Biological Conservation 169 (2014) 100–108

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Conservation genetics of Australasian sailfin lizards: Flagship species threatened by coastal development and insufficient protected area coverage

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ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form 14 October 2013
Accepted 23 October 2013

Keywords:
Black markets
Coastal forests
Conservation
Ecological niche
Hydrosaurus
Mangroves
Phyloforensics

ABSTRACT

Despite rampant coastal development throughout Southeast Asia and the Pacific, studies of conservation genetics and ecology of vulnerable, coastal species are rare. Large bodied vertebrates with highly specialized habitat requirements may be at particular risk of extinction due to habitat degradation and fragmentation, especially if these habitats are naturally patchily distributed, marginal, otherwise geographically limited, or associated in space with high human population densities or heavy anthropogenic disturbance. Particularly telling examples of these conservation challenges are large Australasian reptiles with obligate habitat requirements for lowland, coastal and mangrove forests. Plagued by habitat destruction due to high human densities along coastlines, sprawling rural development, and rapidly developing estuarine fisheries industry, coastal forest reptiles are experiencing rapid declines. And yet studies of population biology, genetics, and habitat requirements of species depending on these environments are few. We undertook the present study in order to take a multifaceted approach to understanding a poignant conservation problem. We identify significant evolutionary units for conservation in large-bodied sailfin lizards (genus *Hydrosaurus*), model suitable habitat in the Philippines from extensive occurrence data and evaluate the efficacy of the current protected area network, and identify the source of hydrosaurs in the illegal pet trade. We determine that the extent of the species' habitat coincident with protected areas is low. Our forensic evaluation of the illegal pet trade in the Philippines determines the existence of a natural population that is at risk of systematic exploitation by traders. Together, this integrative study characterizes a conservation urgency of particular significance: the genetically distinct sailfin lizards of the Bicol faunal region, with suitable habitat virtually unprotected, and clear evidence of heavy exploitation for illegal trade. To the best of our knowledge, our study is the first conservation genetic study to evaluate the potential effectiveness of the protected landscape coverage in the Philippines, a Megadiverse nation and Biodiversity Hotspot.

1. Introduction

Protection of species with obligate requirements of specific, patchily distributed, marginal, or heavily fragmented habitats represents a special challenge for government agencies, wildlife managers, and conservation biologists. Species unable to survive outside of very specific or highly restricted ecological conditions are, by definition, immediately imperiled if their required habitats are altered, degraded, or destroyed by the activities of humans. Celebrated examples of taxa threatened—or even driven to extinction—by anthropogenic destruction of their required habitats include Proboscis Monkeys (Jeffrey, 1982), Northern Spotted Owls (Franklin et al., 2000), Dugongs (Preen and Marsh, 1995), and Ivory Billed Woodpeckers (Schock, 2005).

Lowland and coastal forests, and the species that depend on them, represent poignant conservation challenges of particular concern (Mumby et al., 2004; Millenium Ecosystem Assessment, 2005; Duke et al., 2007), and nowhere is this concern more evident than in the highly degraded littoral zone mangroves and beach or coastal forests of Asia and islands of the Pacific (for review: Polidoro et al., 2010). Throughout the edge of the Asian continent and the adjacent Australasian archipelagoes, coastal and mangrove forests have been systematically cleared for brackish water aquaculture ponds, firewood and timber, or residential and urban development (e.g., Walters, 2000; Blasco et al., 2001). Globally, mangrove biodiversity is highest in the Australasian region (Polidoro et al., 2010), yet in some countries, mangrove and dry

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http://dx.doi.org/10.1016/j.biocon.2013.10.014

coastal forest removal has been near complete (Primavera et al., 2004; Walters, 2004; Samson and Rollon, 2008).

At the same time, the critical importance of coastal forests has been well documented (Millenium Ecosystem Assessment, 2005; Duke et al., 2007; Polidoro et al., 2010). Mangroves and beach forests are recognized to possess a unique flora, which acts at the critical interface between terrestrial, estuarine, and near-shore marine ecosystems (Polidoro et al., 2010). Coastal forests and mangroves buffer against erosion, protect inland communities from damage by inclement weather (Dahdouh-Guebas et al., 2005; Barbier et al., 2008; Das and Vincent, 2009), and represent critical habitat for terrestrial, estuarine and marine species (Robertson and Duke, 1987; Luther and Greenburg, 2009). Finally, mangroves serve as source and sink for nutrients and sediments for inshore marine habitats (i.e., seagrass beds and coral reefs; Dorenbosch et al., 2004; Duke et al., 2007).

In some archipelago nations, the near complete loss of coastal forests may have already occurred. For example in the Philippines, in just the last 75 years, the destruction of the coastal forests and mangroves has been extensive (Primavera, 2000; Walters, 2000, 2004; Primavera et al., 2004; Lewis, 2005; Samson and Rollon, 2008). The archipelago has lost more than 75% (>337,000 ha) of original mangrove forests (Baconguis et al., 1990; Walters, 2000; Samson and Rollon, 2008), with >66% (or 278,657 ha) lost between 1950 and 1990 (Primavera, 2000). The elimination of this unique coastal ecosystem is primarily attributed to urbanization along the coasts and construction of brackish water fishponds (~60%; Samson and Rollon, 2008) and yet its impacts are largely unknown. Despite the extensive documentation of the effects of habitat loss in inland ecosystems (for review: Posa and Sodhi, 2006; Rickart et al., 2011), few, if any, studies have measured the direct effects of habitat destruction on coastal forest obligate species. Due to the paucity of such studies, there is an immediate need for attention to coastal and mangrove forest obligate species in studies of species persistence, habitat availability and suitability, exploitation, and studies of genetic variation in obligate resident species (e.g., conservation genetics).

One such group for which almost no information is known for conservation status assessment is the spectacularly colorful and morphologically distinctive sailfin lizards of the genus *Hydrosaurus*; *Hydrosaurus pustulatus* is already considered vulnerable (A2acd + 4acde; IUCN, 2012) as a result of habitat loss. Sailfin lizards have a Wallacean distribution, occurring from Sulawesi and the Philippines to Papua New Guinea (Fig. 1B), and inhabit vegetation and coastal forests immediately adjacent to beaches, or surrounding low-lying rivers (less than a few hundred meters in elevation), on the banks of estuarine areas, deltas, river mouths, and in mangroves. Tightly linked to riparian habitats in lowland coastal and mangrove forests, hydrosaurs occur in moderate to high densities in suitable habitats but are conspicuously absent when their specific habitats are removed (*personal observations*). With their required habitat severely threatened, sailfin lizards represent an urgent need for conservation genetics studies.

Two additional concerns accentuate the conservation urgency for members of the genus *Hydrosaurus*. First, the taxonomy of the group is unresolved and has never been the subject of a systematic study using genetic data or modern phylogenetic methods. Originally described in the 1800s, the species' original descriptions are brief, based on specimens that were immature at the time of original preservation, and are non-diagnostic by modern standards, further hindering efforts to determine species boundaries and taxonomic management units. Finally, with their striking dorsal crests, conspicuous caudal sail-like structure, and brightly colored, aesthetically pleasing ornate coloration (Fig. 1A), hydrosaurs are heavily exploited in the local and international pet trade (Gaulke, 1998; Welton et al., 2012, 2013a; E. Sy, *personal communication*). This exploitation creates a potentially exacerbating situation given the already tenuous prospects for continued survival of these species.

We undertook the present study in order to provide more information for conservation planning and to characterize the conservation status and urgency for these largely unstudied species of suspected high conservation importance. We (1) screened multilocus genetic data for divergent lineages (likely conservation targets)

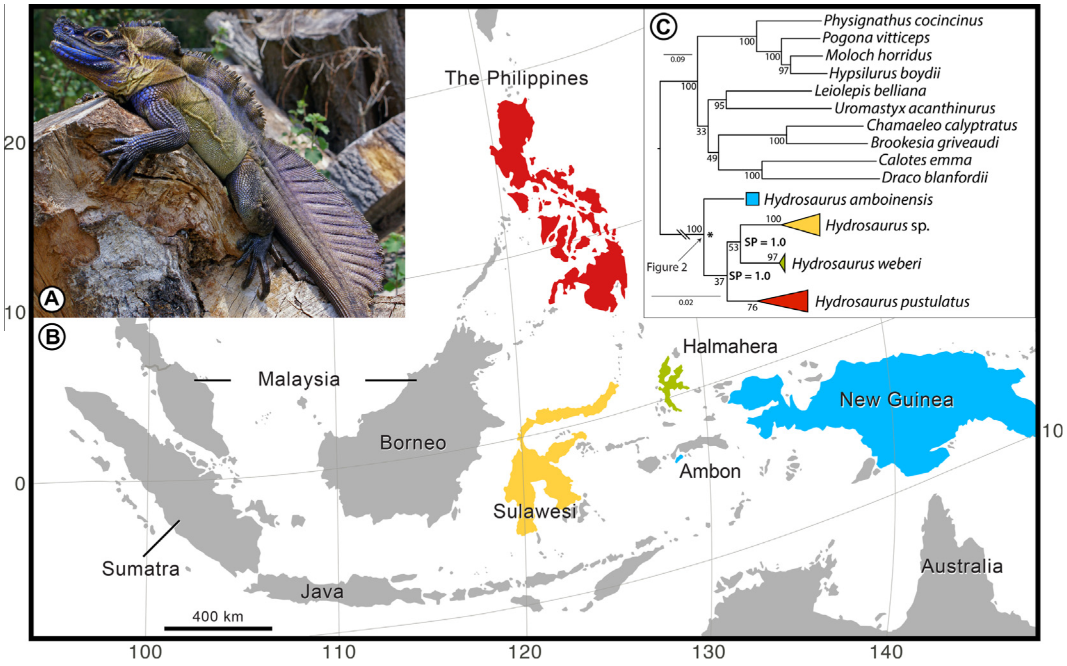

Fig. 1. (A) Photograph of *Hydrosaurus* sp. provided by Scott Corning. (B) The known distribution of sailfin lizards. (C) Simplified phylogenetic estimate inferred from the analysis of one mitochondrial and three nuclear genes, and summary of Bayesian species delimitation (BPP) analyses for the genus *Hydrosaurus* (with BPP speciation probability values on representative nodes). No nuclear data are available for the unvouchered sample of *Hydrosaurus amboinensis*, and therefore, this species was not included in BPP analyses (node highlighted with asterisk). *Hydrosaurus* sp. refers to the sampled population on Sulawesi.

and determine whether these correspond to currently recognized species (*Hydrosaurus amboinensis, H. pustulatus,* and *H. weberi*), and we (2) assessed the distribution of suitable habitat and ecological requirements for *H. pustulatus* in the Philippine archipelago and determined the extent of this habitat's coincidence with protected areas. Finally, we (3) forensically evaluated the illegal pet trade in Manila to determine which natural populations may be at risk of systematic exploitation by black market traders. The results of this pluralistic, multi-tiered approach: (i) clarified putative species boundaries (clear conservation targets, including one lineage from Sulawesi not currently recognized by taxonomists and conservation biologists) throughout the range of the genus *Hydrosaurus*, (ii) quantitatively defined habitat suitability measures for Philippine hydrosaurs (*H. pustulatus*), (iii) quantified status of the species' imperiled habitat (mangroves, coastal forests, and low-elevation riparian corridors) showing suitable environments to be minimally coincident with existing protected areas, and (iv) identified one highly significant, presumably heavily exploited, population (sailfin lizards of the Bicol Peninsula) as the sole source of trade animals sampled from Manila black markets. The integration of these combined results identifies a clear conservation priority (the Bicol region of the Philippines) where law enforcement and conservation specialists could make tremendous strides towards stemming black market trade in Philippine reptiles while taking field-based steps to ensure future survival of these charismatic endemic vertebrates.

2. Methods

2.1. Data collection

Ingroup sampling of wild-caught animals included data for 78 vouchered and 1 non-vouchered individual representing all three currently recognized species of *Hydrosaurus* and 20 individuals sampled from skin and muscle biopsies (from tail tips or crests) from animals in the black market pet trade during five visits from 2011 to 2012 (Appendices S1, S2; Supporting Information). For 81 ingroup samples we sequenced the mitochondrial genes NADH dehydrogenase subunit 2 (*ND2*) and Cytochrome Oxidase I (*COI*), along with their flanking tRNAs. We also sequenced three nuclear loci (nuDNA): two anonymous loci (*L3*,*L52*), and the diacylglyceral lipase alpha (*DGL-∝*) gene (Alföldi et al., 2011; Welton et al., 2013b; Appendix S2; Supporting Information). Additionally, sequence data were available on GenBank for one individual of *H. amboinensis* (*ND2* and *COI*; Okajima and Kumazawa, 2010), eight individuals of *H. weberi* (*COI*; Setiadi et al., 2010), and 10 species used as outgroups (ND2; Appendix S1). All novel sequences were deposited in GenBank (Supporting Information). Primer information, and polymerase chain reaction (PCR) and sequencing protocols are provided in Appendix S2.

2.2. Phylogenetic and population structure analyses

Alignments were made in Se-Al Sequence Alignment Editor (v2.0a11; Rambaut, 2002). Preliminary analyses exploring the impact of missing data on inferred outgroup relationships resulted in identical relationships inferred with and without the inclusion of nuclear data. We therefore chose to include all available data for subsequent analyses. Additionally, following the observation of no strongly supported incongruence between datasets, we conducted subsequent phylogenetic analyses using a combined, partitioned, concatenated dataset. The two datasets, *ND2* and *COI*, were partitioned by codon position, the tRNAs were treated as a single subset, and each of the three nuclear loci, *L3*, *L52,* and *DGL-∝* were analyzed as a single subset. The Akaike information criterion (AIC), as implemented in jModelTest (v2.1.3; Posada, 2008), was used to select the best model of nucleotide substitution for each partition (Table 1).

Table 1
Models of evolution selected by AIC (as implemented in jModelTest) for mitochondrial (*ND2*,*COI*) and nuclear (*L3*,*L52*,*DGL-∝*) data.

Partition	AIC model	Number of characters	Number of variable characters
ND2[a]	GTR + Γ	1035	735
COI[a]	HKY	577	60
tRNAs[b]	HKY + Γ	418	65
L3	F81	281	1
L52	HKY	516	3
DGL-∝	HKY + Γ	480	5

[a] Individual gene codons selected with identical AIC models selected by jModelTest.

[b] tRNAMet, tRNA$^{\mathrm{Trp}}$, tRNA$^{\mathrm{Ala}}$, tRNA$^{\mathrm{Asn}}$, tRNA$^{\mathrm{Cys}}$, and tRNA$^{\mathrm{Tyr}}$.

Partitioned Maximum Likelihood (ML) analyses were conducted in RAxMLHPC v7.0 (Stamatakis, 2006). The more complex model (GTR + I + Γ) was used for all subsets (Table 1), and 100 replicate ML inferences were performed for each analysis. Each inference was initiated with a random starting tree and nodal support was assessed with 100 bootstrap pseudoreplicates (Stamatakis et al., 2008) Alignments and resulting topologies are deposited in Dryad (doi: http://doi.org/10.5061/dryad.1fs7c).

We employed the NeighborNet algorithm (Bryant and Moulton, 2004) in the program SplitsTree (v4.10; Huson and Bryant, 2006) to generate phylogenetic networks for the mitochondrial and concatenated nuclear datasets, respectively. To assess the support for inferred splits in the network, a bootstrap analysis was conducted with 1000 replicates. An analysis of molecular variation (AMOVA) was conducted on sequence data for *H. pustulatus* to investigate the amount of genetic variation explained at different resolutions of geography. All analyses were run with 1000 permutations in Arlequin (v3.5.1.3; Excoffier et al., 2005). The analysis was conducted to elucidate the amount of genetic variation that could be explained by recognized faunal regions versus by islands within faunal regions.

2.3. Species delimitation

We tested the monophyly of *H. amboinensis* (as currently recognized) within a Bayesian framework. A topology was created to constrain all samples of *H. amboinensis* and *H.* sp. (Sulawesi) to be monophyletic. We used the proportion of 16,004 postburn-in trees consistent with this hypothesis as an estimate of the posterior probability of the hypothesis.

We explored support for hypothesized species boundaries within *Hydrosaurus* using the program Bayesian Phylogenetics and Phylogeography (BPP v.2.0; Yang and Rannala, 2010). Due to the single sample of *H. amboinensis* represented by mtDNA only, species delimitation analyses were restricted to the remaining three lineages of *Hydrosaurus* for which nuclear sequence data are available (*H.* sp. [Sulawesi], *H. pustulatus*, and *H. weberi* [Halmahera]). To account for phylogenetic uncertainty, we repeated analyses on all three possible starting topologies: (sp. [Sulawesi], (*pustulatus*, *weberi*)); ((sp. [Sulawesi], *pustulatus*), *weberi*); ((sp. [Sulawesi], *weberi*), *pustulatus*). Samples were assigned to one of the three hypothesized lineages. We phased each nuclear locus, using the program PHASE v.2.1.1 (Stephens and Donnelly, 2003), and retained haplotypes with the highest probabilities for subsequent analyses. Using mtDNA and phased nuDNA data, we ran analyses for 500,000 generations, sampling every 50 generations, with a burn-in of 10,000. We accommodated differential rates of mutation between mtDNA and nuDNA, and explored the impact of both algorithm and prior regime, following the methods of Setiadi et al. (2011) and Leaché and Fujita (2010) (Supporting Information).

2.4. Habitat suitability modeling

We estimated habitat suitability for Philippine populations of *H. pustulatus* using ecological niche modeling and GIS queries of minimum distances to landscape features that correspond to *Hydrosaurus* habitat preferences (e.g., rivers and coastlines) and known collection localities. This procedure allowed us to construct a map of potential habitat suitability throughout the known range of the species. Species occurrence points (localities for which sequence data were collected for vouchered specimens) included 26 unique localities across 13 islands. In order to model suitable habitat for the species we used Normalized Difference Vegetation Index (NDVI) images derived from the Aqua sensor of MODIS at 500 m resolution. We downloaded the NDVI tiles for the study region for the years 2003–2011 and mosaicked them using the MODIS Reprojection Tool 4.1 (USGS, 2012). With these images we generated four sets of layers for each of the years in the following way: (1) mean, (2) maximum, (3) minimum, and (4) minimum of the driest period of the year (estimated to be from November–May).

To estimate areas of suitability, we used the maximum entropy algorithm in MaxEnt (v.3.3.3; Phillips et al., 2006). We generated a model per layer type for unique occurrence points using the raw output with a zero random test percentage. Other parameters remained at default settings. Each model (corresponding to a layer type) was converted to a binary prediction using the minimum training presence value given that all occurrence points come from point localities for which we are confident about their precision. The binary predictions from the four models were then multiplied to obtain a map where all models suggest suitability.

2.5. H. pustulatus suitability map

Because *H. pustulatus* is a species associated with coastal vegetation and mangrove forest, and low elevation fresh water rivers near coasts, we generated raster layers that matched the species habitat suitability in accordance with what is known about its natural history (Supporting Information). This approach restricted our best estimate of suitable habitat for *H. pustulatus* to data from areas that were identified as suitable for the species from the niche modeling map and that coincided with rivers, coastline, mangrove habitat, and known species' occurrence points. Finally, we assessed the degree of protection of the modeled suitable habitats by examining geographic overlap with the current national park system and protected areas network in the Philippines (IUCN and UNEP, 2009). We also compared the suitability models to the geography of current human population density (CIESIN, 2011).

3. Results

3.1. Phylogeny of Hydrosaurus and identification of trade samples

We identified twenty-six unique mtDNA haplotypes and 11 unique nuDNA haplotypes among our 89 samples with complete data for the four genes. Analyses of the combined data (*ND2* + *COI* + *L3* + *L52* + *DGL-*$\propto$) resulted in topologies with high Maximum Likelihood (ML) bootstrap support for major clades (Figs. 1 and 2). All analyses support the monophyly of the genus, however, the relationships among the three recognized species remain uncertain: *H. amboinensis* is recovered as the sister species to a clade of *H. pustulatus* + (*H. weberi* + *H.* sp.) with poor support (Fig. 2). Within *H. pustulatus*, six major clades are supported, corresponding roughly to geographic regions in the Philippines with a few exceptions (Fig. 2, Clades A–F). All trade samples possess high sequence identity with vouchered individuals from the Bicol Peninsula of southeastern Luzon Island (Fig. 2, Clade F).

3.2. Network analyses and distribution of genetic diversity

The results of network analyses corroborate the major results observed from phylogenetic analyses (Fig. 2, Supporting Information). Our mtDNA network (*ND2* + *COI*) revealed eight highly divergent, well-supported groups corresponding to *H. amboinensis*, *H. weberi*, and the six clades of *H. pustulatus* recovered in phylogenetic analyses (Fig. 2). Although the combined nuDNA network revealed little structure (Supporting Information), no strongly supported conflicts with the mtDNA network were apparent. Population samples of *H. pustulatus* from the northern Philippines once again were recovered as members of a distinctly divergent clade among Philippine populations of *Hydrosaurus* (Fig. 2, Clade A). Analyses of molecular variation within *H. pustulatus* samples revealed significant ($P < 0.001$) portions of genetic variation explained by both levels of the AMOVA, with more genetic variation explained among islands within faunal regions than among faunal regions (Fig. 2).

3.3. Species delimitation

Statistical hypothesis tests provided no support (posterior probability approaching 0) for of the monophyly of *H. amboinensis* + *H.* sp. (Sulawesi). Subsequent Bayesian species delimitation analyses aimed at exploring the support for the remaining three lineages within the genus resulted in support for all three lineages as putative species (*H.* sp. [Sulawesi], *H. pustulatus*, and *H. weberi*) with speciation probabilities of 1.0 (Fig. 1C), regardless of starting topology or prior distributions.

3.4. Suitable habitat and protected areas landscapes

The estimate of total suitable habitat for *H. pustulatus* in the Philippines is 127,823 km^2 of which 54,794 km^2 correspond to highly suitable sites for the species after restricting it to areas around mangrove forests, coastlines, and coastal river systems where the species is most likely to be found according to its habitat preferences (Fig. 3). Of the estimated suitable habitat for the full and reduced models, only 11.5% and 9.4%, respectively, are currently represented in the Philippines protected areas (PA) network (Table 2). Within the Bicol Peninsula of Luzon Island, only 2.8% of suitable, coastal habitat currently is protected (Table 2). Considering the country's mangrove forests only, based on recent estimates of this ecosystems occurrence (Giri et al., 2011), 7.5% of this threatened ecosystem is protected in the Philippines under the current PA network (Table 2).

4. Discussion

We define an urgent conservation challenge embodied by sailfin lizards: heavily exploited, large-bodied reptiles with obligate habitat requirements for rapidly disappearing forests, which are insufficiently covered by protected areas. The combination of approaches utilized here is the key to both the characterization of sailfin lizard conservation urgency and an understanding of the way in which human land use patterns along coastal and riparian areas, plus an illegal pet trade's impact on these striking and colorful vertebrates, have conspired to elevate the threat to their continued survival.

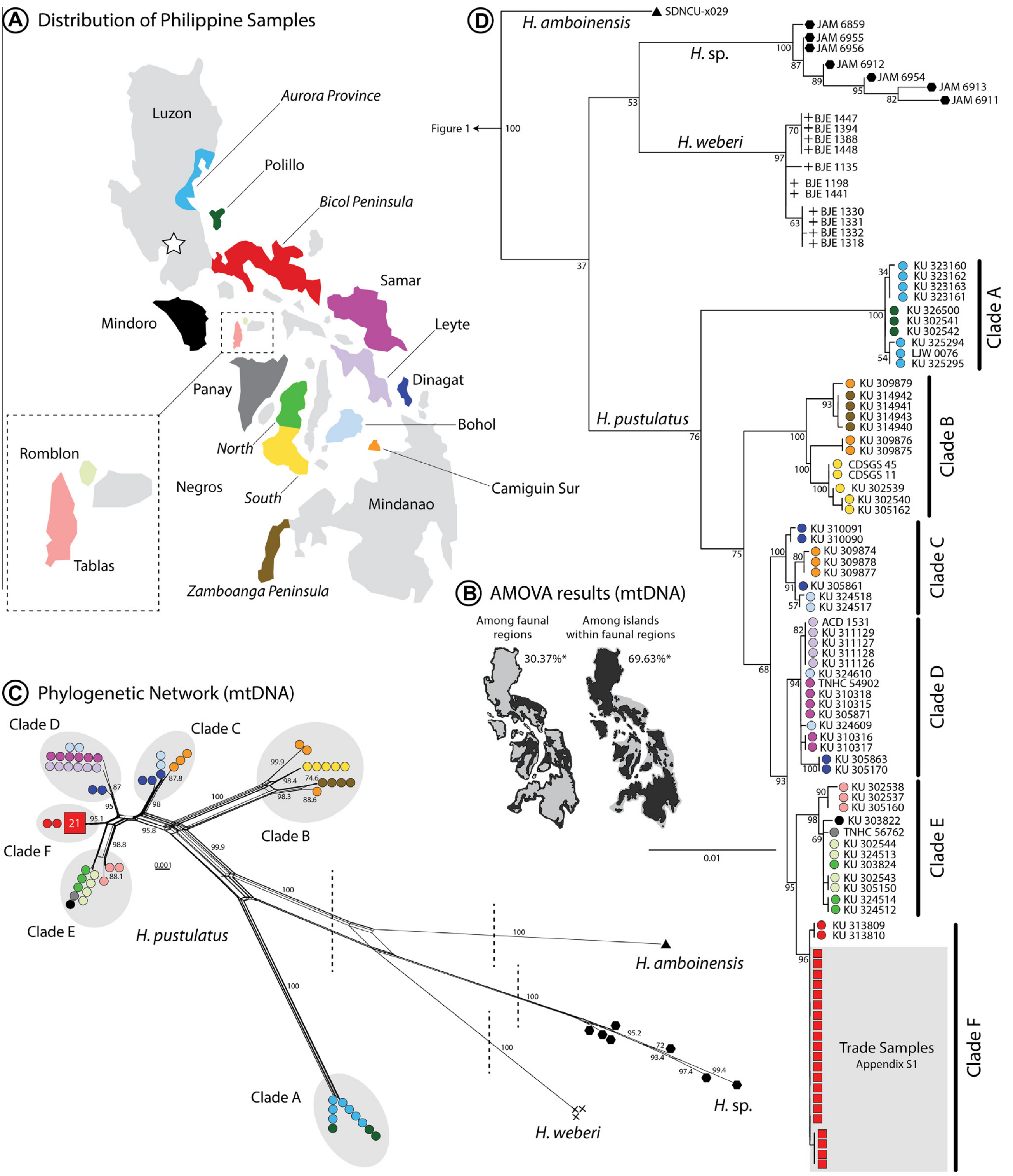

Fig. 2. (A) Color coded map of the Philippine archipelago (note correspondingly colored tree tips). (B) Summarized results of AMOVAs. (C) Mitochondrial haplotype network inferred by SplitsTree (Huson and Bryant, 2006). (D) Preferred Maximum Likelihood phylogenetic tree estimate. Numbers below nodes indicate bootstrap values and clade labels (A–F) are discussed in text. Alphanumerical labeled haplotypes correspond to identifications of vouchered and trade samples (Supporting Information). *Hydrosaurus* sp. refers to the sampled population on Sulawesi.

4.1. Species boundaries and potential unrecognized diversity

Our results partially clarify the current taxonomic arrangement for sailfin lizards and, by implication, suggest four clear targets (probable species) for conservation efforts: *H. amboinensis* of Ambon Island, eastern Maluku, and New Guinea, *H. pustulatus* of the Philippines, *H. weberi* of Halmahera, and a fourth lineage on Sulawesi (for which the name "*H. celebensis*" [Peters, 1872] is available). At a very minimum, these divergent lineages should now be embraced as Evolutionary Significant Units (ESUs; Ryder, 1986), or appropriate targets for conservation to preserve genetic diversity among and within species in an objective (Moritz, 1994), and species-level targets for conservation efforts (IUCN, 2012).

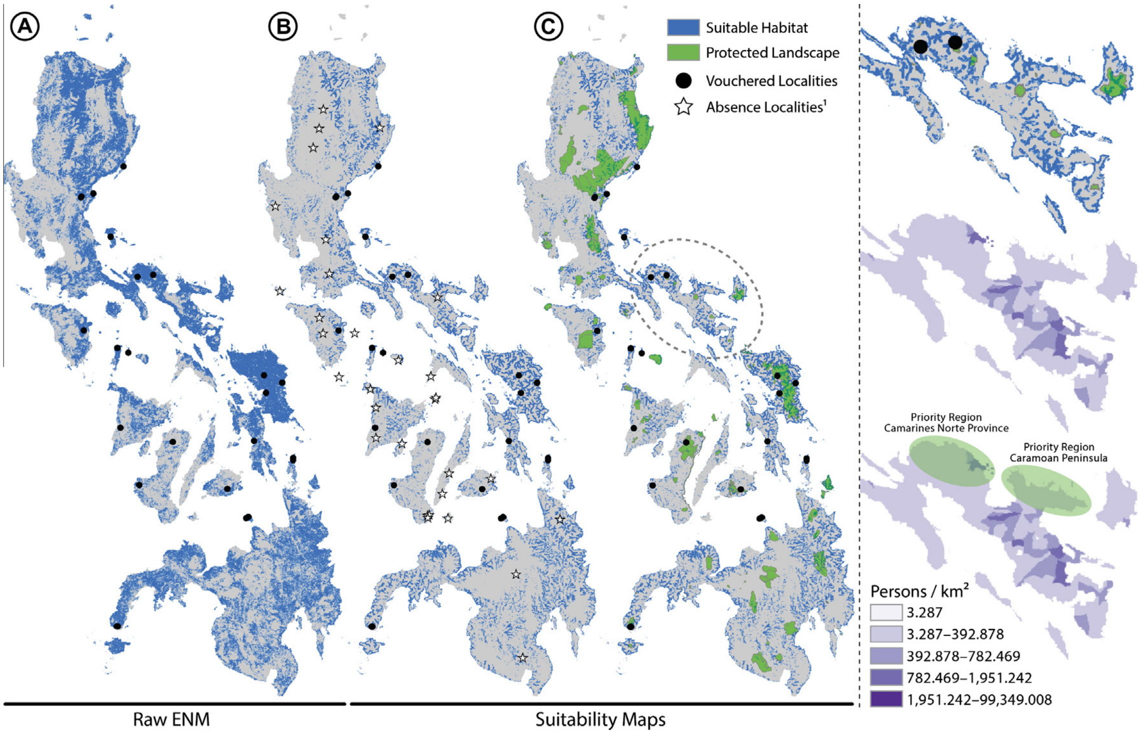

Fig. 3. *Hydrosaurus pustulatus* habitat suitability, with black dots representing vouchered localities sampled for this study, estimated from the full (A) and reduced (B) habitat preference models. Federally protected areas shaded in green (C). The inset panel shows the Bicol Peninsula of Luzon in greater detail (above) with approximate human density projected (see legend; CIESIN, 2011) and suggested priority regions for increased conservation efforts. ¹Stars represent absence localities used in exploratory analyses of presence–absence datasets (shown for reference). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Summary of ecological estimates calculated in this study.

Measure	Model	Region	Total (km^2)	Protected (km^2)	Protected (%)
Pertaining to Hydrosaurus pustulatus					
Suitable habitat	Full	Philippines	127823.535	14719.414	11.5
	Full	Bicol Peninsula, Luzon Island	14217.669	644.135	4.5
Suitable habitat	Reduced	Philippines	54793.873	5152.070	9.4
	Reduced	Bicol Peninsula, Luzon Island	7057.757	197.999	2.8
Suitable habitat	Reduced, Coastal	Philippines	12937.456	732.745	5.7
	Reduced, Coastal	Bicol Peninsula, Luzon Island	2794.537	21.692	0.8
Pertaining to the Philippines					
Mangrove forest		Philippines	1705.481	128.739	7.5
		Bicol Peninsula, Luzon Island	249.501	3.832	1.5

Our results reveal a unique, unrecognized lineage (currently considered *H. amboinensis*) from Sulawesi, Indonesia (Figs. 1 and 2). Historically, sailfin lizards from Sulawesi were described as a unique species, *H. celebensis* (Peters, 1872), but the name was synonymized with *H. amboinensis* (Günther, 1873). Unfortunately, the only published and available sequence of putatively true *H. amboinensis* comes from a genetic pet trade sample (SDNCU-x029; Okajima and Kumazawa, 2010; Okajima and Kumazawa, personal communication) with a reported but unconfirmed region of origin. Although there is general confidence in the samples reported origin from within the range of real *H. amboinensis* (Okajima and Kumazawa, personal communication), there is no vouchered, museum specimen available for comparison. We therefore chose to take a conservative approach in evaluating the results of this study, and have avoided making definitive taxonomic decisions at this time. Regardless of these limitations, the results of all analyses are conclusive in their support for the paraphyly of *H. amboinensis* as currently recognized. Given that the name *Hydrosaurus celebensis* (Peters, 1872) is the oldest available name attributed to the Sulawesi lineage, we find it likely that future studies will result in the resurrection of *H. celebensis*.

Our analyses support a single Sailfin lizard species (*H. pustulatus*) distributed throughout much of the Philippines (Fig. 2); however, haplotype networks and phylogeographic analyses (Fig. 2) reveal six distinct genetic clusters, or haplotype groups, in the Philippines. The most genetically divergent/distinctive appears to be restricted to Aurora and Quezon Provinces of the islands of Luzon and Polillo (Fig. 2, Clade A). With the exception of these distinct northern populations, the remaining clades recovered in phylogenetic analyses do not appear to conform to expected geographic patterns (Fig. 2, Clades B–F), and the observed patterns may be more heavily driven by the persistence of ancestral polymorphism or gene flow. Given the ecology of these coastal species, inter-population gene flow among adjacent regions is most likely a common, ongoing phenomenon—as would be expected with presumably dispersal-prone, large bodied lizards specialized in coastal forests, lowland riparian corridors, and river-mouth habitats. Historically, this may have been especially true during glacial periods when sea levels dropped (100–140 m below current levels) resulting in greater connectivity between adjacent islands previously separated by shallow water (review: Voris, 2000; Siler et al., 2012).

When compared to many other radiations of Southeast Asian agamid lizards (i.e., *Acanthosaura*, *Bronchocela*, *Calotes*, *Draco*, *Gonocephalus*, *Japalura*), the genus *Hydrosaurus* is a relatively species-poor group (Manthey, 2008; Grismer, 2011). Given extensive geographic variation in scale features and body coloration (*personal observations*), conservation workers in the Philippines have long assumed that genetic analyses would reveal a considerable degree of cryptic diversity in the group. Surprisingly, the results of our study demonstrate low genetic diversity across the entire range of the genus (5.7% maximum pairwise sequence divergence), and indicate that the genus *Hydrosaurus* may in fact be composed of a small number of taxa, with relatively widespread geographical distributions. Nevertheless, these species remain a conservation concern because of their dependence on coastal habitats, which have experienced heavy human-mediated development.

4.2. Phylogeographic patterns and future directions

Although the primary focus of this study was a conservation genetic assessment of a flagship conservation genus, and to highlight the importance of reevaluating Philippine protected landscape strategies, phylogeographic patterns elucidated here merit discussion. With *Hydrosaurus amboinensis* supported as sister to all other lineages in the genus, our results indicate that sailfin diversity originated east of Wallace's Line, one of the most celebrated biogeographic barriers separating Asian and Australian biota (Wallace, 1860). The absence of well-supported relationships among the four genetically distinct lineages precludes us from inferring with confidence the number of potential colonization events across this recognized barrier to dispersal; however, it is clear that Wallace's Line was crossed historically at least once, giving rise to *H. pustulatus* (Figs. 1 and 2). Timing of divergences inferred in recent higher-level analyses suggest that the genus is quite old (>55 Ma; Townsend et al., 2011), begging the question of why species diversity is so low.

Within the Philippines, phylogeographical patterns are nonsensical and defy expectation derived from other recent studies (Brown et al., 2013). Shallow divergences and relationships of clades that defy both a south-to-north progression (expected from inferred lineage diffusion from the southeast of the Philippines) and Pleistocene Aggregate Island Complex (PAIC) based expectations of distinct lineages on former Pleistocene landmasses (Brown and Diesmos, 2009; Brown et al., 2013) both suggest the possibility of a recent colonization and expansion through the archipelago.

4.3. Phyloforensics and origins of black market trade in Hydrosaurus

Trade sample sequences were 99.8–100% identical to a single, known-locality sequence from the Bicol Peninsula of Luzon Island. These observations stand in contrast to a recent trade forensic study on monitor lizards of the Philippines (Welton et al., 2013a), which recovered trade samples among seven clades and five species. While Welton et al. (2013a) also found the majority of trade samples (58–68%) to have originated from the Luzon faunal region, only one-third of these samples originated from the Bicol Peninsula. Although we have observed hydrosaurs to be quite common in the Bicol Peninsula in comparison to Luzon proper, where they are rarely encountered, it is not clear at this time whether this observation necessarily is indicative of larger native populations in this region as compared to elsewhere in the archipelago where the species occurs. In fact, the site with the highest density of individuals observed in the wild is located on the small island of Dinagat in the southeastern Philippines. Unfortunately, so little is known about the population sizes and ecology of these species, that this study represents the first approach to synthesize all available information about this unique and threatened vertebrate group. As future studies attempt to better conserve the extant populations of species in this genus, it is critical that researchers focus studies on the ecology of these riparian taxa.

This study adds to a growing baseline of Philippine trade forensic data. However, much remains to be done before we can have a comprehensive understanding of how conservation efforts should be prioritized to address the country's illegal trade. Ideally, future studies will employ more rapidly evolving nuclear loci, possibly incorporating next-generation sequencing technology as a tool for marker discovery (SNP, SSRs; Garvin et al., 2010; Ogden, 2011), as well as more fully explore the power and utility of standard barcoding genes (e.g., COI) for more rapid and cost-effective identification of trade samples for other groups commonly encountered in the black market pet trade. Finally, it is vital that phyloforensic monitoring of the illegal Philippine pet trade should focus on the numerous well-developed trade centers (e.g., Puerto Princesa City, Palawan Island; Cebu City, Cebu Island; Davao City, Mindanao Island).

Although there are clear differences between this and a previous study (Welton et al., 2013a) in terms of collecting locations targeted by poachers, both studies agree on one thing: the Bicol Peninsula of Luzon Island represents a major source of illegally collected individuals for the spectacular, large, and threatened reptiles illegally traded in Manila black markets. Given the moderate to high density of human populations throughout much of the central and southern Bicol Peninsula (CIESIN, 2011; Fig. 3), increased conservation efforts and expansion of protected areas may have the greatest impact in terms of preservation of critical hydrosaur habitat if prioritized in the northern regions of the peninsula (Camarines Norte Province, Caramoan Peninsula; Fig. 2).

4.4. Importance of reevaluating Philippine protected landscape strategies

This work represents the first conservation genetic study aimed at integrating a species-specific, habitat suitability-driven evaluation of the country's protected landscape network with an analysis of a clear conservation threat in the form of illegal commercial exploitation. The question remains: does the non-correspondence between suitable habitat and protected areas represent a serious problem for long-term survival of the *H. pustulatus*? And, expanding this question to other vertebrates with specific habitat requirements, do the majority of land vertebrates in the archipelago inhabit ranges even partially encompassed or overlapped by protected areas? We suspect that although the current protected area system in the Philippines does overlap the geographic ranges of many endemic species, many others possess habitat requirements such that protected areas do not encompass their geographic range. Until future studies continue to address this important conservation issue in a large, comparative framework, we are unable to predict the generality of the apparent mismatch between suitable habitat and protected area network in the Philippines that we have documented here for *H. pustulatus*.

Our results clearly indicate the low extent of remaining habitat for hydrosaurs in the Philippines (Fig. 3; Table 2). Only 5.7% of suitable habitat for *H. pustulatus* is protected under the current protected area network in the Philippines (Table 2). Moreover, in the Bicol Peninsula, identified as the source region for all illegal pet trade samples included in this study, only 0.8% of the coastal suitable habitat is protected (Fig. 3; Table 2) and coastlines are some of the most densely populated human habitations in the region. Our modeled estimates of suitable habitat for *H. pustulatus* likely are conservative and only partially representative of the country's remaining coastal habitat. Unfortunately, we expect that the percentage of protected coastal forest in the archipelago is even lower because most protected areas are inland and do not include coastal forests (Fig. 3). Additionally, we have no doubt that even within protected, suitable habitat, poaching pressures likely exist. Unfortunately, there are no studies to date that have quantified the level of illegal poaching in protected areas in the Philippines. Estimates of the volume of trade would be useful in developing strategic conservation plans for the development and maintenance of protected landscapes in the Philippines. Finally, future field studies should address the degree to which hydrosaurs exhibit tolerance for what appears to be a low habitat connectivity syndrome, given that all coastal habitats throughout the archipelago are experiencing degradation due to encroaching development and/or wholesale removal.

With only 7.5% of all mangrove forest in the country coinciding within protected areas (and a mere 1.5% Bicol region mangroves protected), our study provides a first example of an integrative, multidisciplinary approach to conservation and management of one of the Philippines' major charismatic riparian corridor forest and coastal forest obligate vertebrate species. Unfortunately, the ecological estimates presented in this study likely overestimate the actual percent of mangrove and suitable hydrosaur habitat, as many of these protected regions likely have experienced some degree of habitat degradation, especially at the edges of parks and reserves (*personal observations*). We expect that numerous additional vertebrates will be shown to exhibit similar dependence on coastal and lowland forest habitats and are likely similarly at peril due to transformation of these environments by human activities. We have shown that by integrating multiple lines of evidence, types of data, and analytical approaches, investigators may be able to take advantage of immediately applicable and emergent findings for addressing previously understudied, but at-risk species of high conservation urgency. Great strides towards imperiled mangrove, coastal, and riparian lowland forest obligate species conservation could be made by undertaking similar comparative methods across taxa of varying life history strategies, demographic patterns, and microhabitat requirements.

Acknowledgements

We thank the Protected Areas and Wildlife Bureau (PAWB) of the Philippine Department of Environment and Natural Resources (in particular T.M. Lim, C. Custodio, J. de Leon, A. Manila, and A. Tagtag) for facilitating this study. Thanks are due to A. Diesmos, E. Sy, and J. Barns for logistical support. Sampling efforts followed protocols approved by the University of Kansas (KU) IACUC and MOA between KU and PAWB, as outlined in a GP administered by PAWB. We thank B. Evans and J. McGuire for access to Halmahera and Sulawesi material, respectively. Fieldwork was supported by a National Science Foundation Doctoral Dissertation Improvement grant (DEB 0804115 to CDS), Fulbright and Fulbright-Hayes Fellowships (to CDS); and an NSF Biotic Surveys and Inventories grant (DEB 0743491 to RMB). Jake Esselstyn and three anonymous reviewers provided constructive comments on earlier drafts of the manuscript. A special thanks to L. Campbell and Q. Huijie for help in processing MODIS datasets, and to Scott Corning for providing photographs of adult hydrosaurs.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2013.10.014.

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