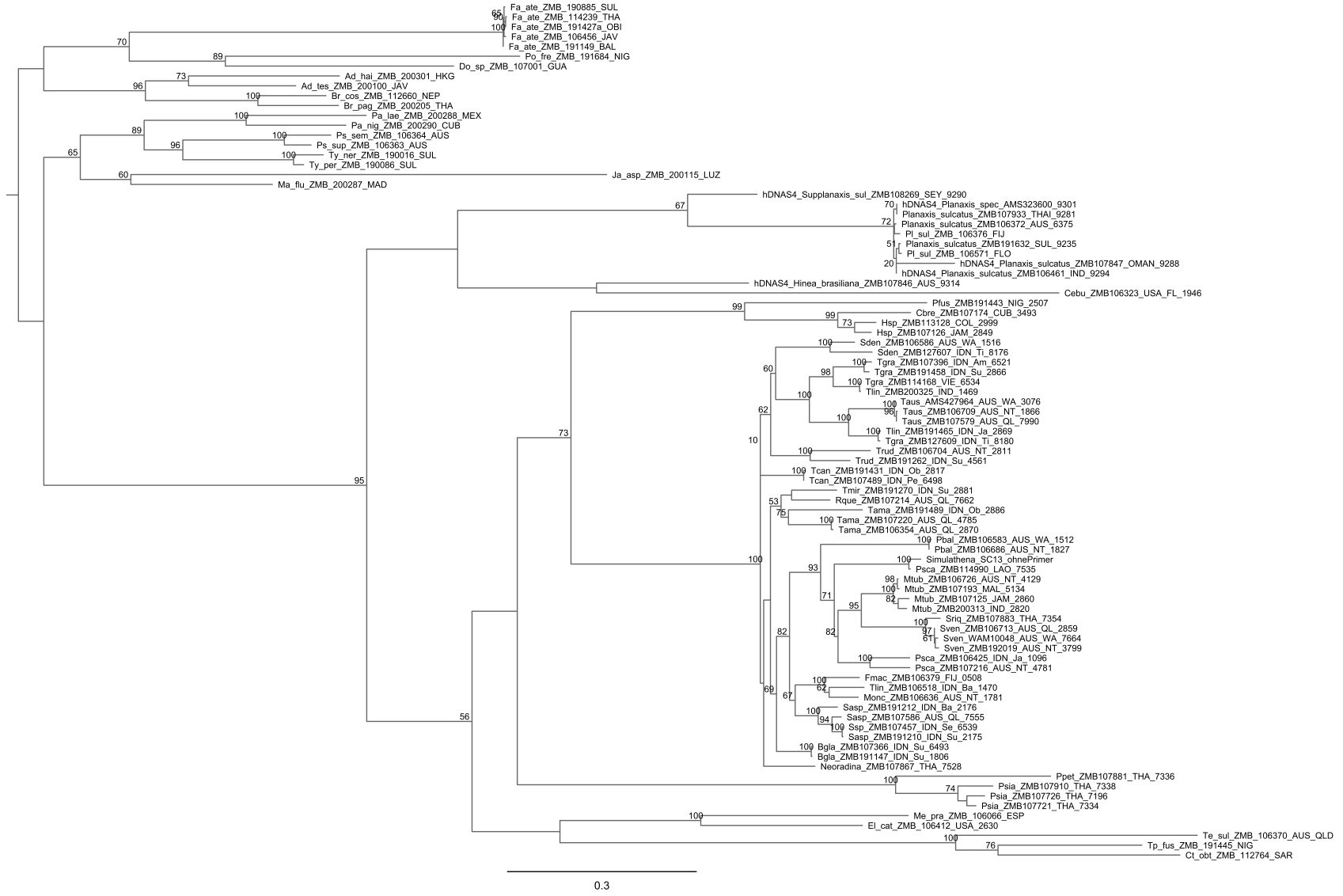


Figure 54 Phylogenetic tree reconstruction by maximum likelihood based on 16S sequences of the hDNA dataset conducted with RAxML (Stamatakis et al., 2008). Numbers on nodes indicate bootstrap support of the shown topology. Historical samples begin with 'SC'. Rque: *Ripalania queenslandica*; Bgla: *Balanochochlis glans*; Psia: *Paludomus siamensis*. For other abbreviations in taxa names see appendix. Four and five-digit numbers represent extraction numbers, numbers with prefix letter code museum numbers.

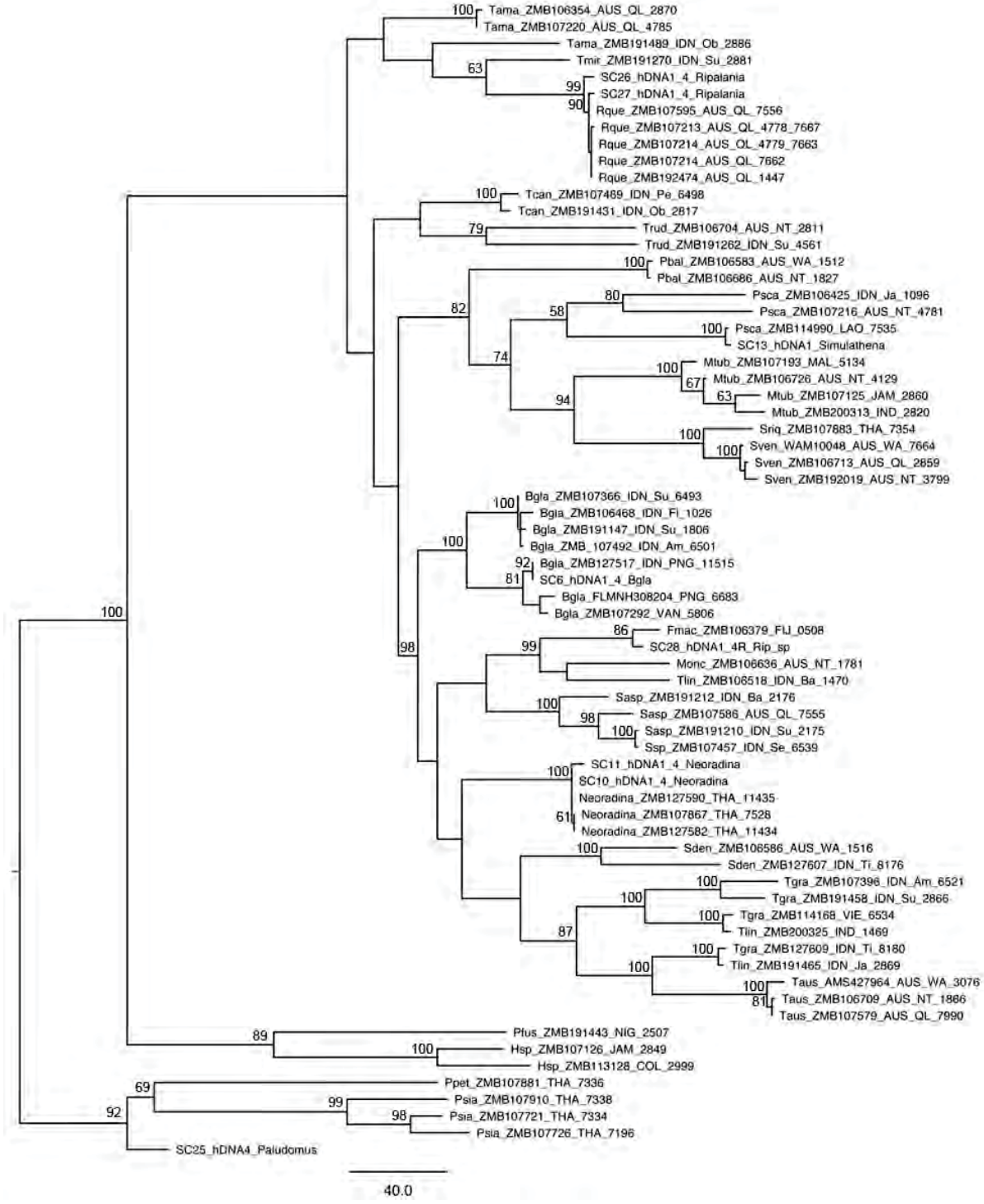


Figure 56 Phylogenetic inference reconstructed by maximum parsimony using the heuristic search algorithm as implemented in PAUP* (Swofford, 2002) based on 16S sequences of the hDNA dataset (compare with fig. 39). Numbers on nodes indicate bootstrap support of the shown topology. For abbreviations in taxa names see appendix. Four and five-digit numbers represent extraction numbers, numbers with prefix letter code museum numbers.

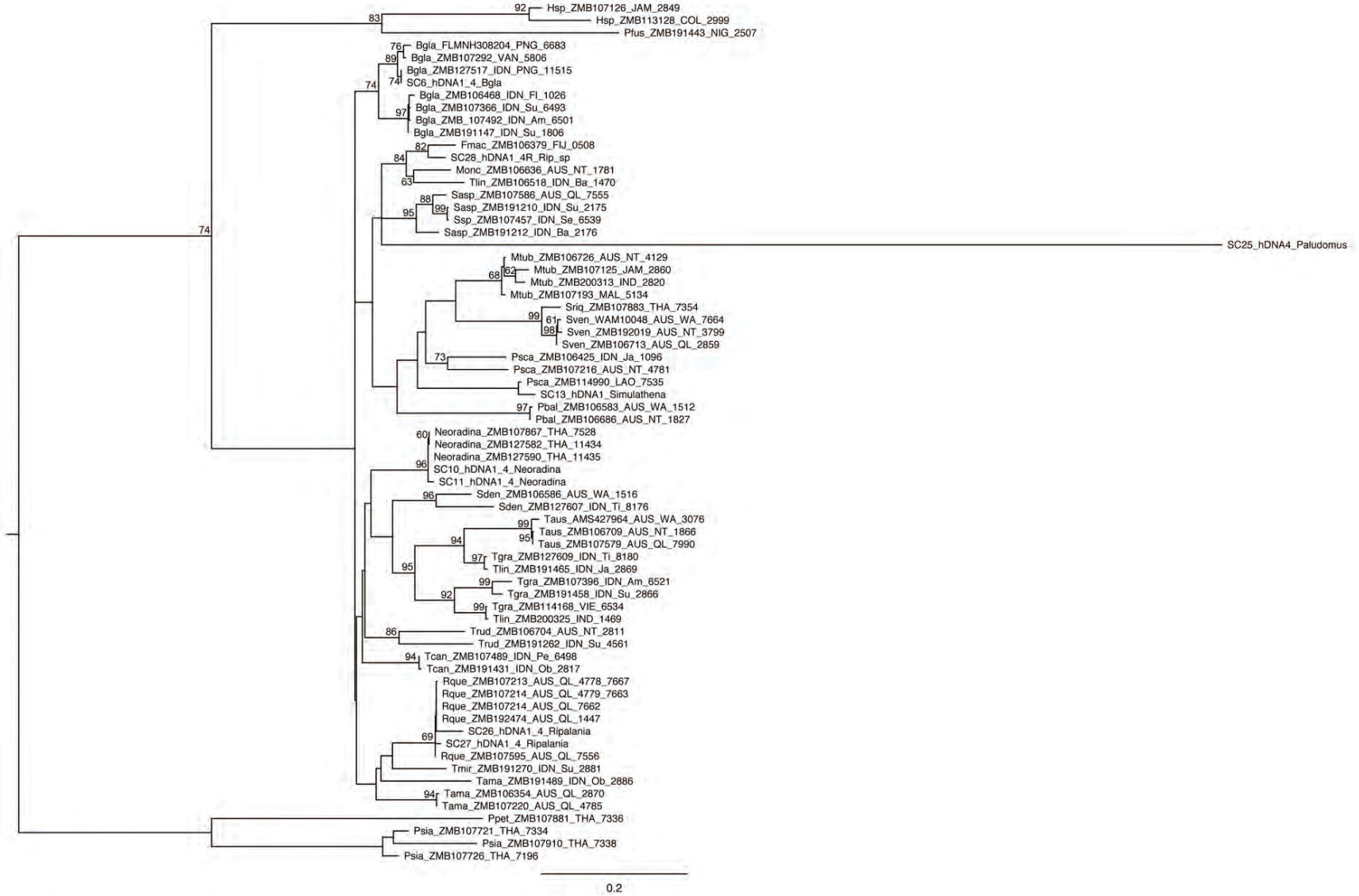
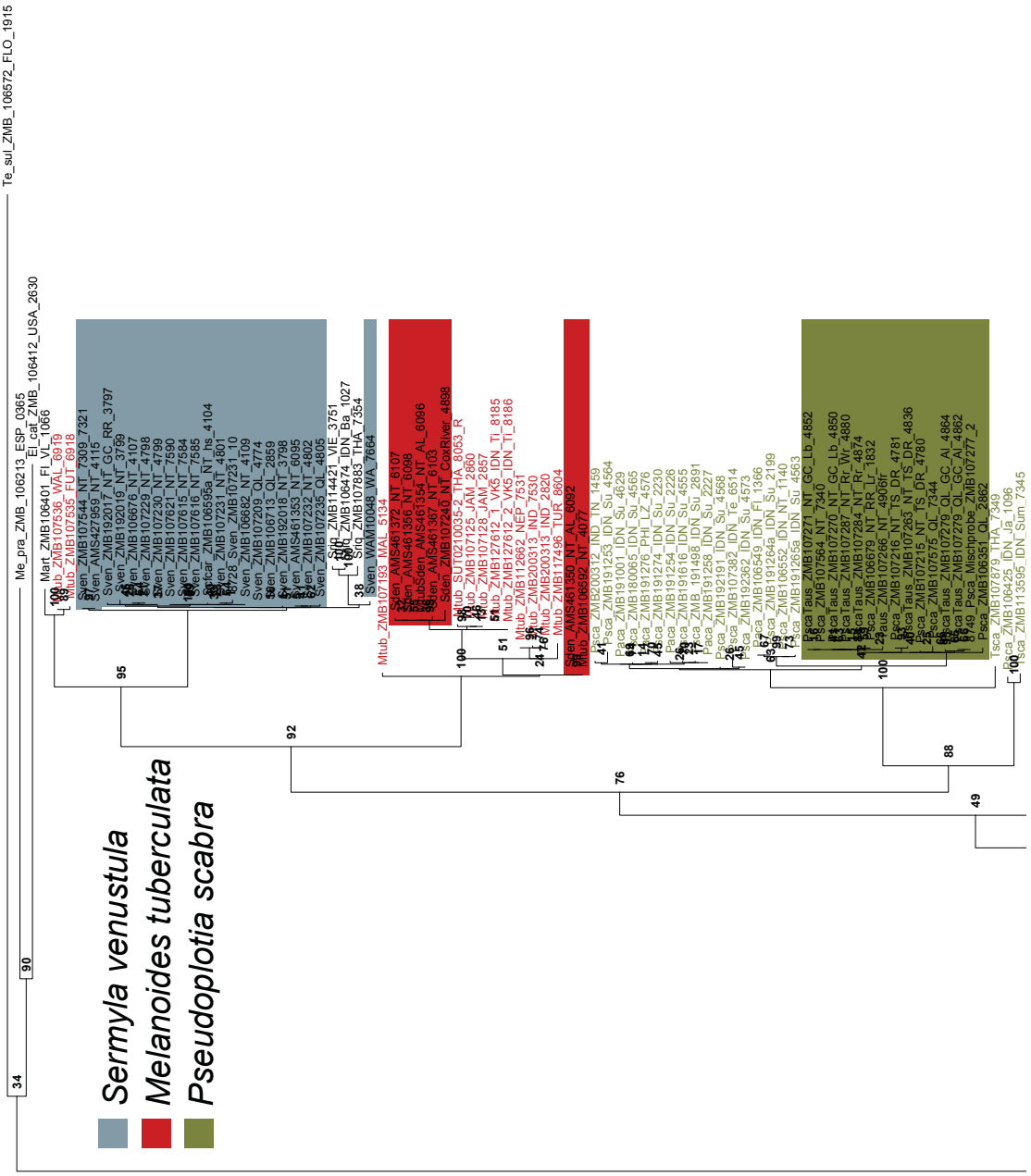
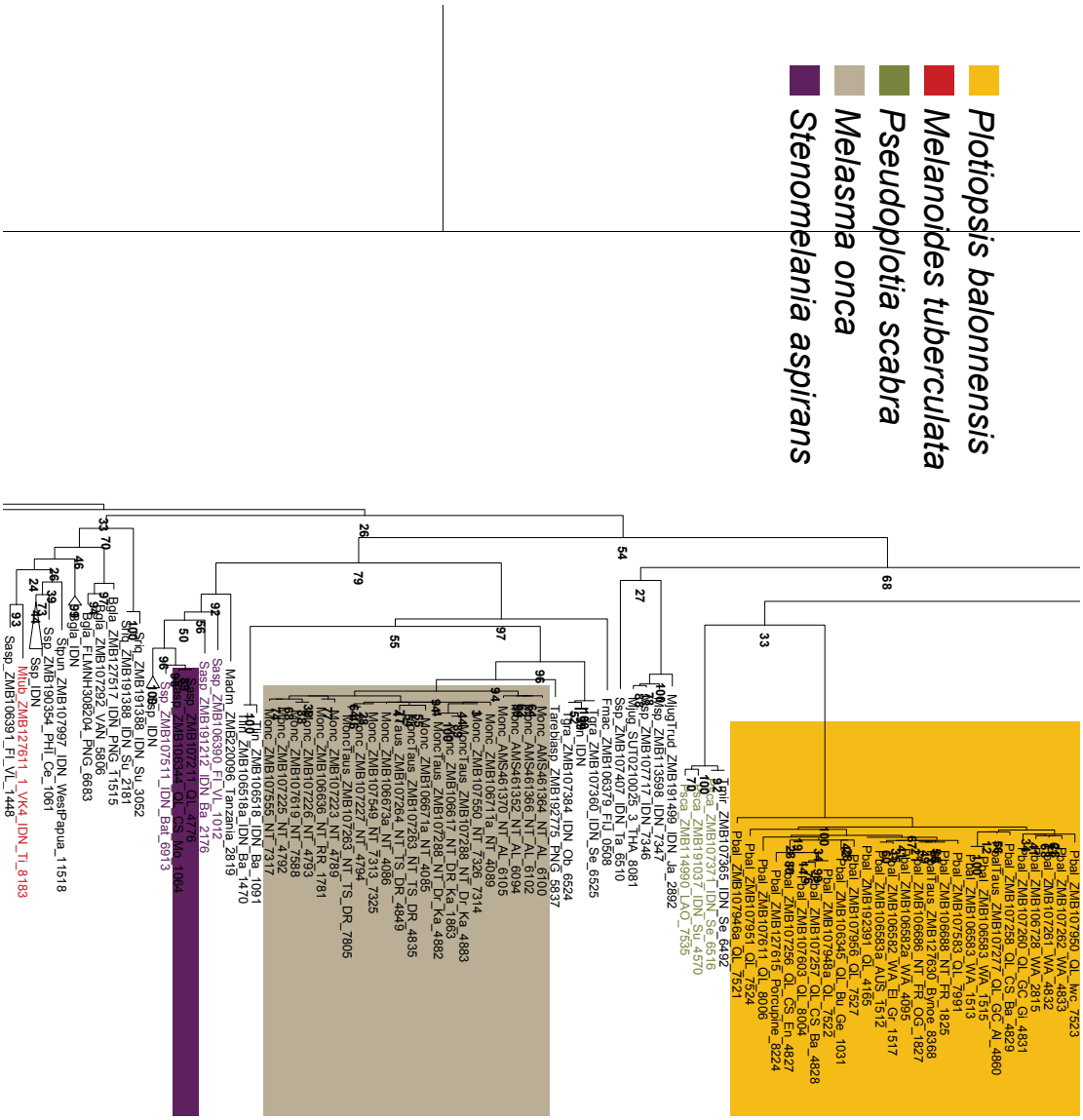
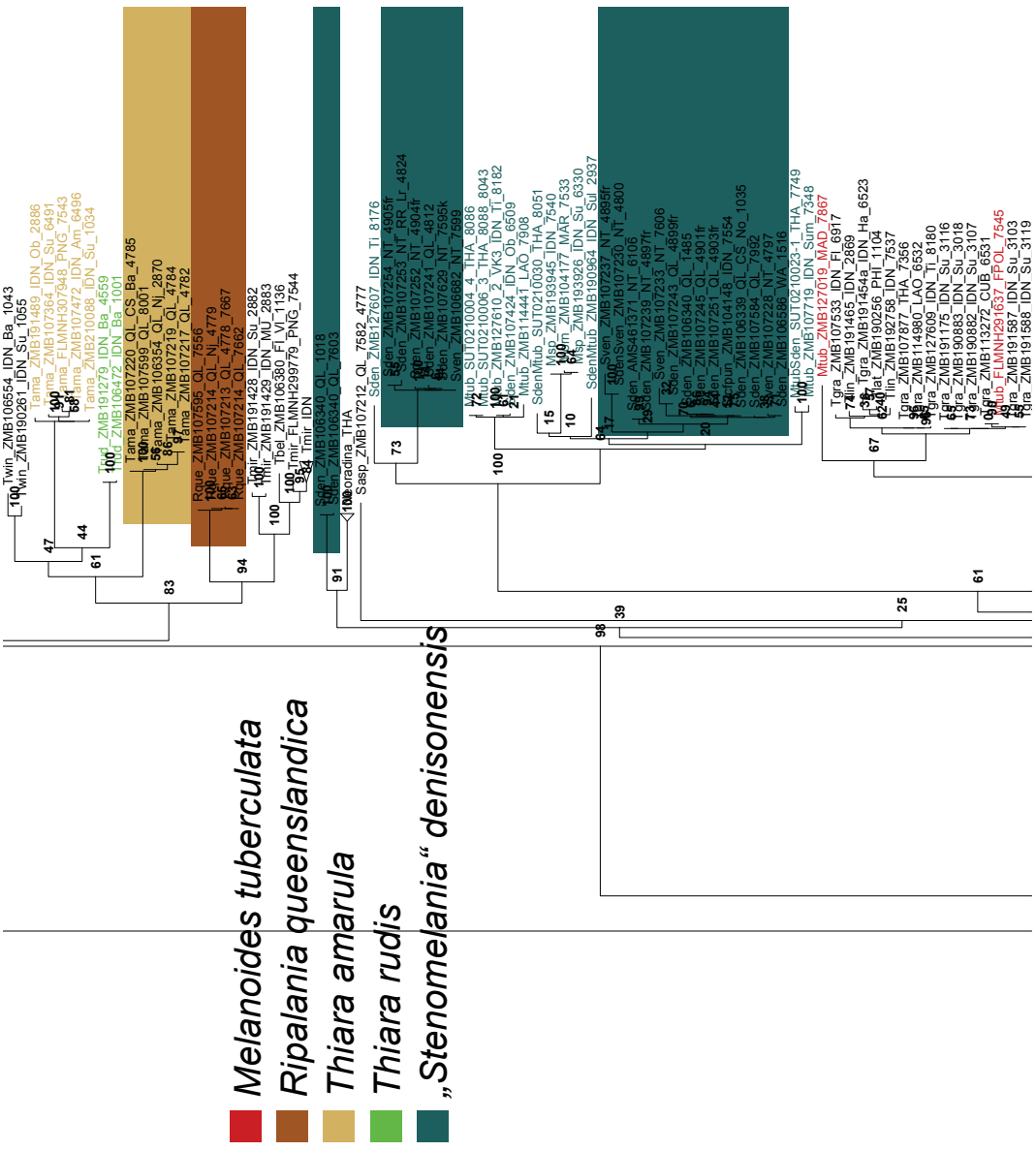


Figure 55 Phylogenetic tree reconstruction by maximum likelihood based on 16S sequences of the hDNA dataset conducted with treefinder (Jobb et al., 2004). Numbers on nodes indicate bootstrap support of the shown topology. Historical samples begin with 'SC'. For abbreviations in taxa names see appendix. Four and five-digit numbers represent extraction numbers, numbers with prefix letter code museum numbers.


Sermyla venustula
Melanoides tuberculata
Pseudoplotia scabra


Plotiopsis balonnensis
Melanoides tuberculata
Pseudoplotia scabra
Melasma onca
Stenomelania aspirans


Melanoides tuberculata
Ripalania queenslandica
Thiara amarula
Thiara rudis
„Stenomelania“ denisonensis

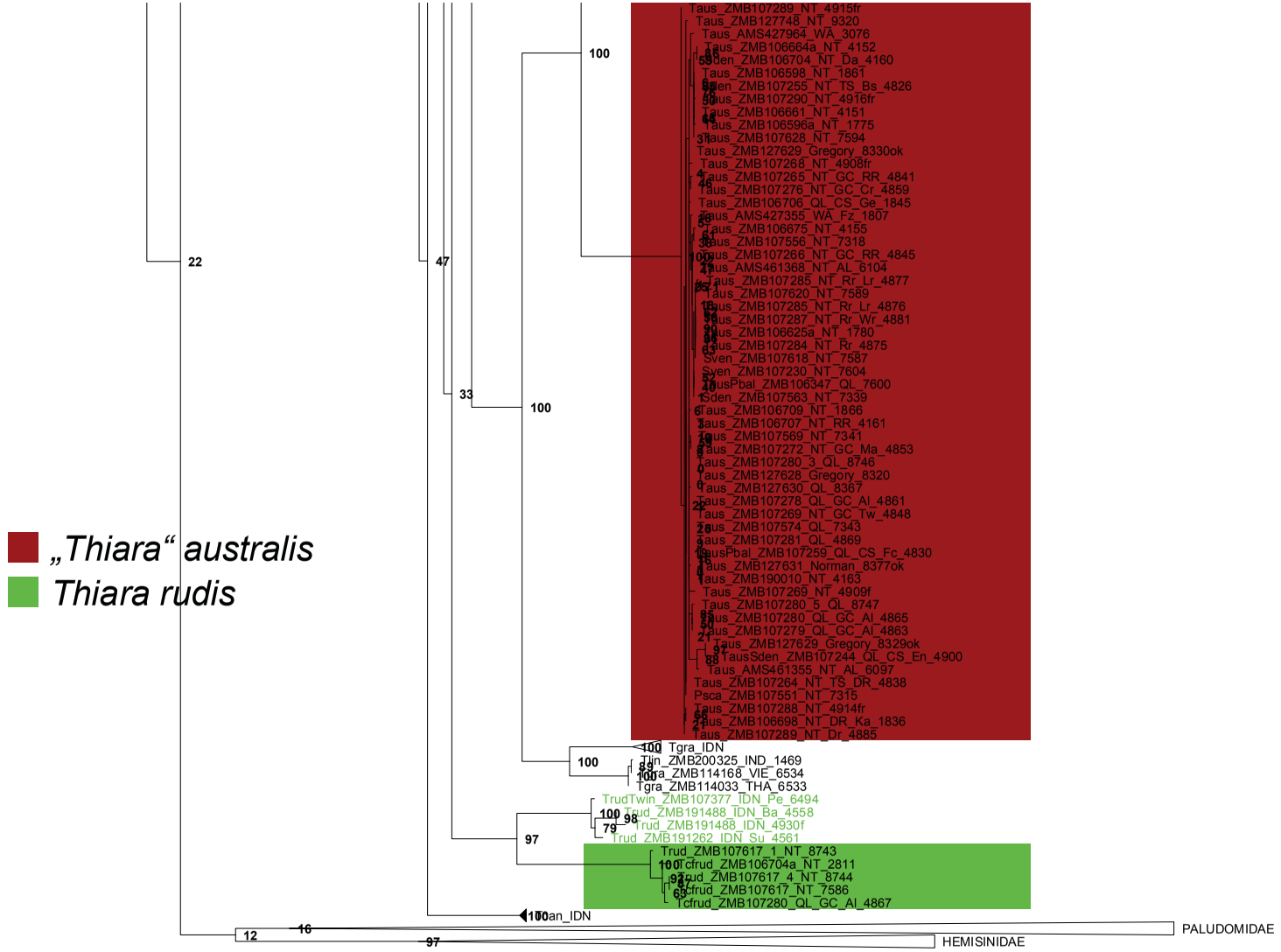


Figure 57 Phylogenetic tree reconstruction by maximum likelihood based on 16S sequences of the haplotype reduced dataset conducted with RAxML (Stamatakis et al., 2008)- to compare with fig. 13 in chapter 4. Numbers on nodes indicate bootstrap support of the shown topology. Terminal labels correspond to the taxon to which the collector assigned each specimen, based primarily on external appearance, and not necessarily to the appropriate taxon.

D AFLP Analyses

Table 26 Mastermix and reaction volume for restriction and ligation

reagent	**volume for 1 reaction**
ddH_2O	3.8 μl
T4 DNA ligase buffer (10x)	1.1 μl
BSA diluted (1mg/ml)	0.55 μl
NaCl (1M)	0.55 μl
MseI adaptor pair kit (50pm/μl)	1.0 μl
EcoRI adaptor pair kit (5pm/μl)	1.0 μl
MseI enzyme (NEB R0525S, 1U)	0.1 μl
EcoRI enzyme (NEB R0101T, 5U)	0.05 μl
T4 DNA ligase (NEB M0202S)	0.1 μl
sum	8.25 μl
DNA (50ng/μl)	2.75 μl
reaction volume	11.0 μl

Table 27 Mastermix and reaction volume for preselective amplification

reagent	**volume for 1 reaction**
ddH_2O	8.85 μl
dNTP (1:4)	2.0 μl
10xPCR buffer	2.0 μl
$MgCl_2$ (25mmol)	2.0 μl
preselective primer pair	1.0 μl
Taq polymerase	0.15 μl
sum	16.0 μl
R/L product	4.0 μl
reaction volume	20.0 μl

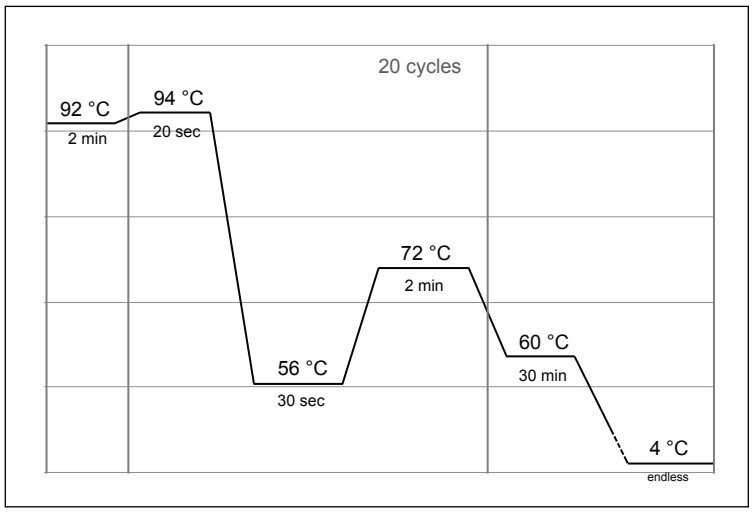


Figure 58 PCR profile for preselective amplification in AFLP analysis

Table 28 Mastermix and reaction volume for selective amplification

reagent	**volume for 1 reaction**
ddH_2O	9.85 μl
dNTPs (1:4)	2.00 μl
10xPCR buffer	2.00 μl
$MgCl_2$ (25mmol)	2.00 μl
MseI selective primer (5μM)	1.0 μl
EcoRI selective primer with dye (1μM)	1.0 μl
Taq polymerase (5U/ μl)	0.15 μl
sum	18 μl
PA product	4 μl
reaction volume	22.0 μl

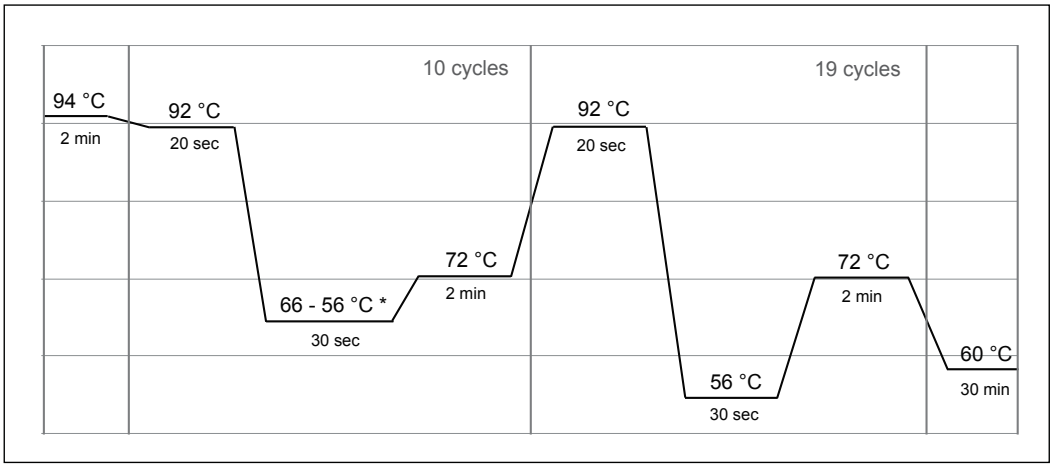


Figure 59 PCR profile for selective amplification; * Reduction in 1 °C steps

Table 29 AMARE output giving an overview of character matrices and quality estimates. Bin and Replicate reliability values are given in percentages. Selected character matrices are indicated in bold.

Bin reliability	Replicate reliability	n°of taxa	n°of markers	n°of characters	Error rate
70.0	0.0 - 80.0	51	678	34578	0.06111
70.0	90.0	36	542	19512	0.06160
71.0	0.0 - 80.0	51	680	34680	0.06038
71.0	90.0	36	547	19692	0.06150
72.0	0.0 - 80.0	51	680	34680	0.06038
72.0	90.0	36	547	19692	0.06150
73.0	0.0 - 80.0	51	682	34782	0.05969
73.0	90.0	36	552	19872	0.06089
74.0	0.0 - 80.0	51	682	34782	0.05969
74.0	90.0	36	552	19872	0.06089
75.0	0.0 - 80.0	51	690	35190	0.05925
75.0	90.0	36	546	19656	0.05881
76.0	0.0 - 80.0	51	690	35190	0.05925
76.0	90.0	36	550	19800	0.05778
77.0	0.0 - 80.0	51	695	35445	0.05829
77.0	90.0	36	550	19800	0.05778
78.0	0.0 - 80.0	51	695	35445	0.05829
78.0	90.0	36	535	19260	0.05317
79.0	0.0 - 80.0	51	695	35445	0.05623
79.0	90.0	37	547	20239	0.05445
80.0	0.0 - 80.0	51	695	35445	0.05623
80.0	90.0	37	547	20239	0.05445
81.0	0.0 - 80.0	51	703	35853	0.05458
81.0	90.0	37	547	20239	0.05405
82.0	0.0 - 80.0	51	703	35853	0.05458
82.0	90.0	37	551	20387	0.05145
83.0	0.0 - 80.0	51	704	35904	0.05239
83.0	90.0	37	551	20387	0.05145
84.0	0.0 - 70.0	51	704	35904	0.05239
84.0	**80.0**	**51**	**704**	**35904**	**0.05239**
84.0	90.0	37	547	20239	0.04674
85.0	0.0 - 80.0	51	702	35802	0.05019
85.0	90.0	37	547	20239	0.04674
86.0	0.0 - 80.0	51	702	35802	0.05019
86.0	90.0	37	547	20239	0.04674

Bin reliability	Replicate reliability	n°of taxa	n°of markers	n°of characters	Error rate
87.0	0.0 - 80.0	51	701	35751	0.04671
87.0	90.0	37	538	19906	0.04165
88.0	0.0 - 80.0	51	701	35751	0.04671
88.0	90.0	37	538	19906	0.04165
89.0	0.0 - 80.0	51	682	34782	0.04244
89.0	90.0	37	538	19906	0.04165
90.0	0.0 - 80.0	51	682	34782	0.04244
90.0	90.0	37	494	18278	0.03201
91.0	0.0 - 80.0	51	623	31773	0.03534
91.0	90.0	37	494	18278	0.03201
92.0	0.0 - 80.0	51	623	31773	0.03534
92.0	90.0	37	441	16317	0.02445
93.0	0.0 - 80.0	51	552	28152	0.02817
93.0	90.0	37	441	16317	0.02445
94.0	0.0 - 80.0	51	552	28152	0.02817
94.0	90.0	37	441	16317	0.02445
95.0	0.0 - 80.0	51	444	22644	0.01974
95.0	90.0	37	335	12395	0.01331

Table 30 AFLP samples. R: Replicate; *sample was included three times. **sample was included twice but did not work as a replicate in AMARE. For other abbreviations see appendix.

Taxa	Museum-Id	Locality		Lab-Id
Balanocochlis glans	ZMB107292	VAN	Santo	5806
Balanocochlis glans	ZMB107493	IDN	Ambon	6506
Hemisinus spec.	ZMB107126	JAM	Middlesex	R2849
Melanoides jugicostris	ZMB127446	THA	Erawan Waterfall	9228
Melanoides jugicostris	ZMB127447	THA	Klong Palian River	9229
Melanoides jugicostris	ZMB191499	IDN	Java	R2892
Melanoides spec.	ZMB190964	IDN	Central Sulawesi	R2937
Melanoides tuberculata	AMS461372	AUS	NT: Arnhem Land	R6107
Melanoides tuberculata	ZMB107125	JAM	Cornwall	2860
Melanoides tuberculata	ZMB107128	JAM	Rio Negro	R2857
Melanoides tuberculata	ZMB107129	JAM	Cornwall	R2855
Melanoides tuberculata	ZMB107180	MAL	Lake Malawi	7632
Melanoides tuberculata	ZMB107193	MAL	Lake Malawi	7623
Melanoides tuberculata	ZMB107194	MAL	Lake Malawi	R7630
Melanoides tuberculata	ZMB107535	FU	Mt. Puke valley	R6918
Melanoides tuberculata	ZMB107536	WAL	Lac Kikila	R6919
Melanoides tuberculata	ZMB107538	FU	Tarodière Nuku	R6920
Melanoides tuberculata	ZMB107875	THA	Wiphawadi Waterfall	7858
Melanoides tuberculata	ZMB127078	MAD	Befandriana	R7876
Melanoides tuberculata	ZMB127442	THA	Khao Thong	R9224
Melanoides tuberculata	ZMB127443	THA	Khao Thong	9225
Melanoides tuberculata	ZMB127511	AUS	WA: Lake Ord	R10421*
Melanoides tuberculata	ZMB127613	AUS	NT: Bitter Springs	R8187
Melanoides tuberculata	ZMB200313	IND	Tamil Nadu	R2820
Melasma onca	ZMB127749	AUS	NT: Daly River	9322
Melasma onca	ZMB127756	AUS	NT: Salt Creek	9331
Neoradina spec.	ZMB107867	THA	West of Krabi	7528**
Paludomus petrosus	ZMB107881	THA	Pranburi River	R7336
Paludomus siamensis	ZMB107721	THA	Sai Yok Yai NP	R7334
Paludomus siamensis	ZMB107909	THA	Ban Pa Koh	R7337
Plotiopsis balonnensis	ZMB106582	AUS	WA: Greenough River	R1517*
Plotiopsis balonnensis	ZMB106583	AUS	WA: Murchinson River	R1512
Plotiopsis balonnensis	ZMB106583b	AUS	WA: Murchinson River	R1515
Plotiopsis balonnensis	ZMB106658	AUS	WA: Walyunga Pool	1809
Plotiopsis balonnensis	ZMB106687	AUS	NT: Finke River	R1826
Plotiopsis balonnensis	ZMB106688	AUS	NT: Finke River	R1825
Plotiopsis balonnensis	ZMB107583	AUS	QL: Barron River	R7991
Plotiopsis balonnensis	ZMB107603	AUS	QL: Salt Water Creek	R8004
Plotiopsis balonnensis	ZMB107611	AUS	QL: Porcupine Creek	8006
Plotiopsis balonnensis	ZMB107946	AUS	QL: Brisbane River	8025
Plotiopsis balonnensis	ZMB107946	AUS	QL: Brisbane River	8027
Plotiopsis balonnensis	ZMB107946	AUS	QL: Brisbane River	8026
Plotiopsis balonnensis	ZMB107948	AUS	QL: South Maroochy River	8039
Plotiopsis balonnensis	ZMB107948	AUS	QL: South Maroochy River	7522
Plotiopsis balonnensis	ZMB107951	AUS	QL: Euri Creek	9333
Plotiopsis balonnensis	ZMB107951	AUS	QL: Euri Creek	9334
Plotiopsis balonnensis	ZMB107951	AUS	QL: Euri Creek	7524
Plotiopsis balonnensis	ZMB107956	AUS	QL: Broken River	7527
Plotiopsis balonnensis	ZMB127507	AUS	WA: De Grey River	10415
Plotiopsis balonnensis	ZMB127507	AUS	WA: De Grey River	10416
Plotiopsis balonnensis	ZMB127509	AUS	WA: Millstream Creek	10417
Plotiopsis balonnensis	ZMB127509	AUS	WA: Millstream Creek	10418
Pseudoplotia cf. *acanthica*	ZMB191487	IDN	Cenral Sulawesi	2885
Pseudoplotia scabra	ZMB107216	AUS	NT: Daly River	4781

Taxa	Museum-Id	Locality		Lab-Id
Pseudoplotia scabra	ZMB107392	IDN	Ambon	R6511
Pseudoplotia scabra	ZMB127775	AUS	QL: Gregory River	9324
Pseudoplotia scabra	ZMB191264	IDN	Sulawesi	2199
Ripalania queenslandica	ZMB107214	AUS	QL: North Johnston River	R7663*
Ripalania queenslandica	ZMB107595	AUS	QL: Daintree River	R7556*
Sermyla riquetii	ZMB106474	IDN	South Bali	1027
Sermyla riquetii	ZMB191388	IDN	South Sulawesi	R2181
Sermyla venustula	ZMB106713	AUS	QL: Norman River	R2859
Sermyla venustula	ZMB127650	AUS	NT: Elsey Creek	R8429
Sermyla venustula	ZMB127654	AUS	NT: Towns River	8447
Sermyla venustula	ZMB127660	AUS	NT: Howard Springs	R8698
Stenomelania aspirans	ZMB107211	AUS	QL: Granite Creek	4776
Stenomelania aspirans	ZMB191212	IDN	South Bali	R2176
"Stenomelania" denisoniensis	ZMB106586	AUS	WA: Ellendale Pool	R1516
"Stenomelania" denisoniensis	ZMB107449	IDN	Ambon	6507
"Stenomelania" denisoniensis	ZMB107584	AUS	QL: Barron River	7992
"Stenomelania" denisoniensis	ZMB127607	IDN	Timor	8176
"Stenomelania" denisoniensis	ZMB127783	AUS	NT: Bitter Springs	9329
"Stenomelania" cf. *denisoniensis*	ZMB127796	AUS	NT: Towns River	9328
Stenomelania spec.	ZMB107457	IDN	Seram	6539
Stenomelania spec.	ZMB107460	IDN	Obi	6540
Stenomelania spec.	ZMB107483	IDN	Ambon	R6508
Tarebia granifera	ZMB107360	IDN	Seram	6525
Tarebia granifera	ZMB107367	IDN	Ambon	6518
Tarebia granifera	ZMB107384	IDN	Obi	6524
Tarebia granifera	ZMB107396	IDN	Ambon	R6521
Tarebia cf. *granifera*	ZMB127445	THA	Erawan Waterfall	R9227
Tarebia granifera	ZMB127609	IDN	Timor	8180
Tarebia granifera	ZMB191458	IDN	Central Sulawesi	2866
Tarebia lineata	ZMB106518	IDN	South Bali	R1091
Tarebia lineata	ZMB106518	IDN	South Bali	R1526*
Tarebia lineata	ZMB191207	IDN	South Bali	2856
Thiara amarula	ZMB107364	IDN	Central Sulawesi	R6491
Thiara amarula	ZMB107585	AUS	QL: Mowbraw River	R7993
Thiara amarula	ZMB107599	AUS	QL: Daintree River	8001
"Thiara" australis	ZMB106698	AUS	NT: Kathrine River	R1836
"Thiara" australis	ZMB106706	AUS	QL: Gregory River	R1845
"Thiara" cf. *australis*	ZMB107277	AUS	QL: Gregory River	4860
"Thiara" australis	ZMB106709	AUS	NT: Blueys Creek	R1866
"Thiara" australis	ZMB107282	AUS	QL: Gilbert River	4870
"Thiara" australis	ZMB107286	AUS	NT: Little Roper River	4878
"Thiara" australis	ZMB107574	AUS	QL: O'Shanassy River	R7343
"Thiara" australis	ZMB107576	AUS	QL: Gregory River	9336
"Thiara" australis	ZMB107579	AUS	QL: Bynoe River	7990
"Thiara" australis	ZMB127505	AUS	WA: Fitzroy River	R10411*
"Thiara" australis	ZMB127505	AUS	WA: Fitzroy River	10412
"Thiara" australis	ZMB127510	AUS	WA: Fitzroy River	10413
"Thiara" australis	ZMB127510	AUS	WA: Fitzroy River	10414
"Thiara" australis	ZMB127728	AUS	NT: Towns River	9326
"Thiara" australis	ZMB127736	AUS	NT: Howard Springs	9318
"Thiara" australis	ZMB127748	AUS	NT: Daly River	9320
Thiara cancellata	ZMB107489	IDN	Central Sulawesi	R6498
Thiara cancellata	ZMB191431	IDN	Obi	2817
Thiara mirifica	ZMB107473	IDN	Ambon	6497
Thiara mirifica	ZMB191270	IDN	Southeast Sulawesi	R2881
Thiara rudis	ZMB106704	AUS	NT: Berry Springs	2811
Thiara rudis	ZMB107614	AUS	NT: Roper River	R7583
Thiara rudis	ZMB107617	AUS	NT: Roper River	R7586

Taxa	Museum-Id	Locality		Lab-Id
Thiara rudis	ZMB127616	AUS	NT: Berry Springs	8198
Thiara rudis	ZMB127619	AUS	NT: Salt Creek	8201
Thiara rudis	ZMB127620	AUS	NT: Roper River	R8202

E Historical DNA sample list

Table 31 List of all samples included in the hDNA approach covering different ages, storage conditions and tissue types.

Museum-Id	Species	Date	Location	Tissue	Storage	Lab no.	PCR band	sequenced
ZMB 107.320-1	*Thiara spec.*	6/2010 (fresh)	Aquarium MfN	foot tissue	ethanol	**SC1**	S4&S5	
ZMB 107.320-2	*Thiara spec.*	6/2010 (fresh)	Aquarium MfN	shell fragment	ethanol	**SC2**	S4&S5	
ZMB 113.346-3	*Planorbis sudanicus*	1907	Rwanda, Mohasi	dry shell fragment	dry	**SC3**	S4&S5	
ZMB 107.840	*Tarebia granifera*	2009	Bougainville Island	complete shell with operculum & body	dry	**SC4**	S4&S5	
ZMB 210.034	*Thiara coacta*	unknown	unknown	unknown	dry storage	**SC5**	S4&S5	
ZMA w/o no.	*Balanocochlis glans*	1903	New Guinea, Mamapiri	foot tissue	ethanol	**SC6**	S4&S5	S4 & S5
ZMB 45.841-1	*Thiara mitra*	1901-1903	Tanzania, Sigi River	soft body	dry	**SC7**	S4&S5	
ZMB 45.841-2	*Thiara mitra*	1901-1903	Tanzania, Sigi River	operculum		**SC8**	S4&S5	
ZMA w/o no.	*Balanocochlis glans*	1948	New Guinea, Sorong	foot tissue		**SC9**	S4&S5	
ZIM 3948-1	*Neoradina prasongi*	1968	Thailand, Kao Tong	foot tissue		**SC10**	S4&S5	S4 & S5
ZIM 3948-3	*Neoradina prasongi*	1968	Thailand, Kao Tong	foot tissue		**SC11**	S4&S5	S4 & S5
WAM 465-80	*Thiara balonnesis*	1979	Australia, Ellendale	foot tissue		**SC12**	S4&S5	
USNM 859456	*Simulathena papuensis*	1970	New Guinea, Yule Island	foot tissue		**SC13**	only S5	S5
ZMB 191.126	*Pachymelania byronensis*	1995	Nigeria, Lagos	foot tissue		**SC14**	only S4	
ZMB 31.148	*Cleopatra bulimoides*	19th century	Egypt, Medinet	foot tissue		**SC15**	S4&S5	
ZMB 220.064	*Cleopatra ferruginea*	1990	Kenya, Kinango	foot tissue		**SC16**	S4&S5	
MRAC w/o no.	*Cleopatra obscura*	1960s (?)	Africa	foot tissue		**SC17**	S4&S5	
DBL w/o no.	*Cleopatra senegalensis*	1960	Ghana	entire shell with animal inside		**SC18**	S4&S5	
NHMW 78.868	*Paludomus negritoides*	1970	Ceylon, Ganga	tissue		**SC19**	/	
MRAC 145-1	*Potadomoides pelseneeri*	1951	Lake Tanganyika	shell fragment		**SC20**	/	
MRAC 341.980	*Potadomoides bequaerti*	1907	Congo River	foot tissue		**SC21**	only S4	
MRAC w/o no.	*Pseudocleopatra dartevellei*	1960s (?)	Africa	entire shell with animal inside		**SC22**	/	
MRAC 47.812	*Potadomoides schoutedeni*	1907	Congo River	foot tissue	dry	**SC23**	/	
DBL	*Tanganciyia rufofilosa*	1957	Lake Tanganyika	foot tissue		**SC24**	S4&S5	
MZB 12.300-1	*Paludomus sp.*	1980	Indonesia, Lombok	foot tissue		**SC25**	only S4	S4
ZMB 86812-1	*Ripalania queenslandica*	1937	Papua New Guinea, Manus	operculum	dry	**SC26**	S4&S5	S4 & S5
ZMB 87263-1	*Ripalania queenslandica*	1937	Papua New Guinea, Ramu estuary	dried shells	dry	**SC27**	S4&S5	S4 & S5
ZMB 87264-1	*Ripalania queenslandica*	1937	Papua New Guinea, Sattelberg	dried shell	dry	**SC28**	S4&S5	S4 & S5
NHM 57038	*Paludomus isseli*	1956	British North Borneo	foot tissue		**SC39**	/	
ZMH 59339	*Paludomus ajanensis*	unknown	Seychelles	dried shell	dry	**SC61**	(S4)	
ZMB 41335-1	*Stomatodon stomatodon*	unknown	Brit. Indien	dried shell	dry	**SC62**	(S5)	
ZMB 114615-3	*Philopotamis sulcatus*	unknown	Sri Lanka	operculum		**SC63**	/	
ZMB 109591	*Paludomus subfasciatus*	1902	Borneo, Sungei Guleh	operculum		**SC64**	/	
NHMW 6-1	*Tanalia aculeata*	unknown	Sri Lanka	dried shell		**SC65**	/	

F Abbreviations

%	percentage
°C	degree Celsius
μ	micro
AFLP	amplified fragment lenght polymorphism
AMARE	AFLP matrix reduction
AMOVA	analysis of molecular variance
AMS	Australian Museum, Sydney
ANSP	Academy of Natural Sciences, Philadelphia
AUS	Australien
Bgla	*Balanocochlis glans*
BMNH	The Natural History Museum, London (formerly British Museum Natural History)
bp	base pair
BT	bootstrap
C	Cytosine
ca.	circa
Carp	Gulf of Carpenteria
CAS	California Academy of Sciences, San Francisco
Cbre	*Cubaedomus brevis*
Cbul	*Cleopatra bulimoides*
Cdec	*Cerithidea decollata*
Cdja	*Cerithideopsilla djadjariensis*
Cebu	*Cerithium eburneum*
cf.	confer
Cjoh	*Cleopatra johnstoni*
Clar	*Cerithideopsis largillierti*
COI	cytochrome *c* oxidase subunit I
CTAB	Cetyltrimethyl ammonium bromide
D	data
ddNTP	di-deoxy-Nucleotide-Tri-Phosphate
df	degree of freedom
DFG	Deutsche Forschungsgemeinschaft
DNA	Desoxyribo-Nuclein-Acid
dNTP	deoxy-Nucleotide-Tri-Phosphate
dsDNA	double stranded DNA
Ecat	*Elimia catenaria*
EDTA	Ethylenediaminetetraacetic acid
Eesp	*Esperiana esperi*

Eint	*Elimia interrupta*
et al.	et alii (and others)
fig.	figure
Fmac	*Fijidoma maculata*
G	Guanin
GPS	global positioning system
Grey	Greyian
GTR	general time reversible
Hcub	*Hemisinus cubanianus*
HPLC	high performance liquid chromatography
Hsp	*Hemisinus spec.*
i.e.	id est (that is)
IBD	isolation by distance
Ind Oc	Indian Ocean
ITS	internal transcribed spacer
IZW	Leibniz Institute for Zoo and Wildlife Research
Jard	Jardinian
k	kilo
km	kilometre
Kref	Krefftian
L Eyre	Lake Eyre
l	litre
Leich	Leichhardtian
Less	Lessonian
ln	natural logarithm
M-Dar	Murray-Darling
m	metre
Ma	Mega annum (one million years)
MAFFT	Multiple Alignment using Fast Fourier Transform
MCMC	Markov Chain Monte Carlo
MCZ	Museum of Comparative Zoology, Harvard University
MFL	minimum fragment length
$MgCl_2$	magnesium chloride
MHNG	Musee d'Histoire Naturelle de Geneve
Mitch	Mitchellian
ml	millilitre
Monc	*Melasma onca*
Mpra	*Melanopsis praemorsa*
Msp	*Melanoides spec.*

mt	mitochondrial
Mtub	*Melanoides tuberculata*
MUSCLE	MUltiple Sequence Comparison by Log- Expectation
MZB	Museum Zoologicum Bogoriense
Ne Coast	North-East Coast
ngen	number of generations
NHMB	Naturhistorisches Museum, Basel
no.	number
Nsp	*Neoradina spec.*
NSW	New South Wales
NT	Northern Territory
NTM	Northern Territory Museum, Darwin
OUT	outgroup
p	probability
PA	preselective amplification
Paca	*Pseudoplotia acanthica*
Pbal	*Plotiopsis balonnensis*
Pbyr	*Pachymelania byronensis*
PCA	principal components analysis
PCR	polymerase chain reaction
Pfus	*Pachymelania fusca*
PHT	peak height threshold
Ppet	*Paludomus petrosus*
Psca	*Pseudoplotia scabra*
Psia	*Paludomus siamensis*
QLD	Queensland
QM	Queensland Museum, Brisbane
RAxML	randomized axelerated maximum likelihood
rfu	relative fluorescence units
RL	restriction and ligation
rpm	rounds per minute
Rque	*Ripalania queenslandica*
SA	South Australia
Sasp	*Stenomelania aspirans*
Sden	*Stenomelania denisoniensis*
Se Coast	South-East Coast
sec	second
Slib	*Semisulcospira libertina*
SMF	Senckenbergmuseum, Frankfurt am Main

sp.	unspecified
Sriq	*Sermyla riquetii*
Ssp	*Stenomelania spec.*
Sturt	Sturtian
SUT	Silapakorn University Thailand
Sven	*Sermyla venustula*
Sw Coast	South-West Coast
tab.	table
Tama	*Thiara amarula*
Taus	*Thiara australis*
Taq	*Thermus aquaticus*
Tcan	*Thiara cancellata*
TE buffer	Tris-EDTA buffer
Tfus	*Tympanotonus fuscatus*
Tgra	*Tarebia granifera*
Tim S	Timor Sea
Tlin	*Tarebia lineata*
Tmir	*Thiara mirifica*
Tpal	*Terebralia palustris*
Tris	tris(hydroxymethyl)aminomethane
Trud	*Thiara rudis*
Ttel	*Telescopium telescopium*
Twin	*Thiara winteri*
UPGMA	unweighted pair group method with arithmetic mean
USNM	National Museum of Natural History, Washington, D.C. (formely United States National Museum)
UV	ultraviolet
V	Volt
VK	private collection of Vince Kessner, Adelaide River
Vlam	Vlaminghian
WA	Western Australia
WAM	Western Australian Museum, Perth
ZMB	Museum für Naturkunde, Berlin (formerly Zoologisches Museum Berlin)
ZMUC	Zoologisk Museum University, Copenhagen
Ztri	*Zemelanopsis trifasciata*