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Phylogenetic position of *Tropidophorus assamensis* Annandale, 1912 with updated morphological data and distributional records

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The poorly known northeastern water skink *Tropidophorus assamensis* is only known from the type locality (Harigaj Range, Sylhet District) in Bangladesh, and few localities in Assam and Mizoram States, north-eastern India. Little is known about the biology including the systematics of the species. In this study, we present for the first time, genetic data (*16s rRNA*) and inferred its phylogenetic position. In addition to this, we provide updated morphological data along with new distributional records of the species from Mizoram State of north-east India.

Keywords: Distribution, morphology, northeastern water skink, systematics

INTRODUCTION

The genus *Tropidophorus* Dumèril and Bibron, 1839 comprises of 29 extant species of semi-aquatic skinks (Uetz et al., 2021). The genus shows a high level of local endemism (Greer & Biswas, 2004), and can be morphologically diagnosed in having exposed tympanum and a single scale at the corner of the eyelid (Greer, 1970; Hikida et al., 2002; Greer & Biswas, 2004). *Tropidophorus* were known to occur in Indochina including the entire Malay Peninsula, Borneo, Sulawesi, and Philippines (Honda et al., 2006).

The northeastern water skink, *Tropidophorus assamensis* Annandale, 1912 is one of the most poorly known species among the congeners from Indochina (see Pawar & Birand, 2001; Honda et al., 2006; Das et al., 2009). Thomas Nelson Annandale (1876–1924), a Scottish Zoologist, one of the founder, and the first director of the Zoological Survey of India (ZSI), originally described the species from Harigaj range (holotype: ZSI 17029), Sylhet hills, Assam, India (at present Sylhet District, north-eastern Bangladesh) (Annandale, 1912; Das et al., 1998). Almost a century after the original description, the species was recorded from Mizoram State, India by Pawar and Birand (2001) at Nengpui Wildlife Sanctuary. A few years later, Mathew (2006) also collected an individual (VR/ERS/ZSI/241) from a bamboo thicket near a stream from Phairuangkai, Lunglei District, Mizoram State. From Assam, the adjacent State of Mizoram, Das (2008) recorded the species based on three individuals (ZSIC 25813; BNHM 1783; AD/BR 05) from Chambuda area and Adakuchi Basti in Barail Range; which were encountered under bryophyte covered rocks on a dry stream bed and under rocks of slow flowing-stream. It was also noted that unlike other skinks, they remained motionless when uncovered (Das et al., 2009). In the recent phylogenetic study of the genus *Tropidophorus* by Honda et al. (2006), a total of 17 representative species were sampled (11 from Indochina; three from Philippines; two from Borneo; one from Sulawesi), but *T. assamensis* was not included in their analyses. The present study provides the first genetic data for *T. assamensis* by sequencing a fragment of the mitochondrial 16S ribosomal RNA (*16s rRNA*) gene. We reconstruct the phylogenetic relationships of *Tropidophorus*, and provide further data about the morphological data of *T. assamensis* based on the recently collected specimens from Mizoram State, India.

This study is based on the museum specimens deposited in the Departmental Museum of Zoology, Mizoram University (MZMU), and Pachhunga University College Zoological Museum (PUCZM). The specimens we collected in this study were anaesthetised using 250 mg/kg of 0.7 % sodium bicarbonate buffered MS-222 (Tricaine Methanesulfonate) solution by intracoelomic injection, followed by euthanasia by a second intracoelomic injection of 0.1 ml unbuffered 50 % (v/v) MS-222 solution following Conroy et al. (2009). Specimens were catalogued and stored in 70 % ethanol.

Genomic DNA was extracted from the liver tissue of MZMU2080 using QIAamp DNA Mini Kit following the standard protocol provided by the manufacturer. *16s rRNA* was amplified using the polymerase chain

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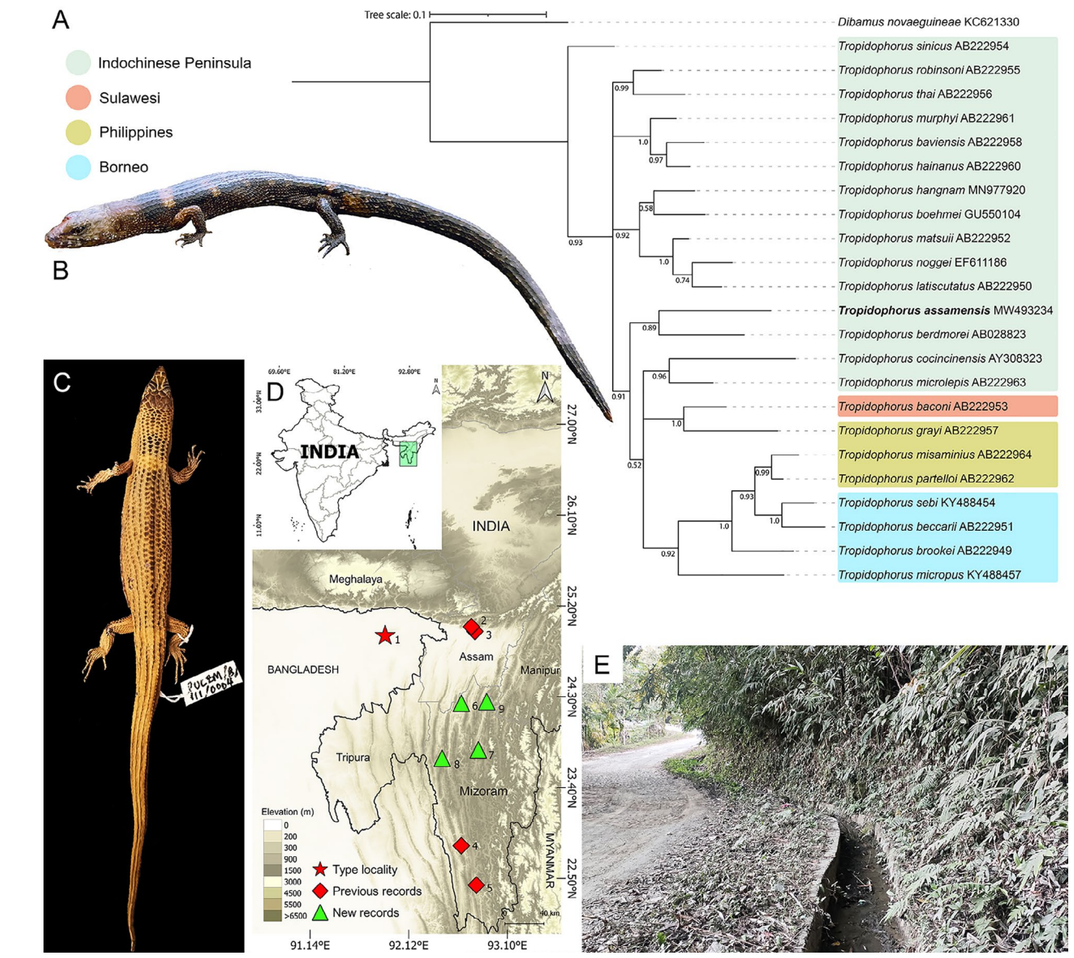

Figure 1. (A) Bayesian Inference phylogeny based on partial *16s rRNA* of *Tropidophorus*. The sample generated in this study is shown in bold. Numbers at each node represent Bayesian posterior probabilities at 20 million generations. **(B)** Adult female *Tropidophorus assamensis* (MZMU2080) in life. **(C)** Preserved specimen of *Tropidophorus assamenis* (PUCZM/B/111/0004). **(D)** Map showing the distribution of *Tropidophorus assamensis*: type locality shown in red star (1. Sylhet District, Bangladesh); previous records from north-east India shown in red diamonds (2–3. Barail Wildlife Sanctuary, Assam State; 4. Phairuangkai, Lunglei District, Mizoram State; 5. Ngengpui Wildlife Sanctuary, Mizoram State); new records from Mizoram State, India shown in green triangles (6. Tuichhuahen river, 7. Tuirial village, 8. Dampa Tiger Reserve, 9. Pualreng Wildlife Sanctuary). **(E)** Microhabitat of *Tropidophorus assamenis* at a roadside drainage in Dampa Tiger Reserve, Mizoram State, India.

reaction (PCR) with the forward L02510 (Palumbi, 1996) and reverse H03063 (Rassmann, 1997) primers in 20 µL reaction volumes, containing 1X amplification buffer, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.2 pM each forward and reverse primer, 1µL genomic DNA, and 1U Taq DNA polymerase. The PCR thermal cycling was performed as 5 min at 95 °C for initial denaturation, followed by 35 cycles of [1 min at 95 °C for denaturation, 30 s for annealing at 50.3 °C, elongation for 1 min at 72 °C], and a final elongation for 5 min at 72 °C. PCR products were checked by gel electrophoresis on a 1.5 % agarose gel containing ethidium bromide. Sample was sequenced using Sanger's dideoxy method, and sequencing reactions were carried using the ABI 3730xl DNA Analyzer at Barcode BioSciences, Bangalore, India. The generated partial *16s rRNA* gene sequence is deposited on the GenBank repository (523 base pairs; accession number MW493234). We included 22 sequences of *Tropidophorus* species available from the NCBI database, and one sequence of *Dibamus novaeguineae* (KC621330) as an outgroup. The nucleotide sequences were aligned using the MUSCLE algorithm (Edgar, 2004) with default parameters, and uncorrected p-distance was calculated in MEGAX (Kumar et al., 2018). The best-fitting model for the nucleotide substitution was selected under the Bayesian Information Criterion (BIC) in ModelTest-NG (Darriba et al., 2020). The Bayesian inference (BI) phylogeny was constructed in MrBayes 3.2.5 by selecting nst=6, rates=gamma and statefreqpr=dirichlet (1,1,1,1) for the GTR + G model. The MCMC (one cold and three hot chains) was run for 20,00,000 generations sampling one tree each 1,000 generations, and the analysis was terminated when the average standard deviation of split frequencies become less than 0.001 (Ronquist & Huelsenbeck, 2003). Burn-in was set to 25 %, and the remaining trees were used to assess Bayesian posterior probabilities (BPP) for nodal support (Fig. 1A). Maximum Likelihood (ML) analysis was performed with 1,000 bootstrap replicates in MEGA X software (Kumar et al., 2018) using the selected model (GTR + G) based on the lowest BIC score (Nei & Kumar, 2000). The ML phylogenetic tree is presented in Supplementary Material.

The first specimen of *T. assamensis* (MZMU613) was collected by H.T. Lalremsanga on 24 August 2011 from the banks of Tuichhuahen river, Kolasib District, Mizoram, India (24°14'10.42"N, 92°38'34.25"E, 62 m/asl.). On 27 November 2020, at ca. 2300, a female individual of *T. assamensis* (MZMU2080) was collected by H.T. Lalremsanga and a team from a roadside water canal located close by the Teirei Forest Guest House, Dampa Tiger Reserve (DTR), Mizoram, India (23°41'26.00" N, 92°27'6.12" E, 260 m/asl., Fig. 1B). In this study, we also examined an individual (PUCZM/B/111/0004) collected by Van Lalhlimpuia on 10 July 2014 from a streamlet at Tuirial village, Aizawl District, Mizoram, India (23°44'13.72"N, 92°47'57.41"E, 370 m/asl., Fig. 1C). Another male individual (MZMU2534) with snout-vent length 61.28 mm was collected on 15 August 2021 by Lal Muansanga from a water-filled muddy crevice of dried pond inside the buffer zone of Pualreng Wildlife Sanctuary, Kolasib District, Mizoram (24˚25'48.51"N, 92˚81'29.78"E, 538 m/asl.). The taxon type locality (Sylhet, Bangladesh), previous records (Mizoram and Assam States, India), and the collection sites of the new specimens (Mizoram State, India) are shown in Fig. 1D. Notably, the partially submerged skink (MZMU2080) within a muddy microhabitat of drainage at Dampa Tiger Reserve (Fig. 1E) not even react or try to flee when encountered and while capturing, instead remained still. Such behaviour is rather similar to the observations of Das et al. (2009).

The specimens agree with the original description (Anandale, 1912) and other literature (Smith, 1935; Mathew, 2006, 2007). Unfortunately, our first collected specimen MZMU613 has possibly been lost during shifting specimens from the old Museum building to the current Departmental Museum of Zoology, Mizoram University. So, the following morphological attributes are solely from the other two specimens, and provided the values as PUCZM/B/111/0004 & MZMU2080, respectively: snout-vent length 70.62 mm & 74.50 mm, exceeding the size of the largest known individual for the species (68.65 mm) previously recorded by Das (2008); tail length 92.42 mm & 90.20 mm; trunk length 41.38 mm & 37.04 mm; snout length 5.60 mm & 5.56 mm; snout width (at nostril) 2.80 mm & 2.85 mm; head length at angle of jaw 11.40 mm & 14.38 mm; head width at angle of jaw 8.61 mm & 9.20 mm; head depth 6.73 mm & 6.84 mm; eye diameter 3.40 mm & 3.90 mm; tympanum exposed and large with diameter 1.86 mm & 2.10 mm; inter-narial distance 1.71 mm & 2.90 mm; eye to nostril distance 3.37 mm & 3.70 mm; tympanum to eye distance 4.73 mm & 5.80 mm; forelimb length 16.58 mm & 18.54 mm; hindlimb length 24.70 mm & 25.92 mm; leg when stretched almost reaches to the wrist; 91 & 80 caudal plates; scales around mid-body 34 & 31; longitudinal mid-dorsal scale from nuchal to level of hindlimb insertion 43 & 46; two large preanal scales; single large frontal; two prefrontal contact each other; supralabials 8 on either sides & 6 on left and 8 on right; infralabials 8 on left and 7 on right & 6 on left and 7 on right; lower eyelids scaly; supra oculars 4 & 4; lamellae on fourth finger 11 & 12, and 18 & 14 on fourth toe; relative finger length F4>F3>F2>F5>F1; and toe length T4>T3>T5>T2>T1; ventrals smooth; dorsal and lateral scales strongly keeled and mucronate; snout sharply pointed; rostrum convex; single nasal; mental large. In life, dorsum dark brownish with obscured yellow patches; prominent yellowish band across hindlimbs, and another across the forelimbs; three or four broken yellowish bands between the forelimbs and hindlimbs; head region including the nape paler than dorsum colour; tail abstrusely banded.

The *16s rRNA* dataset of *Tropidophorus* species consisted of a final alignment of 446 characters after removal of ambiguous aligned sites, of which 141 sites were diagnosed as variable. Our BI and ML analyses showed that *T. assamensis* is forming a sister species with *T. berdmorei* by a considerable nodal support (BPP=0.89; bootstrap value=78), and the clade (*T. assamensis* + *T. berdmorei*) is inferred as sister to the clade consisting of certain Indochinese species (*T. cocincinensis* + *T. microlepis*), Sulawesi (*T. baconi*), Philippines (*T. grayi* + *T. misaminius* + *T. partelloi*), and Borneo species group (*T. sebi* + *T. beccarii* + *T. brookei* + *T. micropus*) (BPP=0.91; bootstrap value=55). The branching patterns from our BI topology largely accorded with the cladogram in Honda et al. (2006), except on the position of *T. berdmorei* and *T. sinicus*. In the studied dataset, the interspecies mean uncorrected p-distance was 10.3 %, ranging from 9.7 % to 13.9 %. The studied sequence of *T. assamensis* showed 9.7 % genetic distance with the sister species *T. berdmorei*, while the maximal genetic distance 13.9 % was diagnosed with *T. beccarii* of the Borneo species group.

This study inferred the phylogenetic position of *T. assamensis* with its congeners, and presents additional distributional localities adjoining previous records with updated morphological and first genetic data (GenBank accession no. MW493234). We assume these new information will help improving the limited knowledge of this rare and poorly known water skink species. As of now, the species is documented from five Districts of Mizoram State and we speculated that it is widespread in the region despite its rarity. Regarding some discordances between our present BI topology and Honda et al.

(2006) in the phylogenetic reconstructions of the genus, we encourage further studies to assess the monophyly of *Tropidophorus* species, and also to determine the definite phylogenetic status of *T. assamensis* with better accuracy.

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Authors' contribution:
Hmar Tlawmte Lalremsanga and Lal Biakzuala collected specimens, conceived the research questions, designed the works, examining specimens, and wrote manuscript. Ht Decemson assists in specimen collection and manuscript writing. Mathipi Vabeiruyreilai assists in specimen collection and molecular works. Fanai Malsawmdawngliana accompanied the field survey and help in examining specimens. Van Lalhlimpuia and Lal Muansanga collected the specimen and help in manuscript revision.

Ethical statement:
The specimens in this study are collected after obtaining permission from Chief Wildlife Warden, Environment, Forest and Climate Change Department, Government of Mizoram, India (Permit No. A. 33011/2/99-CWLW/225).

REFERENCES

Annandale, N. (1912). Zoological results of the Abor Expedition, 1911-1912: II Reptilia. *Records of the Indian Museum* 8, 37–59.

Conroy, C. J., Papenfuss, T., Parker, J. & Hahn, N. E. (2009). Use of tricaine methanesulfonate (MS222) for euthanasia of reptiles. *Journal of the American Association for Laboratory Animal Science* 48, 28–32.

Darriba, D., Posada, D., Kozlov, A. M., Stamatakis, A., Morel, B. & Flouri, T. (2020). ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* 37, 291–294.

Das, A. (2008). Geographic Distribution Notes: *Tropidophorus assamensis*. *Herpetological Review* 39, 110.

Das, A., Saikia, U., Murthy, B. H. C. K., Dey, S. & Dutta, S. K. (2009). A herpetofaunal inventory of Barail Wildlife Sanctuary and adjacent regions, Assam, north-eastern India. *Hamadryad* 34, 117–134.

Das, I., Dattagupta, B. & Gayen, N. C. (1998). History and catalogue of reptile types in the collection of the Zoological Survey of India. *Journal of South Asian Natural History* 3, 121–172.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32, 1792–1797.

Greer, A. E. (1970). A subfamilial classification of scincid lizards. *Bulletin of the Museum of Comparative Zoology* 139, 151–184.

Greer, A. E. & Biswas, S. (2004). A generic diagnosis for the Southeast Asian scincid lizard genus *Tropidophorus* Duméril & Bibron, 1839 (Reptilia: Scincidae). *Journal of Herpetology* 38, 426–430.

Hikida, T., Orlov, N. L., Nabhitabhata, J. & Ota, H. (2002). Three new depressed-bodied water skinks of the genus *Tropidophorus* (Lacertilia: Scincidae) from Thailand and Vietnam. *Current Herpetology* 21, 9–23.

Honda, M., Ota, H., Murphy, R. W. & Hikida, T. (2006). Phylogeny and biogeography of water skinks of the genus *Tropidophorus* (Reptilia: Scincidae): a molecular approach. *Zoologica Scripta* 35, 85–95.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35, 1547–1549.

Mathew, R. (2006). North eastern water skink *Tropidophorus assamensis* Annandale (Reptilia: Sauria: Scincidae) in Mizoram, northeast India. *Cobra* 63, 14–15.

Matthew, R. (2007). Reptilia. In: *Fauna of Mizoram, State Fauna Series*, 14, 545–577. The Director (eds). Kolkata: Zoological Survey of India.

Nei, M. & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. New York: Oxford University Press.

Palumbi, S. R. (1996). Nucleic acids II: the polymerase chain reaction. In: *Molecular Systematics*, 2nd Eds, 205–247. Hillis, D. M., Moritz, C. & Mable, B. K. (eds). Massachusetts: Sinauer Associates Inc.

Pawar, S. S. & Birand, A. (2001). A survey of amphibians, reptiles and birds in northeast India. CERC Technical Report #6. Mysore: Center for Ecological Research and Conservation.

Rassmann, K. (1997). Evolutionary age of the Galapagos iguanas predates the age of the present Galapagos islands. *Molecular Phylogenetics and Evolution* 7, 158–172.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.

Smith, M. A. (1935). The fauna of British India, including Ceylon and Burma. Reptiles and Amphibia, Vol. II. Sauria. London: Francis & Taylor.

Uetz, P., Freed, P. & Hošek, J. (2020). The Reptile Database, http://www.reptile-database.org, accessed [accessed on 21 February 2021].

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Predicted impact of climate change on the distribution of the Critically Endangered golden mantella (*Mantella aurantiaca*) in Madagascar

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The impact of climate change on Malagasy amphibians remains poorly understood. Equally, deforestation, fragmentation, and lack of connectivity between forest patches may leave vulnerable species isolated in habitat that no longer suits their environmental or biological requirements. We assess the predicted impact of climate change by 2085 on the potential distribution of a Critically Endangered frog species, the golden mantella (*Mantella aurantiaca*), that is confined to a small area of the central rainforest of Madagascar. We identify potential population distributions and climatically stable areas. Results suggest a potential south-eastwardly shift away from the current range and a decrease in suitable habitat from 2110 km² under current climate to between 112 km² – 138 km² by the year 2085 – less than 7 % of currently available suitable habitat. Results also indicate that the amount of golden mantella habitat falling within protected areas decreases by 86 % over the same period. We recommend research to ascertain future viability and the feasibility of expanding protection to newly identified potential sites. This information can then be used in future conservation actions such as habitat restoration, translocations, re-introductions or the siting of further wildlife corridors or protected areas.

Keywords: Conservation, SDM, amphibian, montane, rainforest, protected area

INTRODUCTION

Madagascar is one of the world's mega-biodiversity hotspots, with extremely high levels of endemism across the island (Myers et al., 2000; Vieilledent et al., 2013). Amphibians follow the trend with 314 assessed frog species, 99 % of which are endemic (IUCN, 2021), and there are potentially many more yet to be described (Glaw & Vences, 2007). Most species are located within the Eastern rainforest belt (Glaw & Vences, 2007). However, forests across Madagascar are being depleted at an alarming rate, i.e. from 1953 to 2014 forested land cover decreased from 27 % to 15 % (Brown et al., 2015; Vieilledent et al., 2018). Forest fragments that remain are also decreasing in size with mean distance to forest edge dropping from 1.5 km to 300 m respectively (Brown et al., 2015; Vieilledent et al., 2018). Fragmentation of already degraded forest areas may impede the movement of species with low vagility between habitat patches, increase access for loggers or hunters, expose deep forest species to forest edge effects, increase competition for limited resources, or result in habitat patches too small to sustain viable populations (Cushman, 2006; Echeverria et al., 2006; Vieilledent et al., 2018).

Predictions for climate change across Madagascar suggest a rise in temperature of 1.1 ˚C –2.6 ˚C by 2050 (Tadross et al., 2008). Temperatures vary along a gradient from north to south, with the lowest rises predicted in the northern and coastal areas, and highest rises in the southern spiny forest region (Hannah et al., 2008). Rainfall is predicted to increase across the island except along the south-east coast where it will become drier in winter months (Hannah et al., 2008). According to Seidl et al. (2017), climate change has the potential to affect forests in complex ways i.e. an increase in temperature and lower rainfall may lead to higher instances of tree die-off, forest fires, fuel build up, or insect abundance. Under hotter and wetter conditions, soil erosion, runoff and sedimentation become more likely (Seidl et al., 2017). Deforestation and climate change may therefore act synergistically driving species to shift their range to areas with more favourable conditions (Raxworthy et al., 2008). Historically, large tracts of contiguous forest may have made dispersal to higher, cooler or more climatically stable areas easier. However, with many montane forest areas in Madagascar now highly fragmented, dispersal for some species is difficult, if not impossible (Brown et al., 2015).

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Golden mantellas (*Mantella aurantiaca*) are Critically Endangered montane forest dwelling frogs from the Central Eastern Rainforest areas of Mangabe and Analamay in Madagascar (Piludu et al., 2015; Edwards et al., 2019). They are found at altitudes of between 900 m and 1000 m asl and the area of suitable habitat occupied by this species is low at around 10 km^2. A recent survey by Piludu et al. (2015) found 139 breeding sites, many of which were in areas under threat from agricultural expansion, industrial or artisanal mining, or collection for the pet trade, with the majority in areas already classed as protected.

Climate change may exacerbate problems faced by golden mantellas as they are already found at altitudes close to the summits of the slopes they inhabit, leaving no real opportunity for dispersal to higher, cooler altitudes. It is clear there are few in-situ conservation management options remaining: the frogs either adapt to climate change, or alternative suitable habitat needs to be restored in areas where it is required. To this end Species Distribution Modelling (SDM) can play an important part in identifying suitable areas for the possible translocation or reintroduction of golden mantella populations. SDM is the process of exploring the relationships between species distribution and associated environmental and habitat variables, and then predicting spatial relationships (Márcia-Barbosa et al., 2013 Bateman et al., 2013; Cao et al., 2013; Meynard et al., 2013; Rodriguez-Rey et al., 2013). We follow several other authors (Blank & Blaustein, 2012; Chunco et al., 2013; Groff et al., 2014; Sharifi et al., 2017) in using SDM to identify and prioritise optimum habitat requirements, where potential anthropogenic disturbance and climate change impacts are at their lowest. Results can then be used to guide future management decisions regarding the placement of protected areas and the reintroduction or translocation of golden mantellas to favourable sites if needed.

METHODS

Data collection and study area

The aim of modelling was to explore potential suitable habitat to inform broader conservation decisions, in an area around Moramanga Province, Madagascar. Records of golden mantella sightings were collected by Madagasikara Voakajy research teams from ten sites within the protected areas of Mangabe, each containing or bordering known golden mantella breeding ponds. Nine of these sites were surveyed between 28 November 2014 – 12 December 2014, and the tenth earlier on in the year in March 2014. These periods correspond to the main breeding activity periods for this species. All surveys took place between 0700-1400 hrs each day, one visit per forest. The surveys were centered on breeding pools located in shallow depressions within the forest.

Species distribution modelling

A total of 198 golden mantellas were recorded across the ten surveyed sites in Moramanga. In order to meet the assumptions of Maxent with environmental data and reduce spatial bias, we needed to reduce golden mantella presence data to one observation (one frog) per 250 m grid square (See: Elith et al., 2011). In doing so we reduced presence data to 98 *Mantella aurantiaca* locations at a 250 m spatial grain.

Remotely sensed data have greatly improved over recent years and now provide good, useable information to answer ecological questions (Pfeifer et al., 2012). We used remotely sensed data for climate and habitat variables to model current and future distributions for golden mantellas. Four climate variables were selected from Worldclim (Hijmans et al., 2005) due to their biological relevance to frogs and because of low intercorrelation (Pearson's r < 0.7); Temperature seasonality (˚C x 10, standard deviation over monthly values); Mean temperature of the warmest quarter (˚C x 10, any consecutive 3-month period); Mean rainfall of the wettest quarter (mm, any consecutive 3-month period); Maximum water deficit (mm, consecutive months that experience rainfall < monthly PET (Potential Evapotranspiration, Hargreaves method), over which the shortfall in rain is accumulated. Raster development followed Pfeifer et al. (2018). This variable is also defined by Stephenson (1998) as the amount of water by which potential evapotranspiration (PET) exceeds actual evapotranspiration (AET).

Four habitat variables were selected because of their potential relevance to amphibians; Canopy height, Topographic wetness index, Landcover and Enhanced Vegetation Index (EVI). Canopy height (m) was sourced from NASA Earthdata (Simard et al., 2011; ORNL DAAC, 2017). Topographic wetness is a measure of the potential for water to flow into the grid cell and of how likely it is to remain there. We built the raster by using a 30 m filled Aster Digital Elevation Model (NASA/METI/AIST/Japan Spacesystems and U.S./Japan ASTER Science Team, 2001). From this we made two further rasters using ArcGIS 10.3.1 (ESRI, 2015) which described the accumulation of water flow (w) from the surrounding pixels and slope(s). We then used these respective rasters to calculate Topographic index from Ln(900w/tan(s) and values were normalised. Landcover classes are categorical variables such as cropland, forest etc, represented as a percentage of a grid square and interpolated from 1 km to 250 m resolution using bilinear interpolation (weighted distance average) in ArcGIS 10.3.1 (ESRI, 2015) (Arino, et al., 2012); Enhanced vegetation index reflects variation in canopy structure and architecture (Vieilledent et al., 2018). Mean annual Enhanced Vegetation Index is from 16-day 250 m MODIS MOD13Q1 data from the years 2007 – 2017 (Didan, et al., 2015).

Future climate projections (Representative Concentrations Pathways (RCP) 4.5 and 8.5) were sourced from AFRICLIM (Platts et al., 2015). RCP are greenhouse gas concentration projection scenarios adopted by the Intergovernmental Panel on Climate Change so that climate change studies and modelling might use a set of standardised measures (Van Vuuren et al., 2011). RCP 4.5 assumes CO_2 concentrations will continue to rise

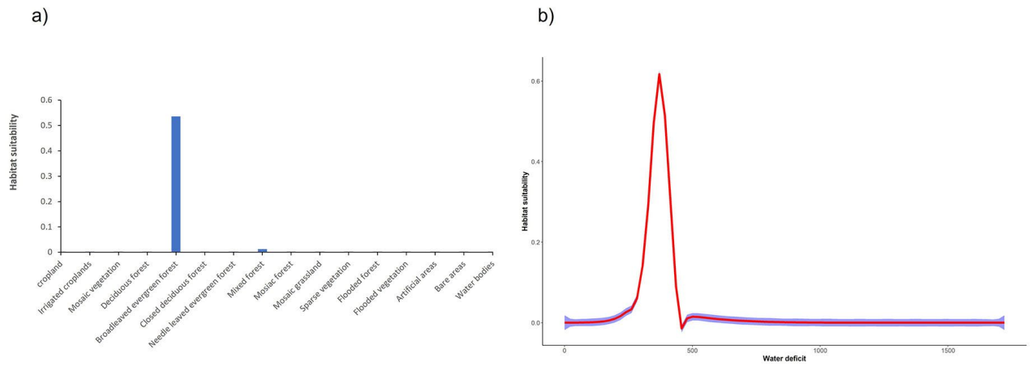

Figure 1. Habitat suitability in relation to **(a)** landcover categories and **(b)** water deficit. Broadleaved evergreen forest and the length and severity of the dry season are the main drivers for the distribution of golden mantellas. Habitat suitability is given as between 0 (unsuitable) and 1 (highly suitable) and is based on variables initially entered in to MaxEnt. Water deficit (Wd) is the amount of water by which potential evapotranspiration exceeds actual evapotranspiration (derived from remote sensed satellite data) and is indicative of the severity of the dry season. The red line is the response curve (fit of the data), the blue line is the standard deviation. Our model suggests habitat suitability is high where water deficit remains low at around 400 mm i.e. associated with a short dry season.

to approximately 650 parts per million (ppm) by 2100 and stabilise thereafter (Van Vuuren et al., 2011). RCP 8.5 assumes rising CO_2 concentrations to approximately 1370 ppm by 2100 (Van Vuuren et al., 2011).

Potential distributions were modelled using Maxent (v. 3.3.3k), a standard SDM technique using presence-only data (Hernández et al., 2006; Pearson, 2007). Climate data were at 1 km resolution and habitat/vegetation data were at 250 m resolution, but for Maxent to work, both sets of data must be at the same scale. All 1 km data were therefore interpolated to 250 m portions, ensuring that values in each grid cell were maintained, e.g. if the 1 km grid square had a temperature of 20 °C , then all of the 250 m grid squares that make up that 1 km grid square would also be at 20 °C. Habitat variables were included as static variables (a variable that may change with climate change, but we are unable to predict the amount of change due to confounding factors such as anthropogenic disturbance within the distribution models for future scenarios). We used static variables as it is difficult to model dynamic variable change (e.g. vegetation growth) along with projected climate change. Although we understand vegetation will alter with climate, preliminary runs of the model suffered from the exclusion of vegetation variables altogether: we therefore chose to keep these static variables (Stanton et al., 2012).

Maxent makes several assumptions that affect the performance of the model (Phillips et al., 2006) and constrain final spatial patterns of species distribution. We therefore used a regularisation multiplier, described by Merow et al. (2013) as placing a Bayesian priori distribution on model parameters (i.e. using current knowledge and reasonable expectation to predict potential distribution). The regularisation multiplier effectively constrains or relaxes the fit around the data balancing the need for both accuracy of predictions and generality (Elith et al., 2011). Prior to running final models, we adjusted the regularisation multiplier and selected the most appropriate model using Akaike Information Criteria (AIC) (Warren et al., 2010; Warren & Seifert, 2011). In addition, the final models were cross-validated ten times, and to determine drivers of distribution, we jack-knifed environmental data (Phillips et al., 2006). All other settings were set to default. We used Albers Africa Equal-area projection to equalise grid cell size (Elith et al., 2011) to ~0.250 m^2 and an appropriately scaled kernel density bias file was used to restrict the placement of pseudo-absences (Fourcade et al., 2014). Maxent is a presence-only modelling system based upon reliable species sightings, which means it does not utilise any known absence information. Instead, it fills the gaps using pseudo-absences (estimated absences). Pseudo-absences are estimated by taking known presence data for large numbers of similar species (kernel density file) and then determining the probability of finding a given species across different areas and habitat. This research used a kernel density file constructed from amphibian sightings across Madagascar. To identify areas of suitable habitat in current and future scenarios, we used maximum test sensitivity plus specificity logistic threshold which minimises error between specificity and sensitivity (false positives and false negatives) (Liu et al., 2005). The Habitat Suitability Index (Fig. 1), i.e. how suitable an area is for a species based upon the variables entered into the model, was calculated using Maxent. To describe the current golden mantella area of occurrence we developed a Minimum Convex Polygon (MCP) based on the raw data for *M. aurantiaca* occurrences and then added a 10 km buffer (e.g. Smith & Green, [2005] suggest maximum dispersal distances for most amphibians would not exceed far beyond 10 km), to create an over-estimate of current area (Fig. 2). Habitat suitability was projected across Moramanga district to identify potential areas

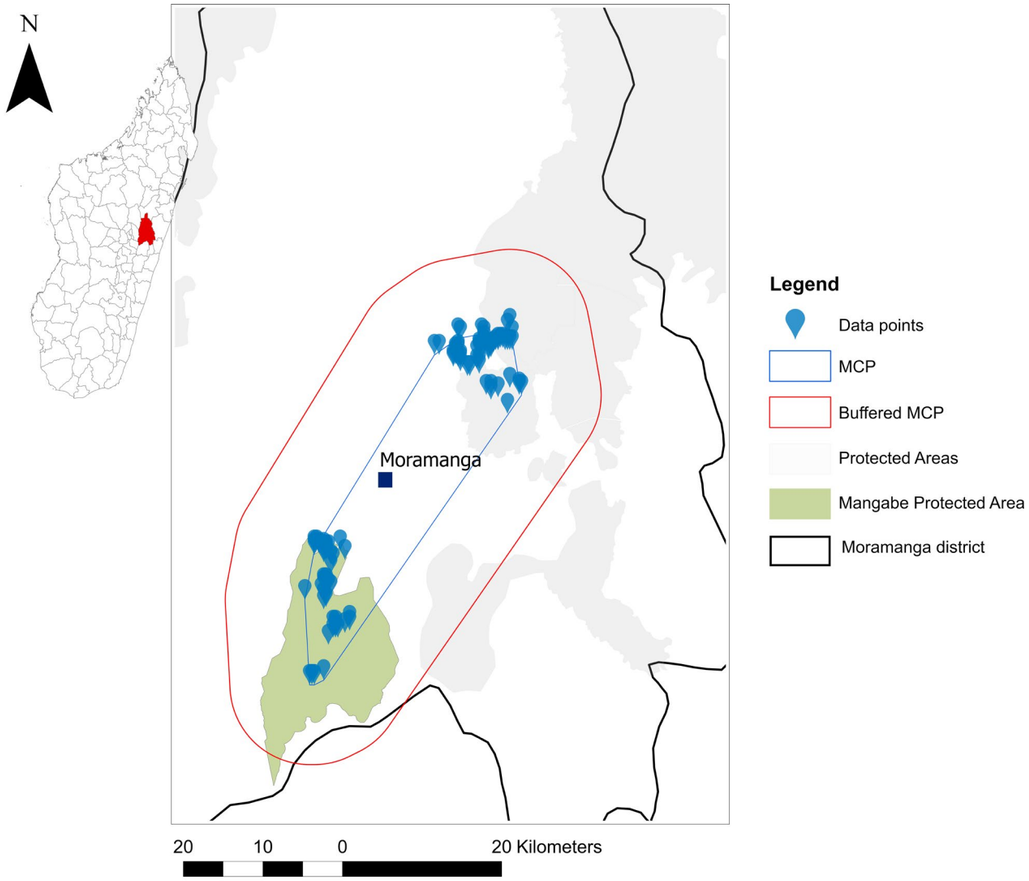

Figure 2. Study area. Data points for golden mantella are shown, from which a Minimum Convex Polygon (MCP) was developed. A 10 km buffer (buffered MCP) was used to account for potential maximum dispersal of frogs when assessing future climate scenarios after Species Distribution Modelling.

of suitable habitat for current conditions and whether suitable habitat fell within the MCP.

For each climate scenario we used a metric from Bungard et al. (2020) to measure the level of imperilment based on the index of net change (*Nc*) in area: *Nc* is calculated for golden mantellas, as the sum of the change for each future scenario; future increase in area (T_{fi}) (km^2) minus future decrease in area (T_{fd}) over the area under current climate conditions (T_c).
Equation 1.

$$Nc = \sum \frac{\left(T_{fi} - T_{fd}\right)}{T_c}$$

We used Protected Planet (2021) to identify the protected areas networks. Finally, we assessed how well the current system of protected area networks surrounding golden mantella area of occupancy accounts for golden mantella distribution in both current and future climate scenarios. To do this, we calculated for each scenario, the simple metric of area of suitable habitat within the protected area network/total area of suitable habitat using ARCGIS proTM.

RESULTS

Our model demonstrated a good fit with the data (AUC = 0.994, SD = 0.001) and showed that two main drivers influence *M. aurantiaca* distributions under current climatic conditions: landcover (contributed 32 % to the final model) and the length and severity of the dry season (water deficit; model contribution: 31 %) (Fig. 1). Mean temperature of the warmest quarter contributed 24 % to the final model, whilst all other variables each contributed < 2 % to the final model except mean rainfall of the wettest quarter (< 9 %). Golden mantellas are found mainly in broadleaved evergreen forest (rainforest) and only have a narrow tolerance of extended dry conditions. The potential distribution of golden mantellas under current climate conditions extends outside the current MCP (Fig. 3) with potentially highly suitable habitat occurring in a narrow south-west to north-east band

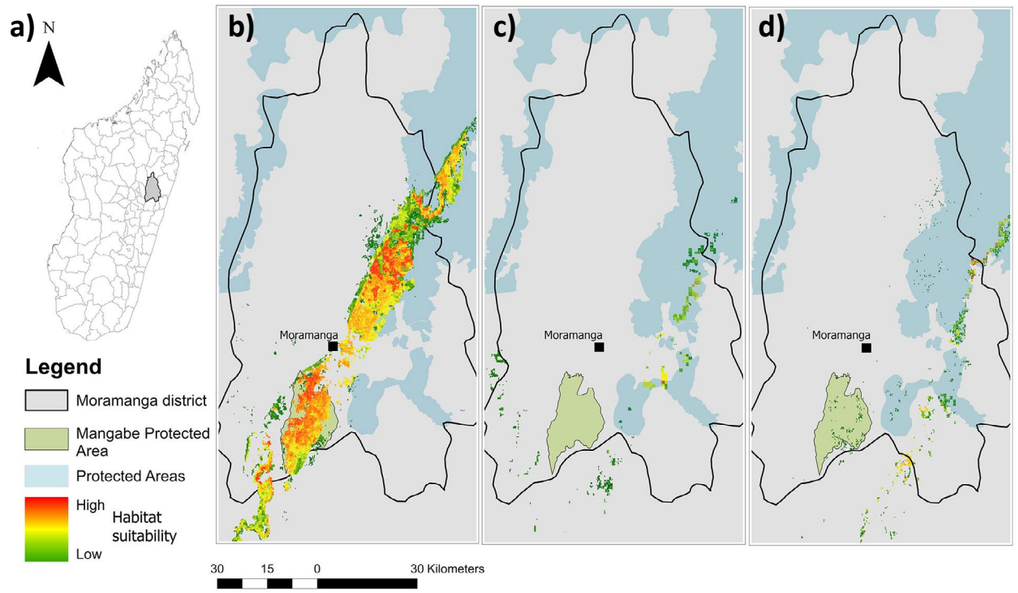

Figure 3. Species Distribution Modelling for the golden mantella showing **(a)** political divisions with Moramanga highlighted in grey with a black border; **(b)** potential distribution under current climate; potential distributions under **(c)** RCP 4.5, 2085 and **(d)** RCP 8.5, 2085, showing decrease in range and shift in a south-easterly direction.

divided into two distinct areas. These areas embrace the two known population centres for golden mantellas: Mangabe in the south and Torotorofotsy/Analamay in the north. From our models, local protected areas currently offer protection to 24 % of potentially suitable habitat for golden mantellas. As climate changes, so does the distribution of golden mantellas, with the area of suitable habitat decreasing from 2,110 km^2 (current climate) to 121 km^2 (= -0.94) and 138 km^2 (= -0.93) (RCP 4.5 and 8.5 respectively; Fig. 3). Furthermore, occupancy of suitable protected area decreases by 86 % for both climate scenarios. Slightly larger areas of suitable habitat predicted under the higher RCP 8.5 scenario would seem counter-intuitive, however it may be that more variation in topography or changes in range and availability of water at higher altitudes increases available area. Equally, although the overall distribution within protected areas is reduced, more of the range is shifted into existing protected areas under RCP 8.5 than under RCP 4.5 (see later discussion). Further, we observed a range shift under scenarios RCP 4.5 and RCP 8.5 to the south-east of the current distribution by 10-15 km (Fig. 3). Within the projected habitat distribution range under RCP 4.5 and 8.5, there are several areas that are predicted to be climatically stable (Fig. 4). By climatically stable we mean consistently provides areas of suitable habitat for golden mantellas across climate scenarios. Assuming landcover is appropriate, the areas predicted here could also provide suitable habitat in terms of water deficit i.e. the range of water deficit stays within the boundaries needed by golden mantellas.

DISCUSSION

We investigated whether projected climate change scenarios would influence current golden mantella population distributions in rainforest habitat in Madagascar. Our results suggest golden mantella population distribution is driven by the type of available habitat and the amount of water retained within those habitats. Our models predict that as the length and severity of the dry season increases, the availability of suitable habitat for golden mantellas decreases by more than 93 %, from 2110 km^2 currently to 121 km^2 under RCP 4.5, and to 138 km^2 under RCP 8.5 by 2085. Consequently, less than 7 % of currently available suitable habitat is likely to remain suitable under these scenarios. We also reveal that local protected areas currently offer protection to 24 % of potentially suitable habitat for golden mantellas. Models predict that the distribution of viable habitat will shift 10 – 15 km away from its current location with the majority (86 %) falling outside of protected areas.

The northern part of the RCP 8.5 projection falls within the Corridor Ankeniheny-Zahamena (CAZ) protected area. Covering some 3691 km^2, CAZ is one of the largest areas of rainforest in Madagascar and comprises a core protected area and sustainable use near the boundary. Likewise, the southern part of the RCP 8.5 projection falls within the Mangabe protected area which also includes a core protected zone and areas of sustainable use. In contrast, the projections of the RCP 4.5 model place the future distribution of golden mantellas outside protected areas.

Increased temperatures and reduced rainfall will

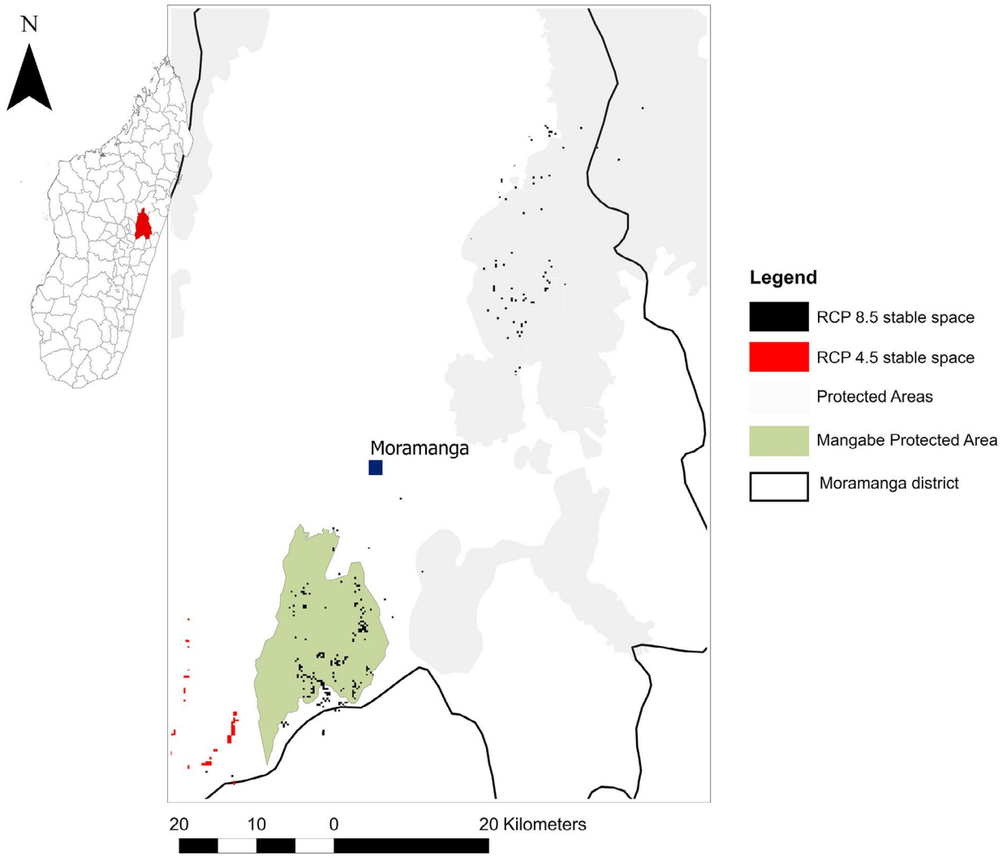

Figure 4. Climate stable spaces predicted within the range of projected distributions for RCP 4.5 and RCP 8.5. Protected areas are shown as light grey, with Mangabe (protected area that covers most of the current distribution of *M. aurantiaca*) highlighted in light green.

change forest habitat by restricting the availability of moisture to vegetation, soil and substrate (Bartelt et al., 2010). As microhabitat becomes warmer and drier the opportunity for thermoregulation and hydroregulation become more challenging. Frogs lose water quickly from the skin by evaporation, and to mitigate this loss they need to find moist habitat in which to take up water at least as quickly as it is being lost (Duellman & Trueb, 1994). Several studies have found that montane amphibians may shift range upslope to cooler areas when exposed to prolonged ambient temperature rises (Raxworthy et al., 2008). However, this is not an option for golden mantellas as they already live at, or close to, the crests of the slopes they inhabit. Further, although golden mantellas are known to migrate a few hundred metres between the crest and breeding ponds (Piludu et al., 2015), rather less is known regarding their long-range dispersal ability. Current mantella forest habitat is also highly fragmented and usually bordered by agricultural land or deforested areas. Consequently, land use other than forest may well prevent range expansion or shift to track preferred environmental variables. Indeed, Harrison et al. (2006) state that where a species is in decline they may not automatically shift or expand their current range to track preferred climatic variables. Willis et al. (2015) advise that if climate suitability changes markedly within a species' current distribution and there is no suitable climate/habitat within realistic colonisation range, then translocation to suitable areas should be considered. Indeed, rigorous habitat assessment should be an essential precursor for any translocation. Equally, any translocation strategy should assess the risks, benefits and cost-effectiveness of alternative approaches, such as whether stock should be sourced from captive breeding populations or non-threatened wild populations (Harding et al., 2016).

SDM results identify several locations considered climatically stable and relatively close (within the Moramanga area) to current golden mantella distributions (Fig. 4). However, most of the predicted stable areas are thought to contain degraded forest or agricultural fields (Pers. Comm. J.Razafimanahaka, 2021). Ideally, we would hope to survey those new sites and other areas in between current and potential distributions to ascertain

if there is a realistic opportunity to develop wildlife corridors, which may facilitate golden mantella range shift.

There is already a programme of survey and research which seeks new areas in which to create, restore or protect breeding ponds and habitat (Piludu et al., 2015); however, in light of our current findings, it may be prudent to consider searching further afield for new potential sites. Our results suggest these new sites should be sought a further 10-15 km south-east from current golden mantella distributions.

The complexity of biological interactions between species, environment and anthropogenic influence over time means there are constraints on the accuracy of any prediction we may make (Harrison et al., 2006). However, climate change is already impacting heavily on species and ecosystems (Hannah et al., 2008; Raxworthy et al., 2008; Tadross et al., 2008), and as such conservation actions should be planned and carried out without delay using the knowledge and techniques we do have, rather than wait until more advanced methods become available (Rowland et al., 2011).

We therefore recommend carrying out surveys to test whether newly highlighted areas identified as climatically stable or within projected distribution under climate change have the potential for translocation of golden mantellas. Where appropriate, this may involve habitat restoration to ensure water bodies for breeding and appropriate associated microhabitat (Edwards et al., 2019). Further research should be conducted into the feasibility of placing wildlife corridors between current and potential golden mantella distribution to facilitate range shift to safer areas. Expanding protection and status to potential climate stable areas and projected population distribution ranges should also be a priority.

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Data Accessibility

Detailed site data for golden mantellas is restricted and sensitive due to their Critically Endangered (CR) status and ongoing susceptibility to collection for the pet trade. Climate data was sourced from Worldclim (See: Hijmans et al., 2005) and AFRICLIM (See: Platts et al., 2015). Data downloaded/used in analysis from Worldclim are given in Table 1. Protected areas shape file for figures were courtesy of Protected Planet (2021).

REFERENCES

Arino, O., Perez, R., Julio, J., Vasileios, K., Bontemps, S., Defourny, P., & Van Bogaert, E. (2012). Global Land Cover Map for 2009 (GlobCover 2009). European Space Agency (ESA) & Université catholique de Louvain (UCL), PANGAEA. DOI: 10.1594/PANGAEA.787668

Bartelt, P.E., Klaver, R.W. & Porter, W.P. (2010). Modeling amphibian energetics, habitat suitability, and movements of western toads, *Anaxyrus* (= *Bufo*) *boreas*, across present and future landscapes. *Ecological Modelling* 221, 2675-2686.

Bateman, B.L., Murphy, H.T., Reside, A.E., Mokany, K. & VanDerWal, J. (2013). Appropriateness of full-, partial-and no-dispersal scenarios in climate change impact modelling, *Diversity and Distributions* 19, 1224-1234.

Blank, L. & Blaustein, L. (2012). Using ecological niche modeling to predict the distributions of two endangered amphibian species in aquatic breeding sites. *Hydrobiologia* 693, 157-167.

Brown, K.A., Parks, K.E., Bethell, C.A., Johnson, S.E. & Mulligan, M. (2015). Predicting plant diversity patterns in Madagascar: understanding the effects of climate and land cover change in a biodiversity hotspot. *PloS one* 10, e0122721.

Bungard, M. (2020). Predictive modelling for anuran responses to climate change in tropical montane ecosystems. PhD thesis, University of York.

Cao, Y., DeWalt, R.E., Robinson, J.L., Tweddale, T., Hinz, L. & Pessino, M. (2013). Using Maxent to model the historic distributions of stonefly species in Illinois streams: the effects of regularization and threshold selections. *Ecological Modelling* 259, 30-39.

Chunco, A.J., Phimmachak, S., Sivongxay, N. & Stuart, B.L. (2013). Predicting environmental suitability for a rare and threatened species (Lao Newt, *Laotriton laoensis*) using validated species distribution models. *PloS one* 8, e59853.

Cushman, S.A. (2006). Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation* 128, 231-240.

Didan, K., Munoz, A.B., Solano, R. & Huete, A. (2015). MODIS vegetation index user's guide (MOD13 series), Vegetation Index and Phenology Lab, The University of Arizona, pp. 1-38.

Duellman, W.E. & Trueb, L. (1994). Biology of amphibians, JHU press.

Echeverria, C., Coomes, D., Salas, J., Rey-Benayas, J.M., Lara, A. & Newton, A. (2006). Rapid deforestation and fragmentation of Chilean temperate forests. *Biological Conservation* 130, 481-494.

Edwards, W.M., Griffiths, R.A., Bungard, M.J., Rakotondrasoa, E.F. Razafimanahaka, J.H., Razafindraibe, P., Andriantsimanarilafy, R.R., & Randrianantoandro, J.C. (2019). Microhabitat preference of the critically endangered golden mantella frog in Madagascar. *Herpetological Journal* 29, 207-213.

Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E. & Yates, C.J. (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions* 17, 43-57.

ESRI (2015). ArcGIS Desktop: Release 10.3.1. Redlands, CA: Environmental Systems Research Institute.

Fourcade, Y., Engler, J.O., Rödder, D. & Secondi, J. (2014). Mapping species distributions with Maxent using a geographically

biased sample of presence data: a performance assessment of methods for correcting sampling bias. *PloS one* 9, e97122.

Glaw, F. & Vences, M. (2007). A field guide to the amphibians and reptiles of Madagascar, Vences & Glaw.

Groff, L.A., Marks, S.B. & Hayes, M.P. (2014). Using ecological niche models to direct rare amphibian surveys: A case study using the Oregon Spotted Frog (*Rana pretiosa*). *Herpetological Conservation and Biology* 9, 354-368.

Hannah, L., Dave, R., Lowry, P.P, Andelman, S., Andrianarisata, M., Andriamaro, L., Cameron, A., Hijmans, R., Kremen, C., MacKinnon, J., Randrianasolo, H.H., Andriambololonera, S., Razafimpahanana, A., Randriamahazo, H., Randrianarisoa, J., Razafinjatovo, P., Raxworthy, C., Schatz, G.E., Tadross, M. & Wilmé, L. (2008). Climate change adaptation for conservation in Madagascar. *Biology Letters* 4, 590-594.

Harding, G., Griffiths, R.A. & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology* 30, 340-349.

Harrison, P., Berry, P., Butt, N. & New, M. (2006). Modelling climate change impacts on species' distributions at the European scale: implications for conservation policy. *Environmental Science & Policy* 9, 116-128.

Hernandez, P.A., Graham, C.H., Master, L.L. & Albert, D.L. (2006). The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* 29, 773-785.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005). Very high-resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25, 1965-1978.

IUCN, (2021). The IUCN Red List of Threatened Species. Version (2020-23). http://iucnredlist.org. Downloaded on 10th March 2021.

Liu, C., Berry, P.M., Dawson, T.P. & Pearson, R.G. (2005). Selecting thresholds of occurrence in the prediction of species distributions. *Ecography* 28, 385-393.

Márcia-Barbosa, A., Real, R., Muñoz, A. & Brown, J.A. (2013). New measures for assessing model equilibrium and prediction mismatch in species distribution models. *Diversity and Distributions* 19, 1333-1338.

Merow, C., Smith, M.J. & Silander, J.A. (2013). A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography* 36, 1058-1069.

Meynard, C.N., Migeon, A. & Navajas, M. (2013). Uncertainties in predicting species distributions under climate change: A case study using *Tetranychus evansi* (Acari: Tetranychidae), a widespread agricultural pest. *PLoS One* 8, e66445.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403, 853-858.

NASA/METI/AIST/Japan Spacesystems and U.S./Japan ASTER Science Team (2001) ASTER DEM Product. distributed by NASA EOSDIS Land Processes DAAC. DOI: 10.5067/ASTER/AST14DEM.003.

ORNL DAAC (2017). Spatial Data Access Tool (SDAT). Oak Ridge, Tennessee, USA: ORNL DAAC. DOI: 10.3334/ORNLDAAC/1388.

Pearson, R.G. (2007). Species distribution modelling for conservation educators and practitioners. *Synthesis American Museum of Natural History*, vol. 50.

Pfeifer, M., Disney, M., Quaife, T. & Marchant, R. (2012). Terrestrial ecosystems from space: a review of earth observation products for macroecology applications. *Global Ecology and Biogeography* 21, 603-624.

Pfeifer, M., Gonsamo, A., Woodgate, W., Cayuela, L., Marshall, A.R., Ledo, A., Paine, T.C.E., Marchant, R., Burt, A., Calders, K., Courtney-Mustaphi, C., Cuni-Sanchez, A., Deere, N.J., Denu, D., de Tanago, J.G., Hayward, R., Lau, A., Macía, M.J., Olivier, P.I., Pellikka, P., Seki, H., Shirima, D., Trevithick, R., Wedeux, B., Wheeler, C., Munishi, P.K.T., Martin, T., Mustari, A. & Platts, P.J. (2018). Tropical forest canopies and their relationships with climate and disturbance: results from a global dataset of consistent field-based measurements. *Forest Ecosystems* 5, 1–14.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006). Maximum entropy modelling of species geographic distributions. *Ecological Modelling* 190, 231-259.

Piludu, N., Dubos, N., Razafimanahaka, J.H., Razafindraibe, P., Randrianantoandro, J.C. & Jenkins, R.K. (2015). Distribution, threats and conservation of a Critically Endangered amphibian (*Mantella aurantiaca*) in Eastern Madagascar. *Herpetology Notes*, 8, 119-123.

Platts, P.J., Omeny, P.A. & Marchant, R. (2015). AFRICLIM: high-resolution climate projections for ecological applications in Africa. *African Journal of Ecology* 53, 103-108.

Protected Planet, UNEP-WCMC & IUCN world database on protected areas. Version (2021). www.protectedplanet.net. Downloaded on 07/03/2021.

Raxworthy, C.J., Pearson, R.G., Rabibisoa, N., Rakotondrazafy, A.M., Ramanamanjato, J., Raselimanana, A.P., Wu, S., Nussbaum, R.A. & Stone, D.A. (2008). Extinction vulnerability of tropical montane endemism from warming and upslope displacement: a preliminary appraisal for the highest massif in Madagascar. *Global Change Biology* 14, 1703-1720.

Rodríguez-Rey, M., Jiménez-Valverde, A. & Acevedo, P. (2013). Species distribution models predict range expansion better than chance but not better than a simple dispersal model. *Ecological Modelling* 256, 1-5.

Rowland, E.L., Davison, J.E. & Graumlich, L.J. (2011). Approaches to evaluating climate change impacts on species: a guide to initiating the adaptation planning process. *Environmental Management* 47, 322-337.

Seidl, R., Thom, D., Kautz, M., Martin-Benito, D., Peltoniemi, M., Vacchiano, G., Wild, J., Ascoli, D., Petr, M. & Honkaniemi, J. (2017). Forest disturbances under climate change. *Nature Climate Change* 7, 395-402.

Sharifi, M., Karami, P., Akmali, V., Afroosheh, M. & Vaissi, S. (2017). Modeling geographic distribution for the endangered yellow spotted mountain newt, *Neurergus microspilotus* (Amphibia: Salamandridae) in Iran and Iraq. *Herpetological Conservation and Biology* 12, 488-497.

Simard, M., Pinto, N., Fisher, J.B. & Baccini, A. (2011). Mapping forest canopy height globally with spaceborne lidar. *Journal of Geophysical Research: Biogeosciences*, 116(4), 1–12. DOI: 10.1029/2011JG001708.

Smith, M.A. & Green, D.M. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* 28, 110-128.

Stanton, J.C., Pearson, R.G., Horning, N., Ersts, P. & Reşit Akçakaya, H. (2012). Combining static and dynamic variables in species distribution models under climate change. *Methods in Ecology and Evolution* 3, 349-357.

Stephenson, N. (1998). Actual evapotranspiration and deficit: biologically meaningful correlates of vegetation distribution across spatial scales. *Journal of Biogeography* 25, 855-870.

Tadross, M., Randriamarolaza, L., Rabefitia, Z. & Zheng, K. (2008). Climate change in Madagascar; recent past and future, Washington DC (World Bank).

Van Vuuren, D.P., Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., Hurtt, G.C., Kram, T., Krey, V. & Lamarque, J. (2011). The representative concentration pathways: an overview. *Climatic Change* 109, 5-31.

Vieilledent, G., Grinand, C., Rakotomalala, F.A., Ranaivosoa, R., Rakotoarijaona, J., Allnutt, T.F. & Achard, F. (2018). Combining global tree cover loss data with historical national forest cover maps to look at six decades of deforestation and forest fragmentation in Madagascar, *Biological Conservation* 222, 189-197.

Vieilledent, G., Grinand, C. & Vaudry, R. (2013). Forecasting deforestation and carbon emissions in tropical developing countries facing demographic expansion: a case study in Madagascar. *Ecology and Evolution* 3, 1702-1716.

Warren, D.L., Glor, R.E. & Turelli, M. (2010). ENMTools: a toolbox for comparative studies of environmental niche models. *Ecography* 33, 607-611.

Warren, D.L. & Seifert, S.N. (2011). Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. *Ecological Applications* 21, 335-342.

Willis, S., Foden, W., Baker, D., Belle, E., Burgess, N., Carr, J., Doswald, N., Garcia, R., Hartley, A. & Hof, C. (2015), Integrating climate change vulnerability assessments from species distribution models and trait-based approaches. *Biological Conservation* 190, 167-178.

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Diversity of herpetofauna at restored cranberry bogs: A comparative survey of herpetofaunal diversity at a restored wetland in comparison to a retired cranberry bog to assess the restoration success

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Wetlands perform critical ecological functions and provide wildlife habitats. Yet, wetland degradation continues at a global scale. In Massachusetts, USA, wetland restoration has reached remarkable heights, partly promoted by the retirement of cranberry bogs. In this study, to assess the effectiveness of cranberry-farm restoration for conservation of native herpetofauna, we surveyed both retired and restored cranberry bogs in south-eastern Massachusetts. Using both visual encounter surveys and baited aquatic traps, we documented herpetofaunal species and their relative abundance. Both survey methods combined, the cumulative herpetofaunal species richness at the restored bogs (16) exceeded that of the retired bogs (11). Our trap surveys indicated that the amphibian species richness at the retired bog was significantly greater than that of the restored bog. In contrast, reptilian species richness as well as the relative abundance of both amphibians and reptiles were significantly greater at the restored bog compared to the retired bog. Subsequent analyses we performed identified that greater habitat heterogeneity emerging from active restoration intervention was the underlying driver of elevated richness and abundance. Most frequently encountered herpetofauna at the restored versus retired bogs were habitat generalists with broader geographic ranges and are not of conservation concern. Our findings suggest that the restored bog we monitored is still in the early-recovery phase after active intervention. We urge the need for long-term herpetofaunal inventories via systematic, standard surveys to assess restoration success.

Keywords: restoration, wetlands, herpetofauna, conservation, community, cranberry bogs

INTRODUCTION

Wetlands are among the world's most productive ecosystems and sustain a myriad of ecosystem functions (Gibbs, 2000; Dudgeon et al., 2006). Despite limited global spatial extent (~6 % of the Earth's land area), wetlands are disproportionately high in biodiversity, hence considered keystone ecosystems (Reis et al., 2017; Gardner & Finlayson, 2018; Figel et al., 2019). Wetlands support a multitude of life-history needs of native fauna and flora. For example, as many as 9.5 % of animal species, including one-third of all vertebrate species, depend on wetlands for at least part of their life cycles (Balian et al., 2007). Despite the multifaceted ecological values, wetlands are among the most threatened habitats (Gibbs, 2000; Dudgeon et al., 2006; Keddy et al., 2009). In the US, draining and filling of wetlands has occurred since the 17th century (Dahl, 1990; Dahl et al., 1991; Gardner & Finlayson, 2018).

Given high conservation potential and functional attributes (stormwater retention, nutrient assimilation, groundwater recharge, and carbon sequestration), wetlands are crucial for global environmental sustainability and resilience (Zedler, 2000; Zedler & Kercher, 2005; Keddy et al., 2009). Therefore, wetland restoration is essential to preserve both wetland biodiversity and ecosystem functions. New sustainability policies and advancements in conservation research have led to commendable efforts in wetland restoration (Postel & Thompson Jr, 2005; Hoekstra et al., 2020). Ecological restoration is a process that recreates, initiates, assists, or accelerates the recovery of a degraded, damaged, or modified ecosystem with respect to environmental health, structural and functional integrity, and ecological sustainability (Ehrenfeld, 2000; Zedler, 2000). The restored state can either resemble the historic community structure and ecosystem processes or an alternative stable state (Suding et al., 2004; Martin, 2017).

While wetland restoration is widely practiced, post-restoration biological monitoring is either largely neglected or limited to opportunistic inventories. Post-restoration appraisals enable critical, comparative evaluation of restoration techniques (Downs & Kondolf,

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2002; Skinner et al., 2008), guide future management decisions, and help reduce uncertainties in contemporary applications (Michener, 1997; Skinner et al., 2008; Loflen et al., 2016). Monitoring is required to track progress along the recovery trajectory, implement corrective actions, and provide feedback on ecosystem state and restoration interventions, thereby inform future actions (Choi, 2004; Klemas, 2013). Although plant communities have been the overwhelming foci in wetland monitoring (Matthews & Spyreas, 2010), floristic diversity alone cannot be considered a universal biodiversity surrogate; thus faunal surveys may provide complementary insights for post-restoration assessments (Lewandowski et al., 2010). Herpetofauna are recognised for their heightened sensitivity to overall environmental quality, and therefore are widely regarded as an indicator of habitat status (Hager, 1998; Welsh Jr & Droege, 2001; Waddle, 2006; Welsh Jr & Hodgson, 2008). Given their shorter generation cycles compared to other tetrapods, herpetofauna can elicit rapid ecological responses to restoration. Many amphibians and reptiles exhibit both ontogenetic and seasonal shifts in habitat associations, thus their community composition can reflect emergent properties of the overall restored wetland complex (Gibbons et al., 2000; Davic & Welsh, 2004). Additionally, in North America, herpetofauna account for a substantial biomass across a wide array of wetlands (Russell et al., 2002a; Russell et al., 2002b; Balcombe et al., 2005). These attributes make monitoring herpetofauna a prudent approach to monitor biological outcomes of wetland restoration.

In this study, we conducted a comparative survey on herpetofauna across two wetland habitat types in south-eastern Massachusetts: an unrestored, retired cranberry bog (hereafter, referred to as the "retired bog") and a former cranberry bog recently restored into a freshwater wetland complex (hereafter, referred to as the "restored bog"). The specific objectives of our study were to (1) compare species richness, (2) overall abundance, and (3) community structure of herpetofauna between retired and restored wetland systems. Our study will elucidate how restoration of retired cranberry bogs into self-sustaining wetlands aid biodiversity conservation.

Study area

Located in south-eastern Massachusetts (Fig. 1) of the North-eastern Coastal ecoregion, our study area abuts Cape Code Pine Barrens, Narragansett and Bristol Lowlands, and southern New England Coastal Plains and Hills. The specific sites we surveyed included (1) Tidmarsh Wildlife Sanctuary, a recently restored (2016) 481-acre freshwater wetland complex (TWS), managed by Mass Audubon, and (2) Foothills Preserve (FP), a 42-acre retired cranberry bog complex, located north-west of TWS and owned by the Town of Plymouth. Both sites were former commercial-scale cranberry farms that operated in unison for over a century. Both TWS and FP are similar in land-use histories, geographical context, and elevation and are in proximity to each other, hence any biological differences are attributable to restoration. Nearly 200 acres of TWS were restored into a mosaic of wetland, aquatic, and upland habitat types (Ballantine et al., 2020). This included: dam removal and partially or completely plugging irrigation canals while reconstructing the meandering lotic systems to reconnect stream channels with floodplains (Norriss, 2018). Introduction of large dead wood and reformation of riffle-pool mesohabitat sequences restored the structural diversity of stream habitats. Additionally, to enhance spatial heterogeneity across the floodplain, a pit-and-mound microtopography was formed throughout the former bogs. Creation of open-water lentic systems also enhanced the habitat diversity across the wetland complex whereas introduction of native trees and shrubs (Atlantic white cedar in particular) assisted in accelerating wetland recovery. The entire restoration process was a collaborative venture between Massachusetts Division of Ecological Restoration, Tidmarsh Living Observatory (a network of academic research institutes), and Mass Audubon. In contrast, FP is neither actively restored nor has been managed as a commercially productive bog since 2010 (by the conclusion of fieldwork); thus, it has underdone secondary succession in the absence of major extrinsic disturbances. As such, the retention ponds, dams, irrigation channels, perimeter ditches, channelised stream flow, and sand layers remained still intact, thus, FP is heavily influenced by farming legacies. In contrast to TWS, no microtopographic complexity exists at FP. TWS contains greater habitat diversity than FP, which is attributable to the restoration process since the pre-restoration habitat stricture at TWS and FP were the same.

METHODS

We conducted our survey from mid-May 2019 to mid-November 2019 and used two standard techniques to adequately survey all habitat types at both TWS and FP, including open waters, wetlands, and uplands: (1) deployment and overnight recovery of non-lethal standard, baited aquatic traps and (2) visual encounter surveys (VES). These techniques have been successfully employed in similar habitats for the same focal taxa elsewhere (Adams et al., 1997; Fellers, 1997; O'Donnell et al., 2007; da Silva, 2010). We conducted sampling between May and August with three consecutive trap nights per week. Our sampling period corresponded with the increased activity of herpetofauna during the growing season. In each trap, we recorded the species, sex (for sexually dimorphic species), age class (adult or juvenile/larvae), and relative abundance of each species. After proper identification, we released all captured animals back to the capture site. In successive deployments, we replaced the bait.

Trap types we used included: (1) funnel traps, (2) minnow traps (large and small Promar Collapsible Traps, Cabela's LLC), and crab traps (Memphis Nets & Twine Co, Inc) placed in shallow water environments, and (3) hoop traps (Memphis Nets & Twine Co, Inc) suspended with stakes and floats, and placed in deeper and open-water habitats of ponds. Upon deployment, we ensured that at least a third of the trap height was above water. Funnel,

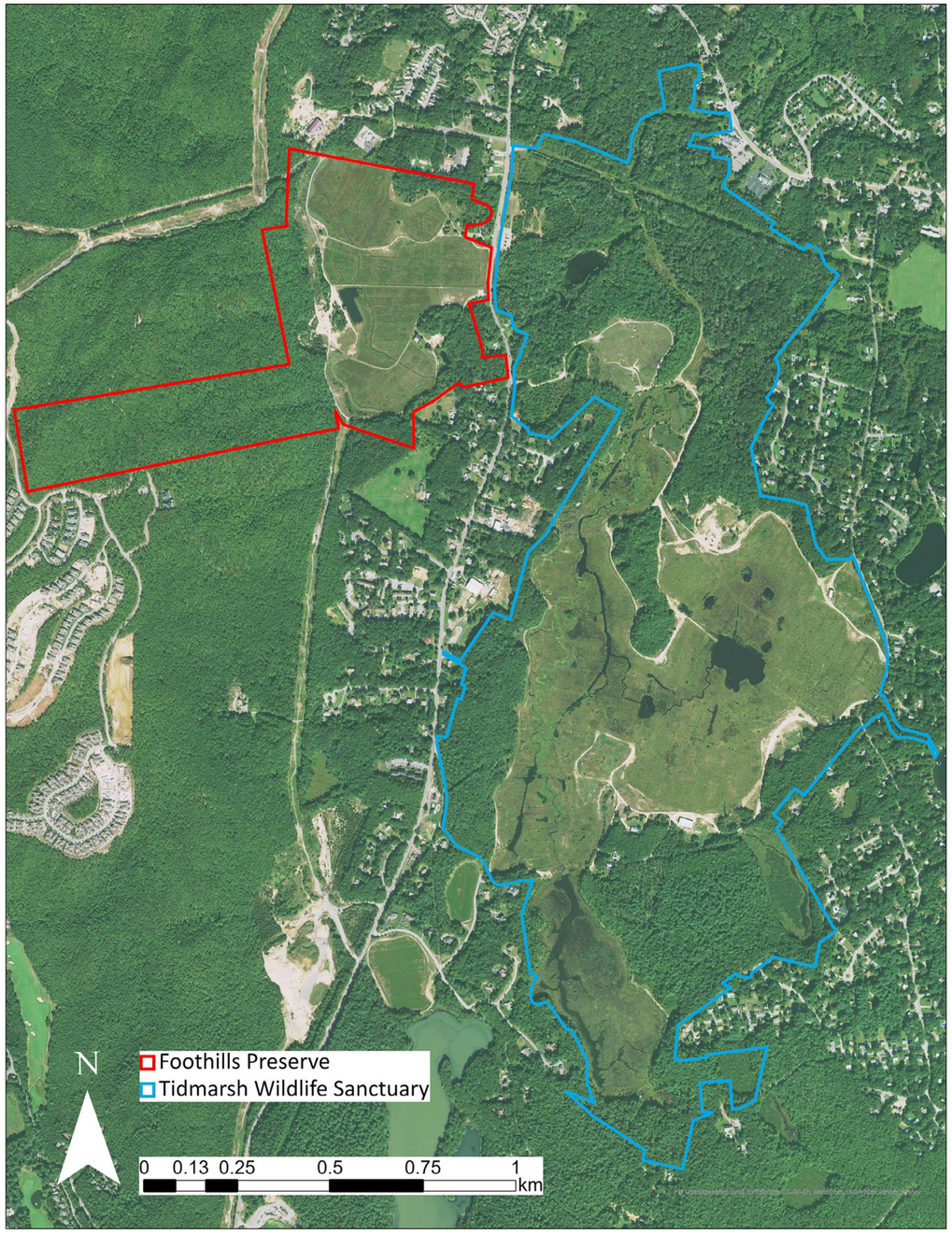
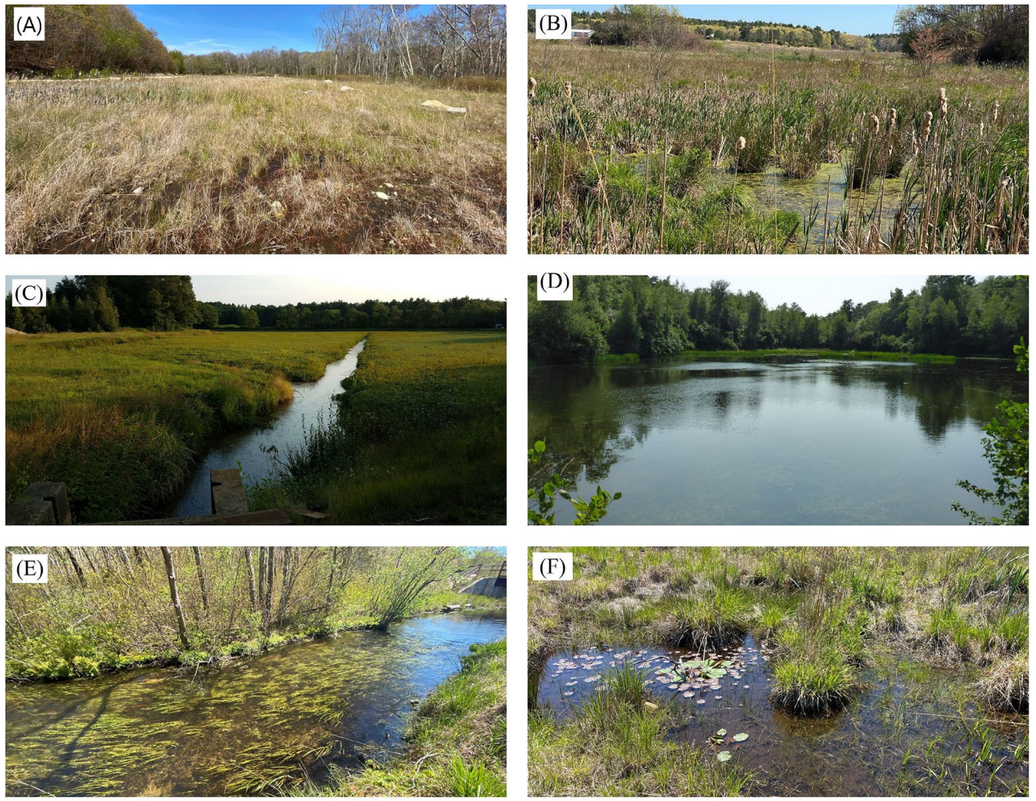


Figure 1. Study sites: Mass Audubon Tidmarsh Wildlife Sanctuary (TWS), a 481-acre restored wetland complex (Blue), and Foothills Preserve (FP), a 42-acre unrestored, retired cranberry bog (Red). Both are located in Plymouth, Massachusetts. Data sources: ESRI World Imagery, ESRI World Street Map.

hoop, and crab traps were baited interchangeably with either oil-immersed sardines or wet cat food whereas minnow traps were baited with dry, beef-based dog food. Use of these trapping methods and baits have been successful in similar research (Adams et al., 1997; Willson & Dorcas, 2004).

Between the restored and retired sites, the number of traps deployed varied as a function of availability of habitat types (Fig. 2), spatial extents, and spatial arrangement. The retired cranberry bog only had two



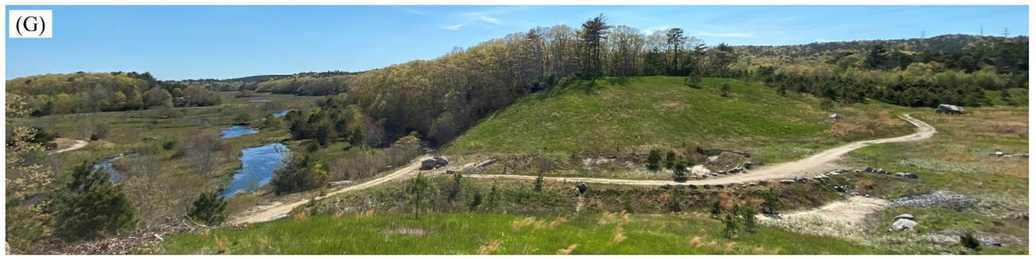


Figure 2. (A-G): Images of the restored bog and unrestored habitat types at the retired cranberry bog: **(A)** floodplain, **(B)** mesic prairie, **(C)** irrigation canal, **(D)** holding pond, **(E)** stream channel, **(F)** ephemeral pool, and **(G)** xeric uplands. All A-G are restored locations. Only C-D are the unrestored habitats.

habitat types that summed to eight distinct trapping sites: irrigation canals (both perimeter and lateral ditches) as lotic systems and holding (retention) ponds as lentic systems. We deployed 2-3 funnel traps and one minnow trap at each retention pond and 1-2 funnel traps per irrigation canal. The restored bog had three different habitat types: freshwater marshes (floodplains, mesic prairies, wet meadows), dugout open-water ponds (lentic systems), and reengineered, meandering streams (lotic systems). We deployed 2-3 funnel (when water levels are high) or 1-2 crab traps (when water levels are lower) within freshwater marshlands. We deployed 3-5 traps per dugout pond, which included 3-4 funnel traps, 2-3 minnow traps, and one hoop trap. At stream habitats, we deployed 2-3 funnel traps. We set traps at a total of 32 sites within the restored bog.

In addition to aquatic trapping, we conducted weekly VES to bolster detection of aquatic herpetofauna in upland habitats and to document semi-aquatic or terrestrial species. During VES, we actively searched throughout upland habitats and dip-netted in areas with standing water while visually scanning for surface-active individuals, and captured animals either manually or by nets. We recorded species identity, sex (if sexually dimorphic), and life-history stage (adult, juvenile/larvae) for all herpetofauna found during VES. If egg masses were found, we also attempted to identify them to the finest taxonomic resolution. The VES varied in person hours and area covered (45-120 mins with 2-6 people) across different habitats. The VES were non-systematic,

therefore, only used to document species presence.

Since the response variables (relative abundance and species richness) did not fit into a Gaussian distribution, had high levels of heteroscedasticity, and our sampling efforts being unevenly distributed between restored versus retired bogs, we opted for non-parametric tests in our statistical analyses. To account for dissimilar trapping efforts among different sampling locations, we calculated the catch per unit effort as number of individuals or species captured per trap night per deployment site to standardise trap data across different habitat types.

$$\text{Species richness} = \frac{\text{Total number of species captured per trapping site per night}}{\text{Number of traps deployed at the site}}$$

$$\text{Relative abundance} = \frac{\text{Total number of individuals captured for a given species per trapping site per night}}{\text{Number of traps deployed at the site}}$$

To account for seasonality and temporal effects of captures, we used the sampling month as covariates. To examine significant differences between the restored and retired sites for herpetofaunal species richness and total abundance, we ran an approximative Wilcoxon-Mann-Whitney (WMW) test where the species richness or relative abundance were considered as the response variables and binary restoration status (actively restored or retired with no active restoration efforts) as the predictor variable. To account for temporal effects, we blocked for the sampling month and ran the same test without blocks.

We ran Permutational Analyses of Univariate Variance (PermANOVA) for modeling species richness and abundance of reptiles, amphibians, and total herpetofauna from trap data (Freeman-Lane algorithm with 5,000 permutations). We also ran Permutational Multivariate Analyses of Variance (PerMANOVA) for modeling overall community structure of herpetofauna from trap data considering the sampling month as a covariate. To determine the environmental drivers of aquatic herpetofaunal community, we first calculated the Bray-Curtis matrix for the herpetofaunal community and considered the distance matrix as the response variable with Euclidified squareroot transformed dissimilarities. We treated sampling month, and restoration status as main effects, habitat type as a nested variable of restoration status and the specific site where the traps were deployed as a nested variable of habitat type. In addition, we included interactions between restoration status × month, habitat type × month, and trapping site × month. For unordered categorical predictor variables, sum contrasts were set-up where coefficients for each categorical variable were constrained to add up to zero while polynomial contrasts were set for ordered categorical predictors (such as sampling months). We used R statistical programme and RStudio Intergraded Development Environment for all statistical analyses (RStudio Team, 2020; R Core Team, 2021).

RESULTS

Combing both VES and trap surveys, we recorded a total 10 and eight amphibian species and four and six reptile species at the restored and retired bogs, respectively (Table 1). Among amphibians, all but spotted salamanders (*Ambystoma maculatum*) and northern leopard frogs (*Lithobates pipiens*) were documented as adults. The spotted salamander was documented based on a single cluster of egg masses found during our VES while the northern leopard frog was found as larvae during our trap surveys; both in marsh habitats of the restored bog. The rest of the anurans were documented as both larvae and adults (Table 1). For both the restored and retired bogs, American bullfrogs (*Lithobates catesbeianus*) and green frogs (*Lithobates clamitans*) were the most abundant amphibian, while Eastern painted turtles (*Chrysemys picta*) were the most abundant reptile. We only found a single non-native species, the red eared slider (*Trachemys scripta elegans*), an encounter limited to a single trap capture at an open-water lentic system at TWS (Table 1).

Herpetofaunal species richness and abundance between the restored versus retired bog

Based on trap surveys, amphibian species richness was significantly less at the restored bog than in the retired bog (WMW test: $z = -4.36$, $p < 0.0001$) even after controlling for the temporal effects (WMW test: $z = -3.99$, $p < 0.0001$) (Fig. 3). In contrast, reptile species richness was significantly greater in the restored bog than in the retired bog (WMW test: $z = 3.54$, $p = 0.0001$), even when accounted for temporal effects (WMW test: $z = 3.63$, $p = 0.0001$) (Fig. 3). Likewise, overall herpetofaunal species richness from trap surveys was significantly greater in the restored bog than in the retired bog (WMW test: $z = -2.76$, $p = 0.003$), even when controlled for the temporal effects (WMW test: $Z = -2.60$, $P = 0.0057$) (Fig. 3). Overall abundance of both amphibians (WMW test: $z = -6.96$, $p < 0.0001$) and reptiles (WMW test: $z = -3.36$, $p < 0.0003$) as well as for all herpetofauna combined (WMW test: $z = -6.35$, $p < 0.0001$) was significantly lower for the retried cranberry bog than the restored bog. These inferences remained unaffected even when controlled for temporal variability (amphibians: $z = -6.98$, $p < 0.0001$; reptiles: $Z = -3.65$, $p = 0.0002$; herpetofauna: $z = -6.42$, $p < 0.0001$).

Drivers of herpetofaunal richness and abundance

Habitat type, specific trapping site, and the interaction terms between habitat type × month as well as trapping site × month were the significant drivers of amphibian species richness (PermANOVA, Table 2). The trapping site × month interaction was the only significant driver for amphibian abundance. Among significant predictor variables, trapping site × month interaction had the strongest influence on amphibian species richness. Neither amphibian richness nor abundance varied significantly as a function of time alone, yet, space × time interaction appeared significant in driving amphibian diversity metrics. Similarly, the restoration status alone (i.e., whether a restored or a retired bog) had no influence on amphibian richness or abundance. Rather, specific

Table 1. The presence or absence of all documented amphibian and reptile species in restored wetlands and retired cranberry bogs as well as different habitat types nested therein. Presence or absence was determined using both trapping and Visual Encounter Survey (VES) data combined. Restored vs retired column indicates where each species was found: restored wetlands only (RS), retired cranberry bogs only (RT); or both (B). Life-history stage column states what life-history stages were found for each species: adult (A), juvenile (J), larval (L), or egg-masses (E).

Scientific Name	Vernacular name	Restored vs retired	Freshwater marshes & floodplains	Irrigation canal	Lentic system	Lotic system	Ephemeral pools	Xeric upland	Life- history stage
Ambystoma maculatum	Spotted salamander	RS			x		x		E
Anaxyrus americanus	American toad	B	x	x	x	x	x	x	A, J
Anaxyrus fowleri	Fowler's toad	B	x	x	x		x	x	A, L
Dryophytes versicolor	Gray treefrog	B	x	x	x				A, L
Lithobates catesbeianus	American bullfrog	B	x	x	x	x			A, L
Lithobates clamitans	Green frog	B	x	x	x	x	x		A, L
Lithobates palustris	Pickerel frog	B	x	x	x				A, L
Lithobates pipiens	Northern leopard frog	RS			x	x			L
Lithobates sylvaticus	Wood frog	B	x			x			A
Pseudacris crucifer	Spring peeper	B			x				A, L
Reptiles									
Chelydra serpentina	Common snapping turtle	B	x	x	x	x		x	A, J
Chrysemys picta	Eastern painted turtle	B	x	x	x	x		x	A, J
Sternotherus odoratus	Eastern musk turtle	RS			x	x			A, J
Storeria occipitomaculata	Northern red-bellied snake	RT		x					A, J
Thamnophis sirtalis	Common garter snake	B	x						A, J
Thamnophis sauritus	Eastern ribbon snake	RS	x			x			A, J
Trachemys scripta elegans	Red-eared Slider	RS			X				A

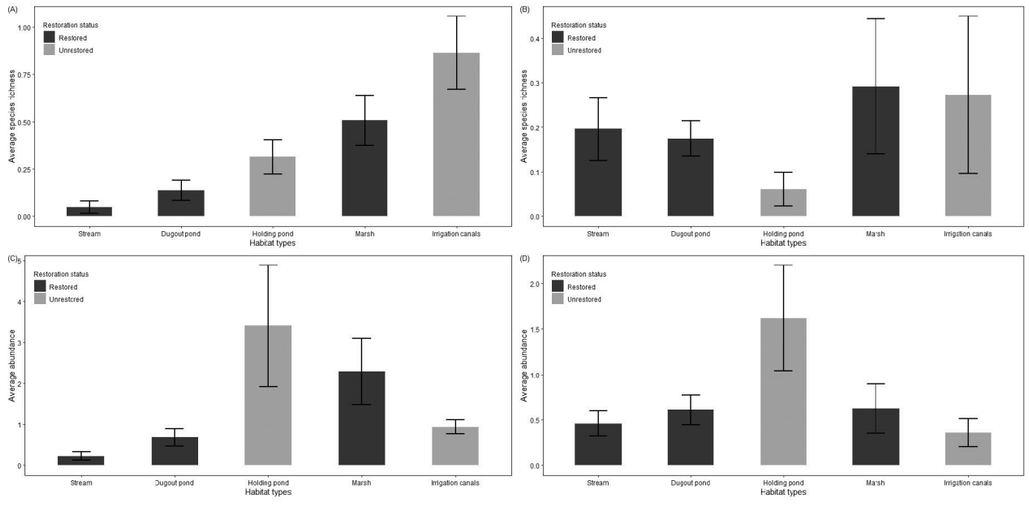


Figure 3. Diversity metrics of herpetofauna from surveys at the restored bog (Mass Audubon's Tidmarsh Wildlife Sanctuary, TWS) and the retired bog (Foothills Preserve, FP). Species richness of amphibians **(A)** and reptiles **(B)** and total abundance of amphibians **(C)** and reptiles **(D)**.

Table 2. (A-F): Results of the Permutational Analyses of Variance (PermANOVA) for modeling species richness and total abundance of both amphibians (A, B) and reptiles (C, D). Species richness and total abundance were included as response variables. Main effects are trap type, restoration status (restored or unrestored), and date of sampling. Habitat type nested within restoration status and trap location nested within habitat type are also included as predictor variables. Interactions include habitat type × sampling month and trap location × month. 0<'***'> 0.001 <'**'> 0.01 <'*'>0.05

Predictor variable	Amphibians						Reptiles					
	(A) Species richness			(B) Abundance			(C) Species richness			(D) Abundance		
	SS	F	p	SS	F	p	SS	F	p	SS	F	p
Trap type	0.43	0.781	0.496	77.902	0.739	0.442	0.537	2.544	0.058	31.456	3.993	0.025*
Restoration status: restored wetlands or retried bogs	0.013	0.072	0.281	53.976	1.536	0.441	0.066	0.932	0.744	0.764	0.290	0.164
Month of survey	0.004	0.019	0.889	9.074	0.258	0.611	0.539	7.667	0.006**	0.070	0.027	0.873
Habitat type	1.539	1.383	0.025*	186.352	0.884	0.165	0.281	0.667	0.604	2.979	0.189	0.102
Trapping site	8.027	0.676	0.001**	1650.651	0.734	0.123	1.494	0.332	0.028*	101.293	0.603	0.214
Habitat type × month	5.491	1.480	0.029*	369.213	0.525	0.136	1.762	1.252	0.824	72.978	1.390	0.209
Trapping site × month	13.564	0.914	0.023*	2672.976	0.950	0.038*	6.835	1.215	0.000***	455.067	2.166	0.021*

Table 3. Permutational Multivariate Analysis of Variance (PerMANOVA) of the overall community structure of herpetofauna from trap surveys. The Bray-Curtis distance matrix was used as the multivariate response variable, restoration status (retired bog or restored wetland), habitat types, trap type, and the interactions between the above main effects were used as predictors while the date of the surveys as a covariate to correct for the temporal effects. Significant codes: 0< '***' >0.001 <'**'> 0.01 <'*'>0.05

	Herpetofauna Community Structure				
	SS	MS	F	R^2	Pr(>F)
Restoration status: restored wetlands or retried bogs	0.1981	0.198085	20.5315	0.0342	0.001***
Date of survey	0.0933	0.018659	1.934	0.01611	0.043*
Habitat type	0.2786	0.092873	9.6262	0.0481	0.001***
Trapping site	0.206	0.017169	1.7795	0.03557	0.014*
Habitat type × month	0.1363	0.010482	1.0865	0.02352	0.048*
Trapping site × month	0.3732	0.008482	0.8791	0.06443	0.609
Trapping site × month	13.564	0.914	0.023*	2672.976	0.950

habitat types that have emerged due to restoration and bog retirement were the drivers of the amphibian assemblage (PermANOVA, Table 2). All traps used seemed to be equally successful in capturing amphibians as trap type had no significant effects on amphibian diversity metrics.

Significant predictors of reptile species richness included: trapping site × month interaction, trapping site, and the survey month while trapping site × month interaction was the only significant environmental predictor of reptile abundance (PermANOVA, Table 2). The trapping site × month interaction was the most influential variable for both abundance and richness of reptiles. This indicated that amphibian species richness varied inconsistently among different habitat types as well as trapping sites across the sampling season, which underpinned the importance of seasonality in structuring the herpetofaunal assemblages. The catch per unit effort seemed to have also varied among different trap types as evident by significance of the trap type as a predictor of reptile abundance. Like amphibians, the restoration status alone influenced neither the reptile richness nor abundance. Rather, specific locations and habitat types that emerged in response to restoration and bog retirement were the drivers of the reptile assemblage (PermANOVA, Table 2).

Drivers of the herpetofaunal community structure and composition

The overall variation in species composition in the entire herpetofaunal assemblage was significantly driven by restoration status, survey month, habitat type, and trapping site while the restoration status accounted for the greatest variation in species composition (PerMANOVA, Table 3). The habitat type × month interaction was also significant, which further underpinned the seasonality effect on structuring the herpetofaunal community.

DISCUSSION

The species inventory we complied for both the restored and retired bogs— VES and traps combined— included 17 species of herpetofauna. Among these, 16 were recorded from the restored bog at TWS, whereas 11 species were recorded from the retired bog (Table 1) at FP. Eleven species were shared between the restored and retired bogs. Five species were unique to the restored bog although only a single species was exclusive to the

retired bogs. Ecological responses of amphibians and reptiles to wetland restoration were different as revealed by our analyses.

Aquatic amphibian communities at the retired bog were significantly greater than that of the restored bog while the opposite was true for aquatic reptiles. Nonetheless, the overall abundance of all herpetofauna in the restored bog exceeded that of retired bogs. The amphibian community at both the restored and retired bogs were dominated by Ranids. Throughout the northern temperate zone, Ranids have been successful at colonising waterbodies in industrial agroecological systems as well as other artificial wetlands (stormwater ponds, cattle ponds, millponds) despite high degrees of nutrient pollution (Homan et al., 2004; Brand & Snodgrass, 2010). Relatively large, hydrologically stable constructed wetlands (such as farm reservoirs) in our study area can support both amphibian reproduction and a greater biomass than smaller ephemeral wetlands (Pechmann et al., 1989; Baber et al., 2004). The reservoirs in the retired bog are larger in size (both surface area and volume) than the restored open-water habitats (Parris, 2006). Therefore, the former offers more niche space and other critical resources for amphibians than the latter. Consequently, the retired bog can accommodate a diverse assemblage of amphibians.

The larval development of large, North-American Ranids usually takes multiple years, therefore, their fitness increases in perennial water bodies such as those found in farmlands (Paton & Crouch III, 2002; Shulse et al., 2010). The reservoirs of retired bogs are comparable to those of farmlands— perennial, nutrient-rich, homogenous in habitat structure, and fish occupied— thus are more suitable for widely distributed Ranids such as bullfrogs and green frogs (Paton & Crouch III, 2002). These Ranids have anti-predatory adaptations (distasteful larvae, avoidance behaviour, or rapid growth), therefore fish presence has no tangible impacts on their survival (Shulse et al., 2010). Further, without active farming, standing water in the retired bog is limited to irrigation canals and reservoirs, which act as refugia for aquatic obligates. This can inflate the amphibian richness in sites we sampled. The agricultural history and homogenised habitat structure at the retired bog can be less suitable for amphibian predators. Anthropocentric landscapes— industrial agroecosystems in particular— undergo biotic homogenisation where human commensals and generalists are accrued at the expense of rare species and habitat specialists (McKinney & Lockwood, 1999; McKinney & Lockwood, 2001; Baeten et al., 2012). Recruitment of tolerant species can elevate absolute species richness in human-modified habitats though such species assemblages are unlikely to include range-restricted and unique species or species of conservation concern (Baber et al., 2004).

The active interventions in restored habitats— pit-and-mound microtopography, reengineered meandering stream channels, ephemeral wetlands with variable hydroperiods, addition of woody debris, and introduction of native flora— have contributed to a much greater structural diversity and overall habitat heterogeneity while increasing the overall wetland acreage (Dimino et al., 2018; McCanty & Christian, 2018). As such, the restored bog provides optimal niche dimensions for a range of life-history functions such as foraging, reproduction, nursing, hibernation, aestivation, and growth (Zedler, 2000; Funk et al., 2013). Importance of niche breadth for herpetofaunal assemblages and other aquatic communities have been substantiated across different geographies (Krzysik, 1979; Behangana & Luiselli, 2008; Marino et al., 2019). Consequently, as predicted by niche diversification and habitat-area concepts, reptile species richness as well as overall abundance of both amphibians and reptiles were greater at the restored bog. The acreage of restored bog is much larger than that of the retired bog, therefore, the former offers a broader resource base, which elevates both the carrying capacity and intrinsic growth rate of herptile populations (Griffen & Drake, 2008). Given the greater habitat area, the restored bog is less burdened with edge effects and more resilient to anthropogenic disturbances emerging from the suburban landscape matrix (Harper et al., 2005). Hence, habitat size can be an important driver of differential herpetofaunal richness and abundance between restored verses retired bogs.

The restored and retired bogs we studied were managed together for commercial cranberry farming for centuries using the same management strategies. When in active production, the restored (TWS) and retired bogs (FP) in our study had comparable habitat structures including cultivated cranberry beds, reservoirs, irrigation channels, perimeter ditches, and surrounding woodlands. Given geographic proximity, both FP and TWS likely have the same source populations and equally accessible by dispersing herpetofauna. Thus, pre-restoration and pre-retirement habitat conditions as well as the original herpetofaunal community structure at TWS and FP are likely similar. Therefore, the observed biological differences can be attributed reliably to wetland restoration. The availability of new habitats and enhanced habitat heterogeneity resulting from restoration can be the primary divers of greater reptile richness and overall herpetofaunal abundance at the restored bog.

Unexpectedly lower amphibian richness at the restored bog can be attributable to several mechanisms. The restoration interventions in wetland environments create a single, prolonged, intensive, pulse disturbance, which includes dramatic changes in the surface topography, hydrologic processes, and nutrient dynamics (McCanty & Christian, 2018; Hoekstra et al., 2020). For instance, dam removal and sand excavation resuspend copious volumes of nutrients into the water column and alters the fluvial processes while microtopographic modifications alter the subterranean microhabitats as well as surface cover structure. These major disturbances in the physical habitat structure, aquatic biochemistry, and hydrology can result in mortality, shrinking the species richness of remnant, post-restoration biological communities (Middleton, 1999; Petranka et al., 2007).

Amphibians are sensitive to environmental perturbations (Blaustein et al., 1994). Restoration

actions can act as a pulse disturbance, which may delay colonisation of disturbance-intolerant amphibians. Amphibians are patchily distributed across their breeding habitats, highly philopatric, and have low vagility (Davic & Welsh, 2004). Therefore, amphibian community in restored habitats may remain species depauperate in early stages of post-restoration (Lehtinen & Galatowitsch, 2001). Particularly given limited home ranges and dispersal and dependency on habitat connectivity, saturation of amphibian richness in the restored bog may take multiple years (Burbrink et al., 1998; Hager, 1998; Grant et al., 2010). Nested in a suburban landscape (Walberg, 2013; Norriss, 2018), TWS may not have sufficient old-growth forest cover to support adult life-history needs of amphibians (Semlitsch, 2002; Petranka et al., 2007; Blomquist & Hunter, 2009). In early phases of colonisation, selection processes favor species with high mobility and generalist traits while species with specialised niche requirements and limited spatial distributions take longer to colonise novel habitats (Mierzwa, 2000; Petranka et al., 2007). Locally abundant, regionally widespread "core species" can readily access and colonise suitable habitats in the landscape (Hanski, 1982; Cadotte & Lovett-Doust, 2007). In contrast, colonisation by satellite species takes longer as they are constrained by landscape permeability, proximity to source habitats, and smaller navigation ranges (Mierzwa, 2000; Lehtinen & Galatowitsch, 2001; Petranka et al., 2007). This explains the greater abundance of generalist species as well as scarcity of rare species and specialists in the restored bog.

All herpetofauna we documented are regionally abundant, habitat generalists with a broad geographic distribution. Herpetofauna we inventoried are listed in neither the US/ Massachusetts Engendered Species Acts nor the IUCN Global Red List. However, the northern leopard frog we recorded at the restored bog has undergone local and regional population declines across a few localities in New England (Gilbert et al., 1994; Pope et al., 2000; Blomquist & Hunter, 2009). Although limited in incidences, presence of northern leopard frogs in the restored bog is noteworthy.

Agricultural legacies and the impacts of the disturbance history are known to persist in aquatic and wetland environments (Harding et al., 1998; Scott, 2006; Ballantine et al., 2017). Hence, century-long farming history is the likely driver of species depauperation at the retired bog. Given the recent restoration intervention, the physical habitat template of the restored bog is temporally dynamic. For instance, the channel geomorphology, streambed heterogeneity, and stream velocity at TWS have undergone dramatic shifts within the first few years of restoration (McCanty, 2020). Similarly, the vegetation structure, composition, and above ground biomass have not reached a stable state at TWS. Since TWS is still passaging through early recovery trajectory, the habitat structure is undergoing dramatic changes. Such environments are better suited for high-plasticity traits and opportunist strategies in contrast to highly specialised life-histories (Russell et al., 2002b; Petranka et al., 2007). Consequently, herpetofauna we found at the restored bogs were largely comprised of generalist species. With sufficient time past the active restoration interventions, as the restored bog reaches a stable state alongside a stable physical habitat structure, taxonomic and trait composition of the herpetofaunal community is likely to diversify (Mierzwa, 2000; Lehtinen & Galatowitsch, 2001; Petranka et al., 2007). Our study also showed tangible influences from short-term temporal covariates on the herpetofaunal community as well. For instance, the significance of month x habitat and month x trapping site interactions on diversity metrics and species composition suggested non-trivial within-season species turnover in the herpetofaunal community.

Evidence for pond-breeding amphibians— such as wood frogs (*Lithobates sylvaticus*) and mole salamanders (*Ambystoma* sp) regardless of their life-history stages— were infrequently found at both restored and retired bogs. Forested vernal pools and small, fishless ephemeral wetlands with small-to-moderate hydroperiods are the primary breeding habitats for these pond-breeding specialists (Cormier et al., 2013). Ephemeral wetlands in the retired bog are limited to tire ruts with unpredictable hydroperiods. Ephemeral marshland depressions in the restored bogs are intermittently connected to perennial waters, thus accessible by predatory fish, which negatively impacts pond-breeding amphibians (Pechmann et al., 1989; Semlitsch, 2002; Petranka et al., 2007). Thus, neither restored nor retired bogs provide ideal habitats for pond-breeding amphibians to sustain long-term viability.

Both richness and abundance of reptiles were greater in the restored bog compared to the retired bog. Restoration efforts at TWS produced a variety of wetlands, meandering stream channels, and semi-perennial ponds. Along with the forest buffers, TWS has morphed into a spatially heterogeneous upland-wetland-aquatic habitat complex forming multiple ecotones, which further enhances both habitat and resource availability for herpetofauna (Norriss, 2018; Ballantine et al., 2020). The process-based restoration has also yielded a diverse range of wetlands with variable flow dynamics, hydrologic features, and vegetation structure, which reinforces the biologically critical resource base at TWS (Briggs et al., 2016; Harvey et al., 2019). In addition, dam removal reconnected the flow-through systems back to the watershed reforming migratory pathways for herpetofauna to navigate through stream networks (Grant et al., 2010). Moreover, reformed stream sinuosity rekindled channel-floodplain interactions, which has widened the resource base (such as foraging opportunities) for freshwater-dependent herpetofauna. In contrast, impoundments and channelised streams of the retired bog not only impede species immigration from source populations but also restricts movement of remnant populations. Restored hydrologic processes— flood pulse between the channel and the floodplain, groundwater discharge that moderates the thermal environment, and watershed-wide stream connectivity— is fundamental to maintain the habitat heterogeneity and to hasten post-restoration trajectory at TWS (Harvey et al., 2019; Ballantine et al., 2020; McCanty, 2020).

Our preliminary findings indicated that restoration was critical for providing habitats for native herpetofaunal communities at a shorter time scale after restoration, at least for those communities that are locally common and regionally abundant. Without active intervention, retired bogs are unlikely to transform into wetlands although the perimeter ditches, irrigation canals, and holding reservoirs can become herpetofaunal refugia. As showed in our study, these refugia offer opportunity for highly resilient human-commensal herpetofauna. Given the underlying sand layers, retried cranberry bogs are likely to undergo upland successions resulting in plant communities dominated by scrub oak, pitch pine, or white pine (Mylecraine et al., 2003; Klee et al., 2019). Retired bogs are also susceptible to exotic invasions, and secondary metabolites released by these invasive plants can result in reduced larval growth and survival (Maerz et al., 2004).

Temporal scale of wetland recovery after active restoration is highly variable. Though wetlands can recover partial functionality within a few years following restoration, regaining full complement of functions requires 5-10 years for low-stress systems whereas diversity-rich or specialised systems take much longer (20-100 years) (Zedler & Callaway, 1999; Matthews & Spyreas, 2010). Further, high latitude, temperate systems that are frequently disturbed by climatic extremities (such as north-eastern US) and stochastic events will require decades to reach the climax community. Hereto, we underscore the need for continued monitoring at TWS to provide further insights into occupancy of rare and conservation-dependent species. Long-term ecological monitoring also opens opportunities for a thorough evaluation of temporal community turnover in restored wetlands. Short-term assessments of biological responses to restoration, such as our study, can help strategise site-specific adaptive habitat management actions, such as headstarting, upland revegetation, and invasive-species management (Kentula, 2000; Zedler et al., 2012). Moreover, long-term, continuous monitoring of retired bogs in comparison to bogs restored following variable designs and trajectories are crucial to determine the most effective restoration procedures.

Unexpected and undesirable developments have been reported in wetland restoration, particularly in high-stress systems (wetlands embedded in dramatically modified river basins) and wetlands with disturbance legacies (Kentula, 2000; Klötzli & Grootjans, 2001). The re-assembly of floral, faunal, and microbial communities to quasi-natural or desired levels at a restored wetland depends on biotic constraints (presence of source populations, metacommunity dynamics), evolutionary histories (phylogenetic constraints and local adaptations), community interactions (competition, trophic dynamics), structural diversity at local scale, landscape-scale processes and connectivity, and current and historical disturbance regimes (land-cover change and hydrological modifications) (Klötzli & Grootjans, 2001; Walker et al., 2004; Klimkowska et al., 2010). Consequently, if the regional species pool is impoverished, local source communities are dispersal limited, or there are impediments to landscape-scale connectivity (outside the restored bog), the restored habitat will have vacant niches, leading to establishment of exotic species. Historical, long-term agricultural land-uses may render some habitats resilient to restoration. In such cases, instead of moving towards the intended trajectory, restored habitats revert to pre-restoration status, as evident from temperate grasslands and middle-to-high order streams of south-eastern US (Harding, 1997; Harding et al., 1998). In our study, evidence for invasive herpetofauna was limited to a single event of capturing a female red-eared slider, a freshwater turtle native to south-eastern US that is competitively superior to those of the north-eastern US. However, this isolated incidence does not suggest any undesirable outcomes. Although both the restored and retired bogs we surveyed shared 10 herptile species in common, since restored sites were significantly greater in herptile abundance and reptile richness, there is no evidence implying resilience or resistance to restoration at TWS.

Conclusive Remarks

The retired bog had been left unmanaged for close to a decade before our survey. Despite lack of active farming for nearly a decade, no rare, threatened, unique species or habitat specialists have colonised therein. As such, retirement from commercial production and subsequent passive restoration alone are insufficient to bolster herptile diversity in retired bogs. Although our observations on unique or conservation-dependent herpetofauna at TWS are infrequent, reptile species richness and herpetofaunal abundance at TWS was greater than that of FP. As TWS continues to recuperate from both century-long framing legacies and pulse disturbance induced via active restoration, exploring turnover in the herpetofaunal community is imperative to determine the suitability for conservation-dependent species. Cranberry farms constitute a critical element in the landscapes of south-eastern Massachusetts. A multitude of economic, ecological, and logistic constraints have led to abandonment of cranberry bogs in Massachusetts. Cranberry bogs taken out of commercial production generate opportunities for wetland restoration. Hereto, our study can serve as a blueprint to develop community-wide surveys to assess biological responses to wetland restoration. Such studies will formulate a scientifically robust knowledgebase that reinforces decision-making in wetland restoration, management, and conservation policies.

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REFERENCES

Adams, M.J., Richter, K.O. & Leonard, W.P. (1997). Surveying and monitoring amphibians using aquatic funnel traps. *Northwest Fauna* 4, 47-54.

Baber, M.J., Fleishman, E., Babbitt, K.J. & Tarr, T.L. (2004). The relationship between wetland hydroperiod and nestedness patterns in assemblages of larval amphibians and predatory macroinvertebrates. *Oikos* 107, 16-27.

Baeten, L., Vangansbeke, P., Hermy, M., Peterken, G., Vanhuyse, K. & Verheyen, K. (2012). Distinguishing between turnover and nestedness in the quantification of biotic homogenization. *Biodiversity and Conservation* 21, 1399-1409.

Balcombe, C.K., Anderson, J.T., Fortney, R.H. & Kordek, W.S. (2005). Wildlife use of mitigation and reference wetlands in West Virginia. *Ecological Engineering* 25, 85-99.

Balian, E.V., Segers, H., Martens, K. & Lévéque, C. (2007). The freshwater animal diversity assessment: an overview of the results.in: Freshwater animal diversity assessment Freshwater animal diversity assessment, 627-637. Springer.

Ballantine, K.A., Anderson, T.R., Pierce, E.A. & Groffman, P.M. (2017). Restoration of denitrification in agricultural wetlands. *Ecological Engineering* 106, 570-577.

Ballantine, K.A., Davenport, G., Deegan, L., Gladfelter, E., Hatch, C.E., Kennedy, C., Klionsky, S., Mayton, B., Neil, C., Surasinghe, T.D. & Valentine, N. (2020). Learning from the Restoration of Wetlands on Cranberry Farmland: Preliminary Benefits Assessment. Massachusetts Division of Ecological Restoration, Cranberry Bog Program, Living Observatory.

Behangana, M. & Luiselli, L. (2008). Habitat niche community-level analysis of an amphibian assemblage at Lake Nabugabo, Uganda. *Web Ecology* 8, 125-134.

Blaustein, A.R., Wake, D.B. & Sousa, W.P. (1994). Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* 8, 60-71.

Blomquist, S.M. & Hunter, M.L., Jr. (2009). A multi-scale assessment of habitat selection and movement patterns by northern leopard frogs (*Lithobates rana pipiens*) in a managed forest. *Herpetological Conservation and Biology* 4, 142-160.

Brand, A.B. & Snodgrass, J.W. (2010). Value of artificial habitats for amphibian reproduction in altered landscapes. *Conservation Biology* 24, 295-301.

Briggs, M.A., Hare, D.K., Boutt, D.F., Davenport, G. & Lane, J.W. (2016). Thermal infrared video details multiscale groundwater discharge to surface water through macropores and peat pipes. *Hydrological Processes* 30, 2510-2511.

Burbrink, F.T., Phillips, C.A. & Heske, E.J. (1998). A riparian zone in southern Illinois as a potential dispersal corridor for reptiles and amphibians. *Biological Conservation* 86, 107-115.

Cadotte, M.W. & Lovett-Doust, J. (2007). Core and satellite species in degraded habitats: an analysis using Malagasy tree communities. *Biodiversity and Conservation* 16, 2515-2529.

Choi, Y.D. (2004). Theories for ecological restoration in changing environment: toward 'futuristic'restoration. *Ecological Research* 19, 75-81.

da Silva, F.R. (2010). Evaluation of survey methods for sampling anuran species richness in the neotropics. *South American Journal of Herpetology* 5, 212-220.

Dahl, T.E. (1990). Wetlands losses in the United States, 1780's to 1980's. U.S. Department of the Interior, Fish and Wildlife Service, Washington D.C.

Dahl, T.E., Johnson, C.E. & Frayer, W. (1991). Wetlands, status and trends in the conterminous United States mid-1970's to mid-1980's. U.s. Department of the Interior, Fish and Wildlife Service, washington, D.C.

Davic, R.D. & Welsh, H.H. (2004). On the ecological roles of salamanders. *Annual Review of Ecology Evolution and Systematics* 35, 405-434.

Dimino, T.F., McCanty, S. & Christian, A. (2018). Local adaptation of fish populations in response to stream habitat restoration at Tidmarsh Farm In: *Connecting Communities and Ecosystems in Restoration Practice*, *Society for Ecological Restoration New England, Southern Connecticut State University, New Haven, CT.*

Downs, P.W. & Kondolf, G.M. (2002). Post-project appraisals in adaptive management of river channel restoration. *Environmental Management* 29, 477-496.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Leveque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J. & Sullivan, C.A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* 81, 163-182.

Ehrenfeld, J.G. (2000). Defining the limits of restoration: the need for realistic goals. *Restoration Ecology* 8, 2-9.

Fellers, G.M. (1997). Design of amphibian surveys. *Northwest Fauna* 4, 23-34.

Figel, J.J., Botero-Cañola, S., Forero-Medina, G., Sanchez-Londono, J.D., Valenzuela, L. & Noss, R.F. (2019). Wetlands are keystone habitats for jaguars in an intercontinental biodiversity hotspot. *PLoS ONE* 14.

Funk, A., Gschöpf, C., Blaschke, A.P., Weigelhofer, G. & Reckendorfer, W. (2013). Ecological niche models for the evaluation of management options in an urban floodplain—conservation vs. restoration purposes. *Environmental Science & Policy* 34, 79-91.

Gardner, R.C. & Finlayson, M.C. (2018). Global wetland outlook: state of the World's wetlands and their services to people. Ramsar Convention Secretariat, Gland, Switzerland.

Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., Tuberville, T.D., etts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S. & Winne, C.T. (2000). The Global Decline of Reptiles, Deja Vu Amphibians. *Bioscience* 50, 653.

Gibbs, J.P. (2000). Wetland loss and biodiversity conservation. *Conservation Biology* 14, 314-317.

Gilbert, M., Leclair Jr, R. & Fortin, R. (1994). Reproduction of the northern leopard frog (*Rana pipiens*) in floodplain habitat in the Richelieu River, P. Quebec, Canada. *Journal of Herpetology*, 465-470.

Grant, E.H.C., Nichols, J.D., Lowe, W.H. & Fagan, W.F. (2010). Use of multiple dispersal pathways facilitates amphibian persistence in stream networks. *Proceedings of the National Academy of Sciences of the United States of America* 107, 6936-6940.

Griffen, B.D. & Drake, J.M. (2008). Effects of habitat quality and size on extinction in experimental populations. *Proceedings of the Royal Society B: Biological Sciences* 275, 2251-2256.

Hager, H.A. (1998). Area-sensitivity of reptiles and amphibians: Are there indicator species for habitat fragmentation? *Ecoscience* 5, 139-147.

Hanski, I. (1982). Dynamics of regional distribution: the core and satellite species hypothesis. *Oikos*, 210-221.

Harding, J.H. (1997). *Amphibians and reptiles of the Great Lakes region*. University of Michigan Press.

Harding, J.S., Benfield, E.F., Bolstad, P.V., Helfman, G.S. & Jones, E.B.D. (1998). Stream biodiversity: The ghost of land use past. *Proceedings of the National Academy of Sciences of the United States of America* 95, 14843-14847.

Harper, K.A., Macdonald, S.E., Burton, P.J., Chen, J., Brosofske, K.D., Saunders, S.C., Euskirchen, E.S., Roberts, D., Jaiteh, M.S. & Esseen, P.A. (2005). Edge influence on forest structure and composition in fragmented landscapes. *Conservation Biology* 19, 768-782.

Harvey, M.C., Hare, D.K., Hackman, A., Davenport, G., Haynes, A.B., Helton, A., Lane, J.W. & Briggs, M.A. (2019). Evaluation of stream and wetland restoration using UAS-based thermal infrared mapping. *Water* 11, 1568.

Hoekstra, B.R., Neill, C. & Kennedy, C.D. (2020). Trends in the Massachusetts cranberry industry create opportunities for the restoration of cultivated riparian wetlands. *Restoration Ecology* 28, 185-195.

Homan, R.N., Windmiller, B.S. & Reed, J.M. (2004). Critical thresholds associated with habitat loss for two vernal pool-breeding amphibians. *Ecological Applications* 14, 1547-1553.

Keddy, P.A., Fraser, L.H., Solomeshch, A.I., Junk, W.J., Campbell, D.R., Arroyo, M.T. & Alho, C.J. (2009). Wet and wonderful: the world's largest wetlands are conservation priorities. *Bioscience* 59, 39-51.

Kentula, M.E. (2000). Perspectives on setting success criteria for wetland restoration. *Ecological Engineering* 15, 199-209.

Klee, R.J., Zimmerman, K.I. & Daneshgar, P.P. (2019). Community Succession after Cranberry Bog Abandonment in the New Jersey Pinelands. *Wetlands* 39, 777-788.

Klemas, V. (2013). Using remote sensing to select and monitor wetland restoration sites: An overview. *Journal of Coastal Research* 29, 958-970.

Klimkowska, A., Van Diggelen, R., Grootjans, A.P. & Kotowski, W. (2010). Prospects for fen meadow restoration on severely degraded fens. *Perspectives in Plant Ecology, Evolution and Systematics* 12, 245-255.

Klötzli, F. & Grootjans, A.P. (2001). Restoration of natural and semi-natural wetland systems in Central Europe: progress and predictability of developments. *Restoration Ecology* 9, 209-219.

Krzysik, A.J. (1979). Resource allocation, coexistence, and the niche structure of a streambank salamander community. *Ecological Monographs*, 173-194.

Lehtinen, R.M. & Galatowitsch, S.M. (2001). Colonization of restored wetlands by amphibians in Minnesota. *The American Midland Naturalist* 145, 388-396.

Lewandowski, A.S., Noss, R.F. & Parsons, D.R. (2010). The effectiveness of surrogate taxa for the representation of biodiversity. *Conservation Biology* 24, 1367-1377.

Loflen, C., Hettesheimer, H., Busse, L.B., Watanabe, K., Gersberg, R.M. & Lüderitz, V. (2016). Inadequate monitoring and inappropriate project goals: A case study on the determination of success for the Forester Creek improvement project. *Ecological Restoration* 34, 124-134.

Maerz, J.C., Brown, C.J., Chapin, C.T. & Blossey, B. (2004). The secondary compounds of invasive plants are toxic to larval amphibians. *Ecological Society of America Annual Meeting Abstracts* 89, 317.

Marino, N.A., Céréghino, R., Gilbert, B., Petermann, J.S., Srivastava, D.S., de Omena, P.M., Bautista, F.O., Guzman, L.M., Romero, G.Q. & Trzcinski, M.K. (2019). Species niches, not traits, determine abundance and occupancy patterns: A multi-site synthesis. *Global Ecology and Biogeography* 29, 295-308.

Martin, D.M. (2017). Ecological restoration should be redefined for the twenty-first century. *Restoration Ecology* 25, 668-673.

Matthews, J.W. & Spyreas, G. (2010). Convergence and divergence in plant community trajectories as a framework for monitoring wetland restoration progress. *Journal of Applied Ecology* 47, 1128-1136.

McCanty, S. 2020. Impact of disturbance regimes on community and landscape biodiversity in atlantic coastal pine barren ecoregion streams. University of Massachusetts Boston, Boston, MA.

McCanty, S.T. & Christian, A. 2018. The effects of ecosystem restoration on community and landscape biodiversity in southeastern Massachusetts headwater streams: A case-study of Tidmarsh Farms cranberry bog restoration. Connecting Communities and Ecosystems in Restoration Practice, Society for Ecological Restoration, Southern Conneticut State University, New Haven, CT.

McKinney, M.L. & Lockwood, J.L. (1999). Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends in Ecology & Evolution* 14, 450-453.

McKinney, M.L. & Lockwood, J.L. (2001). Biotic homogenization: A sequential and selective process. *Biotic Homogenization*, 1-17.

Michener, W.K. (1997). Quantitatively evaluating restoration experiments: research design, statistical analysis, and data management considerations. *Restoration Ecology* 5, 324-337.

Middleton, B.A. (1999). Wetland restoration, flood pulsing, and disturbance dynamics. John Wiley & Sons, New York, NY, USA.388.

Mierzwa, K.S. (2000). Wetland mitigation and amphibians: preliminary observations at a southwestern Illinois bottomland hardwood forest restoration site. *Journal of the Iowa Academy of Science* 107, 191-194.

Mylecraine, K.A., Williams, R., Zimmermann, G. & Kuser, J. (2003). Restoring Atlantic white-cedar on an abandoned blueberry field and cranberry bog in Lebanon State Forest, New Jersey. 213-226 in *Proceedings of the Atlantic white-cedar management and restoration ecology symposium*. Newport News, VA: Christopher Newport University.

Norriss, J. (2018). Cranberry Bog Restoration in Practice Contextualizing the Tidmarsh Farms Restoration using GIS datasets available in Massachusetts. Living Observatory,

Plymouth, MA.
O'Donnell, R.P., Quinn, T., Hayes, M.P. & Ryding, K.E. (2007). Comparison of three methods for surveying amphibians in forested seep habitats in Washington state. *Northwest Science* 81, 274-283.
Parris, K.M. (2006). Urban amphibian assemblages as metacommunities. *Journal of Animal Ecology* 75, 757-764.
Paton, P.W. & Crouch III, W.B. (2002). Using the phenology of pond-breeding amphibians to develop conservation strategies. *Conservation Biology* 16, 194-204.
Pechmann, J.H.K., Scott, D.E., Gibbons, J.W. & Semlitsch, R.D. (1989). Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. *Wetlands Ecology and Management* 1, 3-11.
Petranka, J.W., Harp, E.M., Holbrook, C.T. & Hamel, J.A. (2007). Long-term persistence of amphibian populations in a restored wetland complex. *Biological Conservation* 138, 371-380.
Pope, S.E., Fahrig, L. & Merriam, H.G. (2000). Landscape complementation and metapopulation effects on leopard frog populations. *Ecology* 81, 2498-2508.
Postel, S.L. & Thompson Jr, B.H. (2005). Watershed protection: Capturing the benefits of nature's water supply services. 98-108 in Natural Resources Forum. Wiley Online Library.
R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Reis, V., Hermoso, V., Hamilton, S.K., Ward, D., Fluet-Chouinard, E., Lehner, B. & Linke, S. (2017). A Global Assessment of Inland Wetland Conservation Status. *Bioscience* 67, 523-533.
RStudio Team. 2020. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.
Russell, K.R., Guynn, D.C. & Hanlin, H.G. (2002a). Importance of small isolated wetlands for herpetofaunal diversity in managed, young growth forests in the Coastal Plain of South Carolina. *Forest Ecology and Management* 163, 43-59.
Russell, K.R., Hanlin, H.G., Wigley, T.B. & Guynn, D.C. (2002b). Responses of Isolated Wetland Herpetofauna to Upland Forest Management. *The Journal of Wildlife Management* 66, 603-617.
Scott, M.C. (2006). Winners and losers among stream fishes in relation to land use legacies and urban development in the southeastern US. *Biological Conservation* 127, 301-309.
Semlitsch, R.D. (2002). Critical Elements for Biologically Based Recovery Plans of Aquatic-Breeding Amphibians. *Conservation Biology* 16, 619-629.
Shulse, C.D., Semlitsch, R.D., Trauth, K.M. & Williams, A.D. (2010). Influences of design and landscape placement parameters on amphibian abundance in constructed wetlands. *Wetlands* 30, 915-928.
Skinner, K., Shields Jr, F.D. & Harrison, S. (2008). Measures of success: uncertainty and defining the outcomes of river restoration schemes. *River Restoration: Managing the Uncertainty in Restoring Physical Habitat*. John Wiley & Sons, Chichester, West Sussex, 187-208.
Suding, K.N., Gross, K.L. & Houseman, G.R. (2004). Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology & Evolution* 19, 46-53.
Waddle, J.H. 2006. Use of amphibians as ecosystem indicator species. University of Florida.
Walberg, E. (2013). Tidmarsh Farms, Massachusetts Climate Change Adaptation Plan. Manomet Center for Conservation Sciences, Plymouth, MA.
Walker, K.J., Stevens, P.A., Stevens, D.P., Mountford, J.O., Manchester, S.J. & Pywell, R.F. (2004). The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation* 119, 1-18.
Welsh Jr, H.H. & Droege, S. (2001). A case for using plethodontid salamanders for monitoring biodiversity and ecosystem integrity of North American forests. *Conservation Biology* 15, 558-569.
Welsh Jr, H.H. & Hodgson, G.R. (2008). Amphibians as metrics of critical biological thresholds in forested headwater streams of the Pacific Northwest, U.S.A. *Freshwater Biology* 53, 1470-1488.
Willson, J.D. & Dorcas, M.E. (2004). A comparison of aquatic drift fences with traditional funnel trapping as a quantitative method for sampling amphibians. *Herpetological Review* 35, 148-149.
Zedler, J.B. (2000). Progress in wetland restoration ecology. *Trends in Ecology & Evolution* 15, 402-407.
Zedler, J.B. & Callaway, J.C. (1999). Tracking wetland restoration: do mitigation sites follow desired trajectories? *Restoration Ecology* 7, 69-73.
Zedler, J.B., Doherty, J.M. & Miller, N.A. (2012). Shifting restoration policy to address landscape change, novel ecosystems, and monitoring. *Ecology and Society* 17.
Zedler, J.B. & Kercher, S. (2005). Wetland resources: status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources* 30, 39-74.

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Evolution of sexual dimorphism in the plateau brown frog fails to obey Rensch's rule

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Rensch's rule describes sexual size dimorphism (SSD) that decreases with increasing body size when females are larger than males and SSD that increases when males are larger than females. The plateau brown frog *Rana kukunoris*, a species endemic to the eastern Tibetan Plateau, exhibits female-biased size dimorphism. Using data on body size from 26 populations and age from 21 populations, we demonstrated that SSD did not increase with increasing mean female snout-vent length (SVL) when controlling for sex-specific age structure, failing to support the Rensch's rule. Thus, we suggest that fecundity selection (favouring large female size) balances out sexual selection (favouring large male size), which results in a similar divergence between males and females body size. In addition, sex-specific age differences explained most of the variation of SSD across populations.

Keywords: Age difference, Sexual size dimorphism, *Rana kukunoris*, Rensch's rule

INTRODUCTION

Sexual size dimorphism (SSD) is the intraspecific difference in body size between both sexes, which is a widespread phenomenon in natural populations (Shine, 1989). Rensch's rule states that SSD increases with increasing mean body size among species or populations when males are the larger sex (Rensch, 1950). Interestingly, Rensch's rule is one of the most classic summarisations for the patterns of sexual size dimorphism (Liao et al., 2014). However, the inverse of Rensch's rule postulates that SSD decreases with increasing mean body size when females are larger sex (hypo-allometry; Hedrick &Temeles, 1989).

The evolution of Rensch's rule results from sexual selection, fecundity selection, and ecological divergence all acting concurrently in the same species or population. Sexual selection might favour large male body size because large males have a higher chance of success in male-male competition (Darwin, 1874; Székely et al., 2004; Dale et al., 2007), or smaller male size because of increased mobility or agility (Székely et al., 2004; Kelly et al., 2008), but not in females because reproduction competition is significantly less costly for them (Shine, 1989). In this case, SSD patterns are expected to consistent with Rensch's rule. Thus, Walke et al. (2009) suggested that Rensch's rule may be manifest through the evolution of sex-specific developmental modifiers. Fecundity selection could favour large body size in females, however, as ecological competition between the sexes could then enlarge this difference. For example, limited resources would result in one sex being smaller to compensate for the larger size of the other sex. Thus, if fecundity selection may mainly act in the different species or same species across populations, which would result in SSD patterns obeying the inverse of Rensch's rule. Liao et al. (2014) suggested that Rensch's rule occurs if intense direct selection favours male adult body sizes in both sexes, while the inverse is expected if intense selection favours female adult body sizes. In addition to these two evolutionary explanations, differences in growth rate and/or age structure between sexes can drive the evolution of SSD (Monnet & Cherry, 2002; Fairbairn et al., 2007). For example, females begin breeding later, live longer but grow more slowly than males, resulting in female-larger patterns of SSD in anuran lineages.

Although a few studies of Rensch's rule have begun to test intraspecific patterns in recent years (e.g., Kupfer, 2007; Herczeg et al., 2010; Kelly et al., 2013 Sutter et al., 2008; Polák & Frynta, 2010; Frynta et al., 2012), most studies have concentrated heavily on interspecific tests of Rensch's rule (reviewed by Liao et al., 2014). Most intraspecific tests have concentrated on taxa with male-biased SSD, while few species with female-biased SSD has been studied. In 90 % of anuran species, females are larger than males (Shine, 1979). However, little attention has been paid to intraspecific patterns of Rensch's rule in amphibians (but see Liao & Chen, 2012; Lu et al., 2014).

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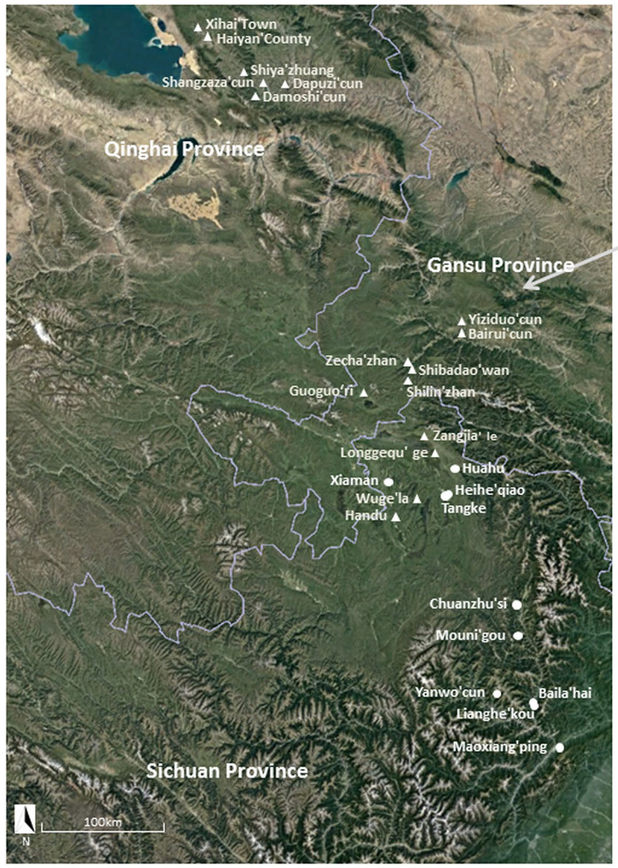
In this study, we examined patterns and possible causes of variation in SSD in the plateau brown frog, *Rana kukunoris*, a species endemic to the eastern Tibetan Plateau (Fei & Ye, 2001). Adult female plateau brown frogs are larger than males in natural populations. Moreover, larger females produce significantly heavier clutches, containing more embryos, indicative of higher fecundity (Chen et al., 2013). Specifically, we tested whether Rensch's rule holds in this species by studying 24 populations. Additionally, we also examined whether there is a correlation between the degree of SSD and operational sex ratio (OSR, the ratio of the sexually competing males to fertilisable females in a breeding aggregation at a given time), sex ratio (SR, the ratio of the number of adult males to the number of adult females in each population), sex-specific age difference (SSAD, the difference between mean male age and mean female age), or elevation.

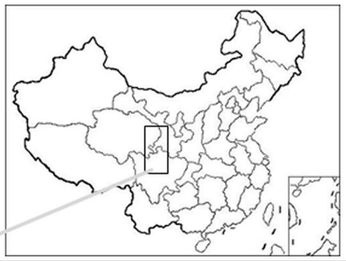
MATERIALS & METHODS

Sample collection

A total of 1,868 adult frogs (1,228 males, 640 females, unpublished data) were collected at sixteen localities with different elevations in the eastern Tibetan Plateau. The body size and age of both sexes in ten different elevations have been surveyed in this species during their breeding seasons (Feng et al., 2015). Thus, from the published literatures and field data we collected for this study, we obtained body size data from 26 male populations and 24 female populations, and age from 21 male populations and 19 female populations (Fig. 1). We recorded population sex ratio and the operational sex ratio in each population.

Frogs were caught by hand from 2008 to 2013 in twelve spawning ponds between March and April, and four feeding sites where frogs may find prey between June and August. We used a caliper to measure snout-to-vent length (snout-to–vent length, SVL, to the nearest 0.1 mm) of both sexes. In *R. kukunoris*, brown-black nuptial pads of front fingers were used to identify males during the breeding season. We randomly removed the longest phalange of the left hindfoot of both sexes in each population which were then preserved in 10 % aqueous solution of formaldehyde for later histological section. All frogs examined were released to original collecting sites. We compiled data on average air temperatures based on data obtained from the Chinese Meteorological





Maoxiang'ping: 103° 55′E, 31° 43′N, 1797 m
Lianghe'kou: 103° 39′E, 32° 04′N, 2296 m
Dapuzi'cun: 101° 38′E, 36° 39′N, 2297 m
Shangzaza'cun: 101° 30′E, 36° 34′N, 2495 m
Baila'hai: 103° 38′E, 32° 09′N, 2526 m
Shiya'zhuang: 101° 20′E, 36° 40′N, 2594 m
Bairui'cun: 103° 11′E, 34° 38′N, 2706 m
Mouni'gou: 103° 38′E, 32° 31′N, 2769 m
Damoshi'cun: 101° 26′E, 36° 29′N, 2789 m
Yanwo'cun: 103° 29′E, 32° 11′N, 2954 m
Haiyan'County: 101° 00′E, 36° 54′N, 2999 m
Chuanzhu'si: 103° 37′E, 32° 47′N, 3022 m
Yiziduo'cun: 103° 14′E, 34° 47′N, 3036 m
Zecha'zhan: 102° 41′E, 34° 29′N, 3049 m
Shibadao'wan: 102° 41′E, 34° 28′N, 3060 m
Xihai'Town: 100° 54′E, 36° 57′N, 3101 m
Shilin' zhan: 102° 41′E, 34° 22′N, 3233 m
Handu: 102° 33′E, 33° 22′N, 3440 m
Guoguo'ri: 102° 19′E, 34° 17′N, 3441 m
Zangjia'le: 102° 33′E, 33° 58′N, 3443 m
longgequ' ge: 102° 55′E, 33° 49′N, 3448 m
Huahu: 102° 52′E, 33° 55′N, 3448 m
Tangke: 102° 55′E, 33° 32′N, 3450 m
Xiaman: 102° 26′E, 33° 43′N, 3450 m
Heihe'qiao: 102° 55′E, 33° 34′N, 3453 m
Wuge'la : 102° 46′E, 33° 31′N, 3454 m

Figure 1. Topographic map showing the location of the 26 *Rana kukunoris* study populations in the eastern Tibetan plateau. Triangle, this study; circles, from Feng et al., 2015.

Table 1. Study site details, including altitude, latitude, longitude, temperature, sample size (n), mean (±SD) body size and age in males and females of 26 populations of plateau brown frog *Rana kukunoris* in Tibet Plateau.

Sites	Altitude (m)	Latitude (degrees)	Longitude (degrees)	Temperature (˚C)	Female SVL (mm)	Male SVL (mm)	Female age (yr)	Male age (yr)	References
Maoxiang'ping	1797	31.71	103.91	10.8	49.50±5.42 n = 3	43.53±3.88 n = 34	4.00±0.00 n = 3	2.97±1.31 n = 34	Feng et al. 2015
Lianghe'kou	2296	32.07	103.65	9.0	63.77±7.81 n = 4	50.20±5.83 n = 17	3.00±0.00 n = 4	2.65±0.70 n = 17	Feng et al. 2015
Dapuzi'cun	2297	36.65	101.65	6.2	61.71±5.19 n = 7	54.70±3.71 n = 17	4.14±1.07 n = 7	2.82±0.53 n = 17	This study
Shangzaza'cun	2495	36.57	101.5	4.1	57.22±4.81 n = 5	52.96±6.93 n = 5			This study
Baila'hai	2526	32.09	103.64	8.6		46.99±5.90 n = 19		3.37±1.07 n = 19	Feng et al. 2015
Shiya'zhuang	2594	36.68	101.34	3.7	58.45±4.77 n = 14	51.44±3.63 n = 39	4.00±0.91 n = 13	2.49±0.60 n = 39	This study
Bairui'cun	2706	34.64	103.19	3.9		48.18±2.89 n = 50		2.68±0.71 n = 50	This study
Mouni'gou	2769	32.51	103.64	7.6	49.93±6.45 n = 8	50.44±6.21 n = 6	3.38±0.74 n = 8	3.67±0.82 n = 6	Feng et al. 2015
Damoshi'cun:	2789	36.49	101.44	2.6	56.33±3.84 n = 43	50.25±4.19 n = 53	3.67±0.78 n = 43	2.87±0.81 n = 53	This study
Yanwo'cun	2954	32.19	103.49	6.9	57.62±7.77 n = 5	53.38±3.99 n = 27	3.20±0.45 n = 5	3.44±0.58 n = 27	Feng et al. 2015
Haiyan'County	2999	36.90	101.01	1.7	44.76±4.60 n = 38	43.82±4.38 n = 150	3.84±1.11 n = 25	3.27±0.87 n = 84	This study
Chuanzhu'si	3022	32.78	103.62	6.2	57.42±2.90 n = 5	51.85±2.76 n = 21	3.80±0.84 n = 5	3.19±0.51 n = 21	Feng et al. 2015
Yiziduo'cun:	3036	34.79	103.23	3.1	59.65±4.30 n = 51	50.13±3.44 n = 106	4.90±0.83 n = 51	3.42±0.65 n = 71	This study
Zecha'zhan	3049	34.49	102.69	3.1	59.70±3.43 n = 115	52.03±3.30 n = 219	4.06± n = 18	3.53±0.70 n = 19	This study
Shibadao'wan	3060	34.47	102.69	2.9	60.42±4.28 n = 9	51.23±2.99 n = 11	4.38±1.51 n = 8	3.45±0.52 n = 11	This study
Xiahai'Town	3101	36.96	100.91	1.6	51.20±0.00 n = 1	43.79±4.65 n = 8	4.00±0.00 n = 1	3.38±0.92 n = 8	This study
Shilin' zhan	3233	34.37	102.68	2.5	58.65±5.00 n = 40	51.90±3.96 n = 88	4.07±0.64 n = 30	2.92±0.65 n = 24	This study
Handu	3440	33.36	102.55	1.8	46.10±4.09 n = 10	43.84±3.40 n = 19			This study
Guoguori	3441	34.29	102.31	1.43	54.84±3.94 n = 270	47.71±3.32 n = 399	4.57±1.17 n = 42	3.95±0.94 n = 73	This study
Zangjia' le	3443	33.97	102.8	1.5	49.10±7.22 n = 10	47.00±2.71 n = 10			This study
longgequ' ge	3448	33.81	102.91	1.5	48.24±7.86 n = 6	44.85±4.12 n = 21			This stud
Huahu	3448	33.92	102.87	1.5	52.11±4.20 n = 7	46.10±3.38 n = 18	3.71±0.76 n = 7	3.22±0.43 n = 18	Feng et al. 2015
Tangke	3450	33.54	102.91	1.8	59.60±5.98 n = 6	50.40±3.97 n = 25	3.50±0.84 n = 6	3.68±0.85 n = 25	Feng et al. 2015
Xiaman	3450	33.72	102.44	2.0	49.54±5.88 n = 5	47.34±3.12 n = 10	3.40±0.52 n = 5	3.20±0.45 n =10	Feng et al. 2015
Heihe'qiao	3453	33.56	102.92	1.8	54.40±6.61 n = 11	50.78±3.42 n = 27	3.64±0.92 n = 11	3.59±0.89 n = 27	Feng et al. 2015
Wuge'la	3454	33.52	102.76	1.8	42.56±5.19 n = 21	42.05±3.80 n = 33			This study

Administration (http://www.cma.gov.cn), Gansu Gahai-Zecha National Nature Reserve Management Bureau and the published literatures (Feng et al., 2015).

Age determination

The paraffin sections and Ehrlich's haematoxylin stain were used to produce histological sections of the phalanges. We counted the number of lines of arrested growth (LAG) in the sections to determine age. Skeletochronologly has been successfully used to age anurans such as *Rana chensinensis* (Lu et al., 2006) and *Bufo minshanicus* (Yu et al., 2019). In this study, 238 females and 449 males were skeletochronologically aged. An index of SSD was calculated with the following equation: log10 (mean female SVL) - $\log_{10}$ (mean male SVL) (Smith, 1999). The sex-specific age difference (SSAD) for each population was calculated with the following equation: $\log_{10}$ (female age) - $\log_{10}$ (male age).

Statistical analyses

To meet the assumption of normality, we $\log_{10}$-transformed body size, age and clutch volume. We fit two generalised linear models (GLMs) to test differences in body size (and age) of sampled individuals between sexes among the 16 (and 11) populations (unpublished data) where population as a random factor and sex as a fixed factor. Then, to investigate variation in SSD among populations, a GLM was used where age was added as covariate together with two interactions between sex and age (fixed effect) and between sex and population (fixed effect). A significant interaction between sex and age would be indicative of differences in growth rates between the sexes, while a significant interaction between sex and population would reveal variation in SSD among-population.

To test Rensch's rule, we regressed mean $\log_{10}$ (female SVL) on the mean $\log_{10}$ (male SVL) across 26 or nine populations. The model I regression (ordinary least squares; OLS) may be yield misleading results because independent variable (female size) is measured without error (Fairbairn, 1997). Hence, the model II regression (reduced major axis; RMA) was also conducted to test for Rensch's rule and to test the null hypothesis of slope = 1 as judged from the overlap of 95 % confidence intervals with a line of isometry (Fairbairn, 1997; for details, see Sokal & Rohlf [1981, p. 219]).

A correlation analysis was used to test the correlation between SSD and SR, OSR, elevation, and temperature, as well as SSAD across populations. Prior to analyses, we removed five populations (1,797 m/asl, 2,296 m/asl and 2,526 m/asl from the published literatures; 2,706 m/asl and 3,101 m/asl from our field studies) because the sample size of one of both sexes was less than five individuals. All analyses were performed with the IBM SPSS Statistics 20.0 (IBM Corp, Armonk, NY, USA).

RESULTS

The mean body size varied significantly among the 14 populations ($F_{13, 1794}$ = 115.179, p < 0.001) and between the sexes ($F_{1, 1794}$ = 967.377, p < 0.001), with females always being larger than males (Table 1). The mean age also varied significantly among the nine populations ($F_{8, 618}$ = 20.784, p < 0.001; Table 1) and between sexes ($F_{1, 618}$ = 189.840, p < 0.001). Frogs at higher elevations were significantly older than those at lower elevations (p < 0.049 for 23 of 36 Fisher's LSD post hoc tests); the opposite results occurred (p < 0.024 for 2 post hoc tests), with non-significant differences occurring between neighbouring populations (p = 0.089–0.978 for 4 post hoc tests) or non-neighbouring populations (p = 0.105–0.904 for the 7 post hoc tests). When controlling for the effects of age ($F_{6, 612}$ = 155.024, p < 0.001), differences in body size among populations ($F_{8, 612}$ = 159.759, p < 0.001) and between the sexes ($F_{1, 612}$ = 127.719, p < 0.001) still remained. A non-significant interaction between sex and age indicated that the relationship between body size and age (≈growth rate) did not vary between the sexes ($F_{4, 600}$ = 1.174, p = 0.321). The interaction between population and sex also was statistically significant ($F_{8, 600}$ = 19.489, p < 0.001), revealing that the degree of SSD vary among the populations.

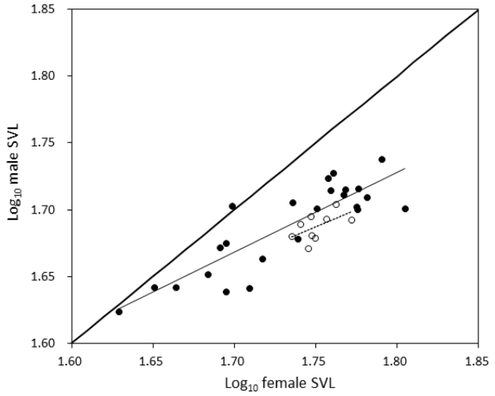


Figure 2. Relationship between mean male SVL and female SVL of *Rana kukunoris*. Black circle, relationship based on raw data from 21 populations [linear regression, β = 0.596 ± 0.075(SE)]; white circle, relationship based on age-corrected data 9 populations [β = 0.514 ± 0.280(SE)]. All data are plotted on logarithm-transformed scale. The thick grey line represents isometry (β = 1).

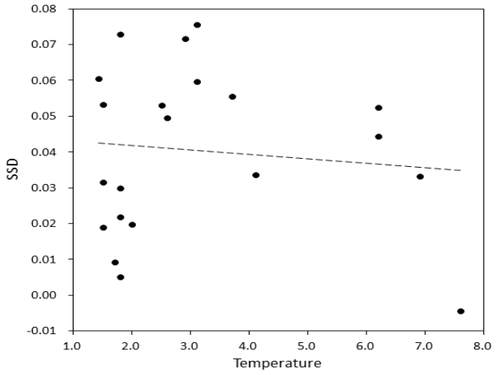


Figure 3. Relationships between (log) SSD and mean annual air temperature in 16 populations of *Rana kukunoris*. Each dot represents a single population. The dotted line shows a non-significant correlation (r =-0.105, p = 0.652).

Model I regression indicated a significant relationship between ($\log_{10}$) male size and ($\log_{10}$) female size among 21 populations ($F_{1, 20}$ = 88.80, slope = 0.607, 95 % CI = 0.472–0.741, p < 0.001; Fig. 2), which conformed to the allometric relationship. Model II regression revealed the same conclusion when RMA regression was used (slope = 0.668, 95 % CI = 0.534–0.803). These results were consistent with the inverse of Rensch's rule. However, there was not a significant allometric relationship (OLS: $F_{1, 8}$ = 3.373, regression slope = 0.514, 95 % CI = -0.148–1.176, p = 0.109; RMA: slope = 0.901, 95 % CI = 0.240–1.563; Fig. 2) between ($\log_{10}$) age-adjusted male size and ($\log_{10}$) age-adjusted female size across nine populations.

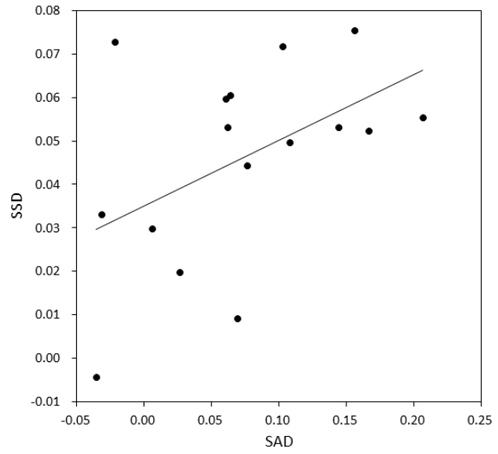


Figure 4. Relationships between (log) SSD and (log) SSAD in 16 populations of *Rana kukunoris*. Each dot represents a single population. The solid line shows a marginally significant correlation (r = 0.481, p = 0.059).

The degree of SSD was not related to either OSR (r = 0.185, n = 17, p = 0.477) or SR (rs = 0.800, n = 4, p = 0.200). Correlations between SSD and elevation (r = -0.105, n = 21, p = 0.650), as well as between SSD and temperature (r = -0.105, n = 21, p = 0.652, Fig. 3) were not significant. The degree of SSD was marginally correlated with SSAD (r = 0.481, n = 16, p = 0.059; Fig. 4).

DISCUSSION

Several hypotheses, including sexual selection, fecundity selection, and sex differences in age have been proposed to explain the evolution of SSD (Shine, 1979; Monnet & Cherry, 2002; Herczeg et al., 2010). In the present study, our results showed that the degree of SSD of *R. kukunoris* varied across different populations, and the degree of female-biased SSD displayed a allometric relationship (slopes < 1.0) with mean female body size when ignoring the influence of age structure, conforming to the inverse of Rensch's rule. This result was consistent with previous studies in owls (Abouheif & Fairbairn, 1997), fish (Herczeg et al., 2010) and amphibians (Liao, 2013; Liao et al., 2014).

The majority of studies suggest that sexual selection in favour of large male size is the primary cause of Rensch's rule because large males are more likely to succeed in male-male competition (Fairbairn, 1997). Conversely, to date a few earlier amphibian studies found evidence for the inverse of Rensch's rule (Fairbairn, 1997; Herczeg et al., 2010; Liao & Chen, 2012; Liao, 2013). The fecundity selection on females favouring large size has been proposed as a hypothesis to explain the inverse of Rensch's rule (Fairbairn & Preziosi, 1994). In this study, we did not find a significant relationship between SSD and OSR or SR, suggesting variation in SSD was not associated with the variation in the strength of male-male competition. Moreover, the allometric relationship was not a significant when adjusting for sex-specific age in the analysis, thus our result showed that SSD size relationships in *R. kukunoris* was inconsistent with Rensch's rule and the inverse of it. This pattern has been found exclusively in taxa with female-biased SSD (by reviewed in Liao et al., 2013). Thus, we suggest that fecundity selection (favouring large female size) balances out sexual selection (favouring large male size) and generates a similar divergence between males and females body size, thus the lack of association between SSD and size.

In indeterminately growing ectotherms, environmental factors (e.g., temperature) are likely to play an important role across ontogeny by sex-specific ways to decide final body size (Ceballos & Valenzuela, 2011; Zhang & Lu, 2013). In this study, we found no significant correlation between the degree of SSD and temperature, as well as SSD and elevation, revealing that temperature and elevation are unlikely to explain part of variation in SSD for *R. kukunoris*. For example, males and females may be exposed to similar temperatures, or similar habitat utilisation in natural populations.

Differences in growth rate and age between the sexes have potential effects on the variation in SSD (Fairbairn et al., 2007; Monnet & Cherry, 2002). For instance, anurans living in low temperatures obtain maturity later and grow slower than those exposed to warm temperatures (Morrison & Hero, 2003). In this study, a non-significant interaction between sex and age across populations suggested that differences in growth rates between the sexes may not explain variation in SSD. However, a relationship between SSD and SSAD was marginally significant. Similarly, previous studies showed that a significant correlation between SSD and SSAD by means of comparisons across species or populations (Monnet & Cherry, 2002; Liao & Chen, 2012; Zhang & Lu, 2012; Liao, 2013; Liao et al., 2013). We also found that variation in SSD across populations and the allometric relationship between sexes across populations were not significant when removing the effects of age. Thus, those results suggested that sex differences in the age structure are likely to explain the variation in SSD in *R. kukunoris*. Similarly, Liao and Chen (2012) suggests that the variation of SSD in Chinese wood frog *Rana chensinensis* can be explained by sex differences in age among populations. Therefore, sex difference in the age structure is one of the mechanisms that most likely contributes to the extent of SSD among populations (Liao & Chen, 2012). In the latest studies, however, variation in SSD of *R. kukunoris* resulted from sex differences in growth rates (Feng et al., 2015). This finding is not consistent with our findings from intraspecific comparisons of the same species because the limited sample size in the latest study may have an effect on the insignificant results.

In conclusion, our results showed hyperallometry in SSD in *R. kukunoris* when females are larger, indicating a pattern consistent with the inverse of Rensch's rule. Fecundity selection is the more likely to explain this pattern because reproductive output increases significantly with increasing female body size within and among populations. However, the allometric relationship

was not significant when adjusting for sex-specific age in the analyses, thus our result showed that SSD size relationships in *R. kukunoris* was inconsistent with Rensch's rule and the inverse of it. We suggest that the interplay between natural and sexual selection on females and males have generated a similar divergence between male and female body size, thus the lack of association between SSD and size. Additionally, sex differences in age for populations are a likely explanation for the variation of SSD in *R. kukunoris*.

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REFERENCES

Abouheif, E. & Fairbairn, D.J. (1997). A comparative analysis of allometry for sexual size dimorphism: assessing Rensch's rule. *The American Naturalist* 149, 540–562.

Ceballos, C.P. & Valenzuela, N. (2011). The role of sex-specific plasticity in shaping sexual dimorphism in a long-lived vertebrate, the snapping turtle *Chelydra serpentina*. *Evolutionary Biology* 38, 163–181.

Chen, W., Tang, Z. H., Fan, X. G., Wang, Y. & Pike, D. A. (2013). Maternal investment increases with altitude in a frog on the Tibetan Plateau. *Journal of Evolutionary Biology* 26, 2710–2715.

Darwin, C. (1874). The Descent of Man, and Selection in Relation to Sex. Reprinted by Rand, McNally, and Co., Chicago, 1974.

Dale, J., Dunn, P. O., Figuerola, J., Lislevand, T., Székely, T., & Whittingham, L. A. (2007). Sexual selection explains Rensch's rule of allometry for sexual size dimorphism. *Proceedings of the Royal Society B: Biological Sciences* 274, 2971-2979.

Fairbairn, D. J., Blanckenhorn, W. U. & Székely T. (2007). Sex, size, and gender roles: Evolutionary studies of sexual size dimorphism. Oxford: Oxford University Press

Fairbairn, D.J. & Preziosi, R.F. (1994). Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, *Aquarius remigis*. *The American Naturalist* 144, 101–118.

Fairbairn, D.J. (1997). Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annual review of ecology and systematics* 28, 659–687.

Fei, L. & Ye. C.Y. (2001). *The Colour Handbook of Amphibians of Sichuan*. China Forestry, Beijing

Feng, X.Y., Chen, W., Hu, J.H. & Jiang, J.P. (2015). Variation and sexual dimorphism of body size in the plateau brown frog along an altitudinal gradient. *Asian Herpetological Research* 6, 291–297.

Frynta, D., Baudyšová, J., Hradcová, P., Faltusová, K. & Kratochvíl, L. (2012). Allometry of sexual size dimorphism in domestic dog. *PLoS ONE* 7, e46125.

Hedrick, A.V. & Temeles, E.J. (1989). The evolution of sexual dimorphism in animals: hypotheses and tests. *Trends in Ecology & Evolution* 4, 136–138.

Herczeg, G., Gonda, A., & Merilä, J. (2010). Rensch's rule inverted female driven gigantism in nine-spined stickleback *Pungitius pungitius*. *Journal of Animal Ecology* 79, 581–588.

Kelly, C.D., Bussiere, L.F. & Gwynne, D. (2008). Sexual selection for male mobility in a giant insect with female-biased size dimorphism. *The American Naturalist* 172, 417–423.

Kelly, C.D., Folinsbee, E.K., Adams, C.D. & Jennions, D.M. (2013). Intraspecific sexual size and shape dimorphism in an Australian freshwater fish differs with respect to a biogeographic barrier and latitude. *Evolutionary Biology* 40, 408–419.

Kupfer, A. (2007). Sexual size dimorphism in amphibians: an overview. In: Fairbairn DJ, Blanckenhorn WU, Székely T (eds) *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. Oxford University Press, Oxford, pp 50–59.

Liao, W.B., Liu, W.C., & Merilä, J. (2014). Andrew meets Rensch: Sexual size dimorphism and the inverse of Rensch's rule in Andrew's toad (*Bufo andrewsi*). *Oecologia* 177, 389–399.

Liao, W.B. (2013). Evolution of sexual size dimorphism in a frog obeys the inverse of Rensch's rule. *Evolutionary Biology* 40, 493–499.

Liao, W.B. & Chen, W. (2012). Inverse Rensch-rule in a frog with femalebiased sexual size dimorphism. *Naturwissenschaften* 99, 427–431.

Liao, W.B., Zeng, Y., Zhou, C.Q. & Jehle, R. (2013). Sexual size dimorphism in anurans fails to obey Rensch's rule. *Frontiers in Zoology* 10, 1-7.

Lu, D., Zhou, C.Q. & Liao, W.B. (2014). Pattern of sexual size dimorphism supports the inverse Rensch's rule in two frog species. *Animal Biology* 64, 87–95.

Lu, X., Li, B. & Liang, J.J. (2006). Comparative demography of a temperate anuran, *Rana chensinensis*, along a relatively fine elevational gradient. *Canadian Journal of Zoology* 84, 1789–1795.

Monnet, J.M. & Cherry, M.I. (2002). Sexual size dimorphism in anurans. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269, 2301–2307.

Morrison, F.C. & Hero, J.M. (2003). Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology* 72, 270–279.

Polák, J. & Frynta, D. (2010). Patterns of sexual size dimorphism in cattle breeds support Rensch's rule. *Evolutionary Ecology* 24, 1255–1266.

Rensch, B. (1950). Die Abhängigkeit der relativen sexualdifferenz von der Körpergröße. *Bonner Zoologische Beiträge* 1, 58–69.

Shine, R. (1979). Sexual selection and sexual dimorphism in the amphibia. *Copeia* 1979, 297–306.

Shine, R. (1989). Ecological causes for the evolution of sexual size dimorphism: a review of the evidence. *Quarterly Review of Biology* 64, 419–46.

Smith, R.J. (1999). Statistics of sexual size dimorphism. *Journal of Human Evolution* 36, 423–459.

Sokal, R.R. & Rohlf, F.J. (1981). Biometry: the principles and practice of statistics in biological research. Freeman, San Francisco.

Sutter, N.B., Mosher, D.S. & Ostrander, E.A. (2008). Morphometrics within dog breeds are highly reproducible and dispute Rensch's rule. *Mammalian Genome* 19, 713–723.

Székely, T., Freckleton, R.P. & Reynolds, J.D. (2004). Sexual selection explains Rensch's rule of size dimorphism in shorebirds. *Proceedings of the National Academy of Sciences of the United States of America* 101, 12224–12227.

Walker, S. P., & McCormick, M. I. (2009). Sexual selection explains sex-specific growth plasticity and positive allometry for sexual size dimorphism in a reef fish. *Proceedings of the Royal Society B: Biological Sciences* 276, 3335-3343.

Yu, T. L., Wang, D. L., Busam, M., & Deng, Y. H. (2019). Altitudinal variation in body size in *Bufo minshanicus* supports Bergmann's rule. *Evolutionary Ecology* 33, 449-460.

Zhang, L.X. & Lu, X. (2013). Sexual size dimorphism in anurans: ontogenetic determination revealed by an across-species comparison. *Evolutionary Biology* 40, 84–91.

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Acanthosaura meridiona sp. nov. (Squamata: Agamidae), a new short-horned lizard from southern Thailand

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A new short – horned lizard species of the genus *Acanthosaura* from southern Thailand, is described herein. The species was previously recognised as *Acanthosaura crucigera* and has been reported to present a wide distribution across mainland south-east Asia. The combination of modern morphological studies of *Acanthosaura meridiona* **sp. nov.** allows its separation from closely related species *A. crucigera*, on the basis of presenting more nuchal scales, more scales between diastema, more scales bordering rostral scales and more midline ventral scales. Mitochondrial DNA analysis also indicated a sister relationship between *A. meridiona* **sp. nov.** and *A. crucigera* with a 100 % probability according to Bayesian and maximum – likelihood analyses. The pairwise distance between *A. meridiona* **sp. nov.** and *A. crucigera* ranges from 9.9 – 11.1 %, while the distance between *A. meridiona* populations ranges from 0 – 0.9 %. This new discovery contributes to the redescription of the distribution of *A. crucigera* under Kra Isthmus and its replacement by *A. meridiona* **sp. nov.**

Keywords: crucigera complex, tropical rainforest, Thai – Malay Peninsula, ND2, taxonomy

INTRODUCTION

Agamid lizards of the genus *Acanthosaura* Gray, 1831 are distributed in south-east Asia with a range extending from Myanmar, eastward through Thailand, Cambodia, Laos, Vietnam, and Yunnan and southward through the Indochinese and Thai – Malay Peninsula, Sumatra, and Anambas and Natunus Archipelagos (Taylor, 1963; Grismer et al., 2008; Manthey, 2008; Das, 2010; Wood et al., 2010; Ananjeva et al., 2011, 2020; Pauwels et al., 2015; Trivalairat et al., 2020; Liu et al., 2020). Previously, *Acanthosaura crucigera* (Boulenger, 1885) was described from the type locality in Tavoy with a distribution, covering the distribution of the entire genus (Boulenger, 1912; Taylor, 1963; Pauwels et al., 2003; Grismer et al., 2006; Manthey, 2008). However, recent morphological and molecular studies of the *A. crucigera* complex have revised the complex identifying several undescribed and cryptic species, and its members have also been separated into distinct species with different geographic distributions. For instance, two montane populations from Peninsular Malaysia are likely *A. bintangensis* (Wood et al., 2009) and *A. titiwangsaensis* (Wood et al., 2009); one population from the eastern Thailand and Cambodia populations consists of *A. cardamomensis* (Wood et al., 2010); and the species from Phuket Island and south – western Thailand are *A. phuketensis* (Pauwels et al., 2015) (Chan – ard et al., 1999; Pauwels et al., 2002; Pauwels & Iskandar 2010; Wood et al., 2009, 2010; Grismer, 2011; Pauwels et al., 2015).

In the 19th century, within the geographic distribution on the Thai – Malay Peninsula, only two species of *Acanthosaura* were considered to be present (*A. crucigera* and *A. armata* (Hardwick & Gray, 1827)) before being separated into five species recently, as mentioned above (Wood et al., 2009; Pauwels et al., 2015). In addition, Boulenger (1885) and Taylor (1963) had described specimens No. 3885 (female) and No. 3887 (male) from Na Pradoo Sub – district, Khok Pho District, Pattani Province and No. 192 (female) from Nabon District, Nakhon Si Thammarat Province from southern Thailand and designated these specimens as *A. crucigera*. However, some of the described characteristics of these specimens and the *A.* cf. *crucigera* population from southern Thailand and Malaysia of Wood et al. (2010) were confused with the true *A. crucigera* population from western Thailand and southern Myanmar. To clarify the taxonomic confusion of these *A.* cf. *crucigera* populations, *Acanthosaura* lizards in these southern regions were collected and compared with other *Acanthosaura* lizards through genetic and morphological analysis. The results showed that this population represents an undescribed species.

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MATERIALS & METHODS

Sampling and specimen preparation

Fieldwork for *A. crucigera* was conducted in four localities of Thailand: seven specimens from the western region of Taksin Maharat National Park, Mueang, Tak Province on 26 April 2016; eight specimens from the southern region of Na Yong District, Trang Province on 27 April 2017; one specimen from the southern region of Ton Lat Waterfall, Nathavee District, Songkhla Province on 15 May 2017; and four specimens from southern region, Wang Hip River, Thung Song District, Nakhon Si Thammarat Province on 3 June 2017. Specimens were collected by hand, photographed, euthanised by freezing at – 10 ˚C for a few days, fixed in 10 % formalin, and later transferred to 70 % ethanol. Fresh liver samples were stored in absolute ethanol prior to formalin fixation. Specimens were deposited at the Natural History Museum, National Science Museum, Technopolis, Pathum Thani Province (THNHM) and Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok Province, Thailand (QSMI).

Morphological characters

A total of 34 specimens of six *Acanthosaura* species in Thailand, including one from Vietnam, were examined from THNHM and QSMI (Appendix 1). All data of currently recognised *Acanthosaura* species were obtained from Günther (1861), Boulenger (1885), Ananjeva et al. (2008), Wood et al. (2009), Wood et al. (2010), Ananjeva et al. (2011), Nguyen et al. (2018), Pauwels et al. (2015), Nguyen et al. (2019), Trivalairat et al. (2020), Ananjeva et al. (2020), and Liu et al. (2020). Meristic and measured morphological characters were noted for each specimen of the type series on the left side followed Pauwels et al. (2015), Liu et al. (2020), and Ananjeva et al. (2020). Measurements were performed with callipers to the nearest 0.01 mm.

The following morphometric and meristic data were collected: SVL – snout – vent length, measured from the tip of the snout to the tip of the vent; TaL – tail length, measured from the posterior margin of the vent to the tip of the tail; TBW – tail base width, maximum width at tail base; HL – head length, measured from posterior edge of the rectis of the jaw to the tip of the snout; HW – head width, maximum head width, the width at the level of the tympanum; HD – maximum head height, measured across the parietal region; SL – snout length, measured from the anterior edge of the orbit to the tip of the snout; ORBIT – orbit diameter, measured from the posterior to the anterior edge of the orbit; EYE – eye diameter, measured from the posterior to the anterior edge of the eye; TD – tympanum diameter, measured horizontally from the anterior to the posterior border of the tympanum; TN – scales absent on tympanum (0) or present (1); PS – postorbital spine length, measured from the base to the tip of the spine; NS – number of nuchal scales; NSL – maximum length of the largest spine in the nuchal crest measured from the base to the tip; DS – maximum length of the largest spine in the dorsal crest measured from the base to the tip; WNC – maximum width of the spines in the nuchal crest, measured at the base; DIAS – length of the diastema, measured from the posterior end of the nuchal crest to the anterior end of the dorsal crest; DIASN – number of scales in the vertebral crest scale diastema counted from the posterior end of the nuchal crest to the anterior end of the dorsal crest; FOREL – forelimb length, measured from axilla to the proximal edge of the palmar region; HINDL – hindlimb length, measured from groin to the proximal edge of the plantar region; SUPRAL – number of supralabials; INFRAL – number of infralabials; VENT – number of ventral scales counted at the midline from the anterior edge of the shoulders to the edge of the vent; FI – number of subdigital lamellae on the fourth finger; TO – subdigital lamellae on the fourth toe; OS – length of the occipital spine, measured from the base to the tip; NSSOS – number of scales surrounding the occipital spine; CS – number of canthus rostralis – supraciliary scales, counted from the nasal scale to the posterior end of the ridge at the posterior margin of the orbit; RW – rostral width; RH – rostral height; RS – number of scales bordering the rostral scale; NS – number of scales between the nasals; NCS – number of scales between the fifth canthals; NSCSL – number of scales from the fifth canthal to the fifth supralabial; NR – number of scales between the nasal and the rostral scales; NSSLC – number of scales between the seventh supralabial and the sixth canthal; MW – mental width; MH – mental height; PM – number of scales bordering the mental; YAS – presence (1) or absence (0) of a Y – shaped arrangement of enlarged scales on the snout; ND – presence (1) or absence (0) of a black, diamond shaped, nuchal collar; LKP – presence (1) or absence (0) of light knee patch; BEP – presence (1) or absence (0) of a black eye patch; ESBO – presence (1) or absence (0) of elliptical scales below the orbit; GP – size of gular pouch scored as absent, small, medium or large; OF – presence (1) or absence (0) of oblique fold anterior to the fore limb insertions.

Molecular analysis

Three specimens of *A. crucigera* from each region (western and southern regions, total six specimens) were examined for molecular data comparing with other taxa of *Acanthosaura* species from GenBank (Macey et al., 1997, 2000; Zug et al., 2006; Okajima & Kumazawa, 2010; Wood et al., 2010; Yu et al., 2015; Trivalairat et al., 2020) (Table 1). DNA was extracted from liver samples with a TIANamp Genomic DNA Kit (catalog number DP304 – 02; TIANGEN Biotech (Beijing) Co., Ltd., Beijing). The samples were lysed using proteinase K for three hours at 56 ˚C. DNA was eluted from the spin column with 150 µl of buffer.

Polymerase chain reaction (PCR) was performed using EP0402 TAQ DNA POLYMERASE. The samples were amplified using two primers, METF6 (L4437a; 5’ – AAG CTT TCG GGC CCA TAC C – 3’) and ACANTHND2.833. R1 (5’ – AGG GAG GTT ATT GTT GCT AG – 3’), for a 698 bp fragments of the NADH dehydrogenase subunit 2 (ND2) gene (Wood et al., 2010). PCR protocol for the amplification of genomic DNA began with an initial denaturation for 2 min at 95 ˚C, followed by 95 ˚C for 35 s, annealing at 50 ˚C for 35 s, and extension at 72 ˚C

for 154 s per cycle for 32 cycles (Jackman et al., 2008). Successful PCR products were cleaned and sequenced at Macrogen Co., South Korea.

Phylogenetic analysis

DNA sequences were cleaned and aligned using ClustalW v. 1.83 (Thompson et al., 1994) with default parameters using MEGA6 (Tamura et al., 2013). All DNA sequences were translated into amino acids to confirm the absence

Table 1. GenBank accession numbers for agamid sequence used in phylogenetic analyses of *Acanthosaura*.

Taxon	Voucher	Locality	Coordination	GenBank	References
				ND2	
Ingroup					
Acanthosaura meridiona **sp. nov**	QSMI1594	Na Yong, Trang, Thaiand	7°34'12.0"N, 99°46'48.0"E	MH777404	This study
Acanthosaura meridiona **sp. nov**	THNHM28061	Na Yong, Trang, Thaiand	7°34'12.0"N, 99°46'48.0"E	MH777407	This study
Acanthosaura meridiona **sp. nov**	THNHM28062	Na Yong, Trang, Thaiand	7°34'12.0"N, 99°46'48.0"E	MH777405	This study
Acanthosaura crucigera	QSMI1592	Muang, Tak, Thailand	16°46'48.0"N, 98°55'12.0"E	MH777408	This study
Acanthosaura crucigera	QSMI1593	Muang, Tak, Thailand	16°46'48.0"N, 98°55'12.0"E	MH777403	This study
Acanthosaura crucigera	THNHM28057	Muang, Tak, Thailand	16°46'48.0"N, 98°55'12.0"E	MH777402	This study
Acanthosaura crucigera	CAS229582	Kawthaung, Tanintharyi, Myanmar	-	GU817389	Wood et al. (2010)
Acanthosaura crucigera	CUMZR2008.05.26.1	Petchaburi, Thailand	-	HM143889	Wood et al. (2010)
Acanthosaura armata	NSMT-H4595	Asia	-	AB266452	Okajima and Kumazawa (2010)
Acanthosaura armata	-	Asia	-	NC014175	Okajima and Kumazawa (2010)
Acanthosaura aurantiacrista	THNHM28064	Mae Sariang, Mae Hong Son, Thailand	18°09'02.8"N, 97°58'50.2"E	MH777406	Trivalairat et al. (2020)
Acanthosaura aurantiacrista	QSMI1446	Sop Khong, Omkoi, Chiang Mai, Thailand	17°39'45.4"N, 98°11'53.6"E	MK798128	Trivalairat et al. (2020)
Acanthosaura aurantiacrista	THNHM28521	Sop Khong, Omkoi, Chiang Mai, Thailand	17°39'45.4"N, 98°11'53.6"E	MK798129	Trivalairat et al. (2020)
Acanthosaura aurantiacrista	THNHM28522	Sop Khong, Omkoi, Chiang Mai, Thailand	17°39'45.4"N, 98°11'53.6"E	MK798130	Trivalairat et al. (2020)
Acanthosaura aurantiacrista	QSMI1447	Sop Khong, Omkoi, Chiang Mai, Thailand	17°39'45.4"N, 98°11'53.6"E	MK798131	Trivalairat et al. (2020)
Acanthosaura aurantiacrista	QSMI1448	Sop Khong, Omkoi, Chiang Mai, Thailand	17°39'45.4"N, 98°11'53.6"E	MK798132	Trivalairat et al. (2020)
Acanthosaura aurantiacrista	THNHM28523	Sop Khong, Omkoi, Chiang Mai, Thailand	17°39'45.4"N, 98°11'53.6"E	MK798133	Trivalairat et al. (2020)
Acanthosaura capra	MVZ222130	Vietnam	-	AF128498	Macey et al. (1997)
Acanthosaura cardamomensis	FMNH263225	Kampot, Cambodia	10°37'19"N, 104°02'52"E	GU817397	Wood et al. (2010)
Acanthosaura cardamomensis	FMNH263261	Kampot, Cambodia	10°37'19"N, 104°02'52"E	GU817400	Wood et al. (2010)
Acanthosaura lepidogaster	MVZ224090	Vinh Thu, Vietnam	-	AF128499	Macey et al. (2000)
Acanthosaura lepidogaster	MD001	Hainan, China	-	KR092427	Yu et al. (2015)
Outgroup					
Calotes emma	CAS223062	Rakhine State, Myanmar	-	DQ289460	Zug et al. (2006)

Table 2. Pairwise distances of ND2 within and among six species of *Acanthosaura*, including outgroup *Calotes emma*: *A. meridiona* **sp. nov**- MH777405 (THNHM 28062, Holotype), MH777404 (QSMI 1594, Paratype), MH777407 (THNHM 28061, Paratype); *A. crucigera*- MH777402 (THNHM 28057), MH777403 (QSMI 1593), MH777408 (QSMI 1592).

Taxa	1	2	3	4	5	6
Calotes emma	-	-	-	-	-	-
Acanthosaura armata	0.332	-	-	-	-	-
Acanthosaura cardamomensis	0.344-0.354	0.165-0.172	-	-	-	-
Acanthosaura meridiona **sp. nov.**	0.340-0.346	0.189-0.192	0.147-0.155	0.000-0.009	-	-
Acanthosaura crucigera	0.348-0.367	0.196-0.215	0.140-0.148	0.099-0.111	-	-
Acanthosaura capra	0.344	0.173	0.199-0.210	0.215-0.222	0.224-0.233	-
Acanthosaura lepidogaster	0.363	0.182-0.189	0.182-0.222	0.199-0.231	0.208-0.237	0.163-0.185

of premature stop codons in the sequences. Average pairwise distance between individuals and mitochondrial clades were generated in MEGA6. The Maximum Likelihood analysis (ML) was performed using MEGA6 with 2,000 tree search replicates, 25 initial GAMMA rate categories and final optimisation using four GAMMA shape categories.

Bayesian Inference was performed in MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003), based on best – fit models of sequence evolution selected by MrModetest 2.3 (Nylander, 2004) under the Akaike Information Criterion (AIC). To calculate Bayesian posterior probabilities (BPP), 2,000 pseudo – replicates of the rapid bootstrap algorithm were run for 20 million generations with tree sampling every 100 generations implementing a General Time Reversible model (GTR) and GAMMA distribution of nucleotide rates. Bayesian posterior probabilities were then estimated using a Markov Chain Monte Carlo (MCMC) sampling approach after the average standard deviations once reached 0.002. A 50 % majority consensus tree was generated after discarding 20 % of initial samples as burn – in. Bootstrap values 70 % for ML and BPP of ≥ 95% were considered as indicators of strongly – supported nodes (Hillis & Bull, 1993; Felsenstein, 2004).

RESULTS

Molecular analysis

Molecular comparison of 698 nucleotides of ND2 revealed a difference of 0 – 0.9 % among three specimens of *A.* cf. *crucigera* from the southern region (GenBank references MH777404, MH777405 and MH777407) (Table 2). The ND2 analyses among the three specimens of *A.* cf. *crucigera* from revealed differences of 9.9 – 11.1 % compared to five specimens of *A. crucigera* from western region (GenBank GU817389, HM143889, MH777402, MH777403, and MH777408); differences of 14.7 – 15.5 % compared to two specimens of *A. cardamomensis* (GenBank GU817397 and GU817400); differences of 18.9 – 19.2 % compared to two specimens of *A. armata* (GenBank AB266452 and NC014175); differences of 19.9 – 23.1 % compared to two specimens of *A. lepidogaster* (Cuvier, 1829) (GenBank AF128499 and KR092427); and differences of 21.5 – 22.2 % compared to one specimen of *A. capra* Günther, 1861 (GenBank AF128498). The phylogenetic relationships within the genus *Acanthosaura* revealed through Bayesian inference and maximum – likelihood analyses of the ND2 gene showed multiple, strongly supported lineages (Fig. 1). In both analyses *Acanthosaura* cf. *crucigera* from southern Thailand form a clade that is distinct from other populations.

Taxonomy

Acanthosaura meridiona **sp. nov.**

(ZooBank: BAD96710-9B36-4E22-BE76-C126E2D1DF13)

Acanthosaura armata: Blanford 1879: 130. (part)

Acanthosaura crucigera: Taylor 1963: 870–874. (No. 192, 3885, 3887)

Holotype: THNHM28062, ethanol – preserved whole adult male individual, collected by Poramad Trivalairat (formerly TP.RE000013SO) on 28 April 2017 (Fig. 2).

Type locality: Na Yong District, Trang Province, southern Thailand (7°57'57.9"N, 99°78'65.8"E), at 195 m asl.

Paratype: Six ethanol – preserved whole individuals. Two adult males, QSMI1594 and THNHM28059 (formerly TP.RE000004SO and TP.RE000003SO, respectively), with the hemipenis everted and four adult females, QSMI1595, QSMI1596, THNHM28060, and THNHM28061 (formerly TP.RE000005SO, TP.RE000010SO, TP.RE000009SO, and TP.RE000011SO, respectively), from the same location, collection date and collector as the holotype (Fig. 3 – 4).

Diagnosis: A medium – sized species (maximum SVL 115.1 mm for males and 118.1 mm for females) with a single short conical spine above the posterior margin of the eye; small spine on the occiput between the tympanum and the nuchal crest; tympanum scaled, large, roundish; moderately developed gular pouch; small scales intermixed with medium keeled scales on the flanks with a random distribution; nuchal crest with slightly equal rows of 8 – 10 tiny semi – conical spines; large diastema of 10 – 16 scales between the nuchal and vertebral crests; vertebral crest composed of small equally sized saw – like scales beginning in the shoulder region and decreasing in size until the base of the tail; tail 1.07 – 1.61 of SVL; black collar and black eye patch present, extending posteriorly to reach the nuchal crest. Description of the holotype: Adult male. SVL 109 mm; TL 176 mm, tail complete; HL 20.8 mm; head is one – fifth the length of the body (HL/SVL 0.19), narrow (HW/SVL 0.15), moderately tall (HD/HL 0.60), triangular in dorsal and lateral views; snout moderately long (SL/HL 0.45); rostrum moderately wide (RW/RH 2.44), steeply sloping anteriorly; canthus rostralis prominent, forming a large projecting shelf extending above the eye, composed of 14 large scales; shelf terminates with a notch anterior to the postorbital spine; rostrum moderate in size, rectangular, bordered laterally by the first supra labials and posteriorly by six smaller scales; nasal roundish, surrounded by one prenasal anteriorly, three postnasals posteriorly and one subnasal; six scales between the nasal scales; elongate supra nasal scales; moderate scales above the orbit weakly keeled; three rows of slightly keeled scales below the orbit extending from the posterior margin of the nasal to half of the eye; eye very large (EYE/HL 0.30), orbit very large (ORBIT/HL 0.50); prefrontal and frontal slightly keeled and smaller than the scales between the orbit and supralabials occipital scales weakly keeled; large parietal; short conical epidermal spine above the posterior margin of the eye, posteriorly pointed, surround by five small lanceolate scales; suborbital scales small, slightly keeled, extending in a row of five equal large scales from below the posterior margin of the eye to the anterior margin of the tympanum, decreasing in size posteriorly; short conical epidermal spine equal to the postorbital spine, laterally pointing outward, surrounded by a rosette of four small lanceolate scales; tympanum exposed, roundish similar to half of the eye, surrounded by tiny conical scales; 13 rectangular supralabials of similar size; mental pentagonal similar in size to the adjacent infralabials; two postmentals similar in size, four scales contacting the mental; chin shield large, extending

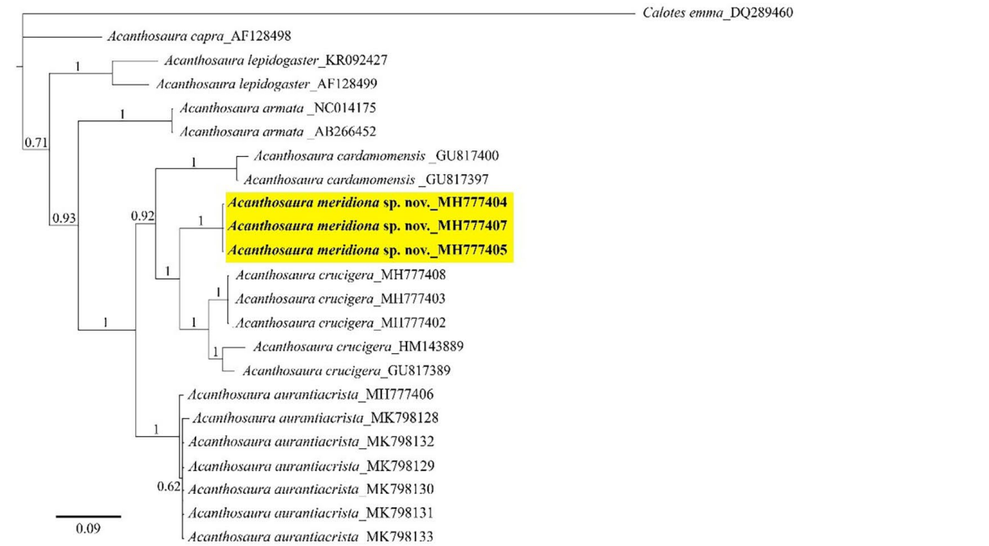

Figure 1. Phylogenetic analyses of mitochondrial NADH dehydrogenase subunit 2 gene (ND2) of *Acanthosaura*. The above phylogeny represents the analysis using Bayesian inference and the lower phylogeny was generated using maximum likelihood. Codes after sequences represent GenBank accession numbers. Highlighted tips represent *Acanthosaura meridiona* **sp. nov.**

posteriorly to the angle of the jaw, separated from the infralabials by one scales row anteriorly and four at the angle of the jaw; 11 rectangular infralabials, scales slightly decreasing in size posteriorly; gular sharply keeled and spinose with a creamy, small midventral row; dewlap and gular pouch very small and melanistic; nuchal crest composed of eight short semi – conical scales similar in size to the postorbital and occipital spine, bordered on each side by two rows of keeled, triangular scales; nuchal crest followed by a diastema of 13 scales at the base of the nape; dorsal body crest is half of the nuchal crest, extending from the posterior margin of the diastema onto the sacrum; vertebral crest composed of small, epidermal, flat, triangular scales, bordered by three rows of smaller paravertebral triangular scales; vertebral crest slightly decreasing to the sacrum, then fading progressively.

Moderate sized body, laterally compressed triangular in cross – section; dorsal body scales small and moderate keeled, randomly arranged, keels projecting posteriorly; scales of the pectoral region and abdomen larger than the dorsal scales, keeled, semi – transverse rows arranged; keeled scales anterior to the vent large; limbs relatively long, dorsal and ventral scales of forelimbs

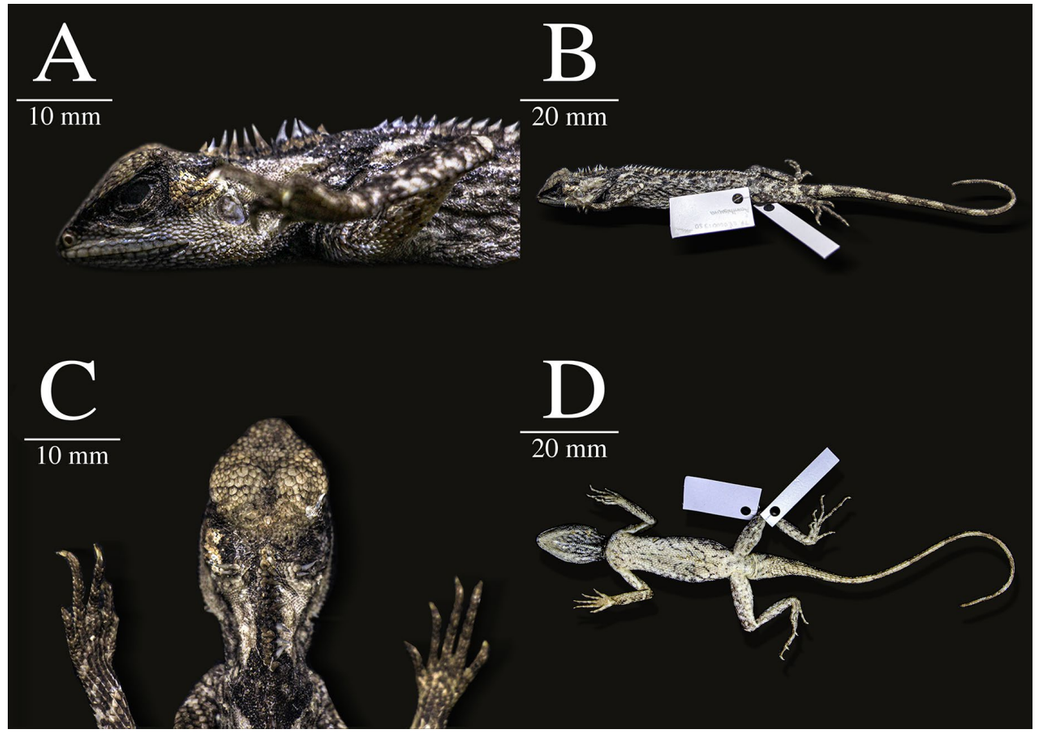

Figure 2. Adult male holotype (THNHM28062) of *Acanthosaura meridiona* **sp. nov.** from Wang Nam Rab Resort Na Yong District, Trang Province, southern Thailand (7°57'57.9"N, 99°78'65.8"E) at 195 m above sea level. **(A)** Lateral view of head. **(B)** Dorsal view. **(C)** Dorsal view of head. **(D)** Ventral view.

sponged rosette shape. In preserved ethanol, the penises exhibited creamy yellow coloration.

Coloration in life: Front of head with transverse bars of black and green, the most prominent bar crossing the orbital region; lips whitish yellow, areas where black lines radiate from the eye; black eye patch, body with a deep black marbled reticulum with some light – brown enclosing yellowish to brownish yellow spots, or greenish spots; whitish or whitish yellow ocellated spot at the knee and elbow, with others indicated on the arm and leg; ventral coloration creamy, with irregular black stripes in some cases; arms with darker and lighter marks above; legs darker above with brown bars below; tail banded with dark brown and dirty light – brown (Fig. 5).

Natural history: This species usually lives near streams, waterfalls or moist areas with rocks and logs and in areas covered with high trees shading evergreen rainforests (Fig. 6). It is active during the day on various substrates such as the ground, logs, rocks, ferns of approximately 0.5 m in height, or trees 1 – 2 m above the ground. It sleeps at night approximately 1 – 2 m above the forest floor, in a log holes or under rocks on the ground. When awakened by approach or provocation, the lizards quickly climb upward, while others may drop to the ground and seek refuge under rocks or hollow logs. Our observations showed that some individuals had eaten earthworms on the ground.

Distribution. *Acanthosaura meridiona* **sp. nov.** occurs in southern Thailand according to personal field observations, including records in Na Yong District, Trang Province; Khao Bantad Wildlife Sanctuary, Trang – Phattalung Province; Krabi Province; Wang Hip River, Thung Song District, Nakhon Si Tammarat Province; Ton Lat Waterfall, Nathavee District, Songkhla Province. In addition, specimens were collected from Natural History Museum, National Science Museum, Technopolis, Pathum Thani Province at the following locations: Khanom Waterfall, Lan Saka District, Nakhon Si Tammarat Province; Tak Ta Khan, Ban Ta Khun District, Surat Thani Province; and Thale Ban National Park, Khuan Don District, Satun Province (Fig. 7).

Etymology: The specific name meridiona comes from the Latin word meridionalis, meaning southern. It is a reference to the distribution of the species in the southern region of Thailand. We suggested the following common names: kingkakhaownaamsunn tai (Thai), southern short- horned lizard (English), süd-kurzhorn nackenstachler (German), and Acanthosaurus à cornes courtes du sud (French).

Comparisons: Table 4 summarises the comparisons of the morphometric measurements and meristic data for all currently recognised species in comparison with *A. meridiona* **sp. nov.** and other recognised *Acanthosaura* species.

Acanthosaura meridiona **sp. nov.** differs from *A. armata* in having smaller ORBIT/HL ratio (0.44 – 0.53 vs

Table 3. Morphological (in mm) and meristic data for the type series of *Acanthosaura meridiona* **sp. nov**. For character abbreviations see Materials & Methods.

	Holotype THNHM28062Adult male	Paratype THNHM28059 Adult male	Paratype QSMI 1594 Adult male	Paratype QSMI 1595 Adult female	Paratype QSMI 1596 Adult female	Paratype THNHM28060 Adult female	Paratype THNHM 28061 Adult female
SVL	109.0	100.6	115.1	109.7	116.3	116.3	118.1
Tal	176.0	>107.6	171.6	140.7	>112.0	156.7	159.9
Tal/SVL	1.61	NA	1.49	1.28	NA	1.35	1.35
TBW	11.0	12.7	13.3	10.8	9.0	14.4	10.8
HL	20.8	21.4	23.2	22.0	23.4	24.1	21.2
HL/SVL	0.19	0.21	0.20	0.20	0.20	0.21	0.18
HW	16.7	19.3	18.8	18.2	18.0	22.0	18.4
HW/SVL	0.15	0.19	0.16	0.17	0.15	0.19	0.16
HD	12.4	14.7	16.5	14.8	18.0	19.6	17.2
HD/SVL	0.11	0.15	0.14	0.13	0.15	0.17	0.15
SL	9.4	9.2	12.2	9.9	10.0	12.7	9.2
SL/HL	0.45	0.43	0.53	0.45	0.43	0.53	0.43
ORBIT	10.4	9.7	12.4	9.7	10.9	12.0	10.5
ORBIT/HL	0.50	0.45	0.53	0.44	0.47	0.50	0.50
EYE	6.2	6.6	7.5	6.7	7.4	7.6	3.0
TD	3.1	3.7	3.4	3.6	3.9	4.3	3.0
TD/HD	0.25	0.17	0.15	0.16	0.22	0.18	0.14
TN	0	0	0	0	0	0	0
PS	4.0	4.6	5.9	5.0	5.0	5.3	3.4
PS/HL	0.19	0.22	0.25	0.23	0.21	0.22	0.16
NSL	5.2	3.8	5.3	3.9	4.9	5.7	3.5
NSL/HL	0.25	0.18	0.23	0.18	0.21	0.24	0.17
NS	10	9	9	10	9	9	8
DS	2.5	2.1	2.1	2.6	3.1	2.2	2.2
DS/HL	0.12	0.10	0.09	0.12	0.13	0.09	0.10
WNC	0.8	1.0	1.3	0.9	1.2	1.6	1.0
DIAS	7.5	6.6	7.6	8.1	8.1	5.5	7.7
DIAS/SVL	0.07	0.07	0.07	0.07	0.07	0.05	0.07
DIASN	13	10	13	14	16	10	14
FOREL	49.5	43.8	54.1	56.1	41.0	49.1	44.2
HINDL	59.4	56.9	65.6	46.7	57.8	59.6	56.8
SUPRAL	13	12	12	13	13	13	13
INFRAL	11	12	11	13	13	12	11
VENT	65	66	67	64	63	60	60
FI	18	18	17	17	17	18	16
TO	23	22	22	23	>14	22	22
OS	4.1	5.3	3.1	3.8	3.8	4.4	3.4
OS/HL	0.20	0.25	0.13	0.17	0.16	0.18	0.16
NSSOS	4	4	5	4	4	4	4
CS	14	12	13	14	15	13	12
RW	2.2	3.0	2.9	2.7	3.5	3.4	3.1
RH	0.9	1.2	1.4	1.1	1.7	1.4	1.5
RS	6	6	5	5	6	5	5
NS	6	7	7	7	9	7	7
NCS	10	13	13	15	15	13	14
NSCSL	9	10	9	9	10	9	9
NR	1	1	1	1	1	1	1
NSSLC	12	12	10	10	9	10	12
MW	1.0	1.4	1.1	1.2	1.0	1.2	1.0
MH	0.5	1.2	0.9	1.0	0.9	0.9	0.7
MW/MH	2.00	1.17	1.22	1.20	1.11	1.33	1.43
PM	4	4	4	4	4	4	4
YAS	1	1	1	1	1	1	1
ND	1	1	1	1	1	1	1
LKP	1	1	1	1	1	1	1
BEP	1	1	1	1	1	1	1
ESBO	0	0	0	0	0	0	0
GP	1	1	2	1	1	1	1
OF	**1**	**1**	**1**	**1**	**1**	**1**	**1**

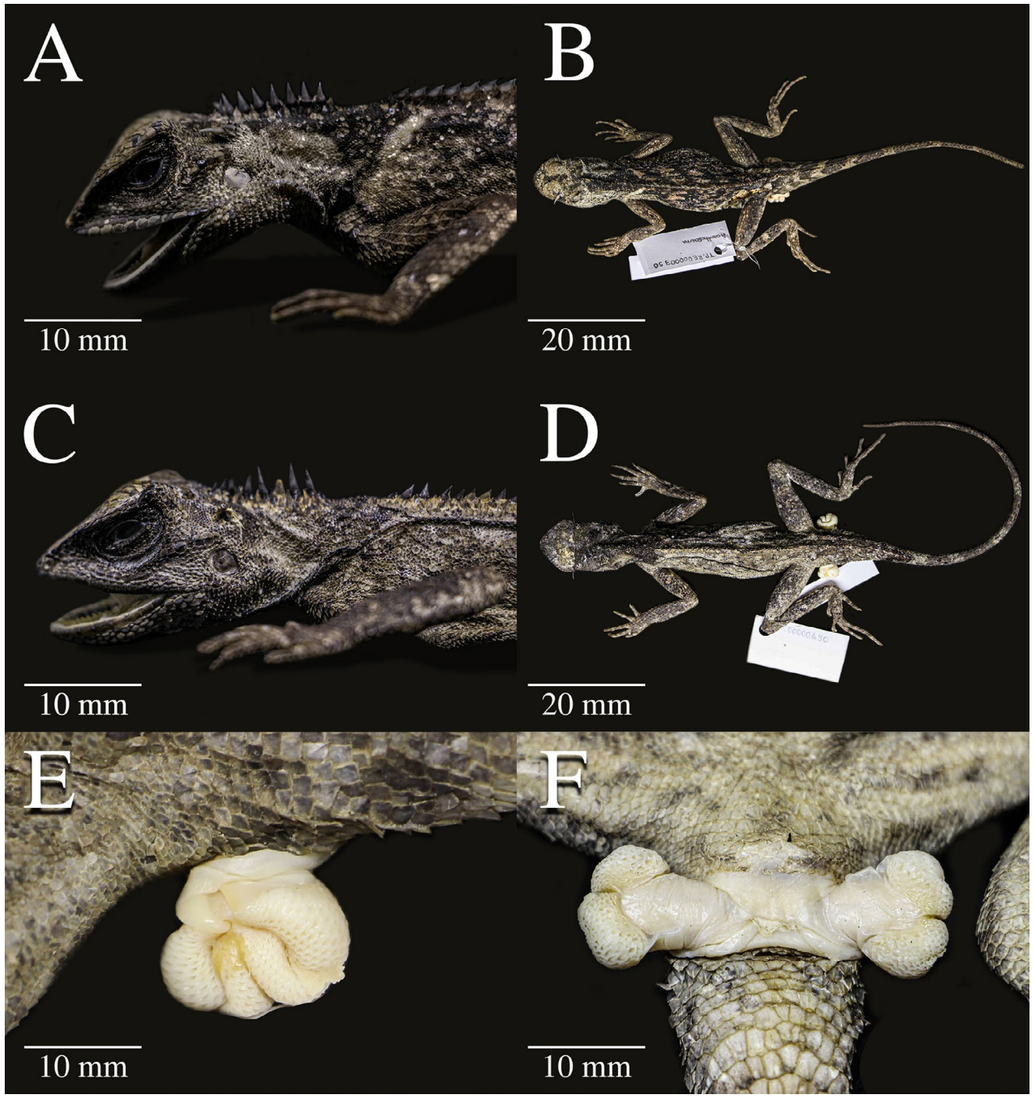

Figure 3. Paratype specimens of male *Acanthosaura meridiona* **sp. nov. (A)** Lateral view of head of THNHM28059. **(B)** Dorsal view of THNHM28059. **(C)** Lateral view of head of QSMI1594. **(D)** Dorsal view of QSMI1594. **(E)** Everted left hemipenis. **(F)** Cloaca opening with everted hemipenis.

slightly keeled, proximal scales smaller than the distal scales; five digits on the manus; subdigital scales keeled, subdigital lamellae under the fourth finger 18. Scales on the hindlimb keeled, femoral scales slightly keeled and smaller than those on the tibia; five digits on the pes; subdigital scales keeled, subdigital lamellae under the fourth finger toe 23; tail length 1.6 times SVL, tail covered with keeled spinose scales, keels on subcaudals directed posteriorly; subcaudals much longer than supracaudals; base of the tail 11 mm wide.

Variation: The paratypes resemble the holotype in all the characters except that THNHM28059 (male), THNHM28061 (female) and QSMI1594 (female) differ from the holotype in lacking stripes in the dorsal head region. All specimens present varied nuchal scales 8 – 10. THNHM28061 (female), QSMI1594 (male), QSMI1595 (female) and QSMI1596 (female) differ from the holotype in lacking a faint dark marbled pattern on the dorsum bearing small randomly distributed yellow markings. QSMI1594 (male) and QSMI1596 (female) differ from the holotype in exhibiting creamy ventral coloration without black stripes. The paratypes except for THNHM25089 (male) differ from the holotype in presenting a darker gular region. Morphometric and meristic data for the type series are shown in Table 3.

The hemipenis of two specimens (THNHM28059 and QSMI1594) were everted and showed lengths of 10 – 13 mm, and each penis side diverged to a symmetrical

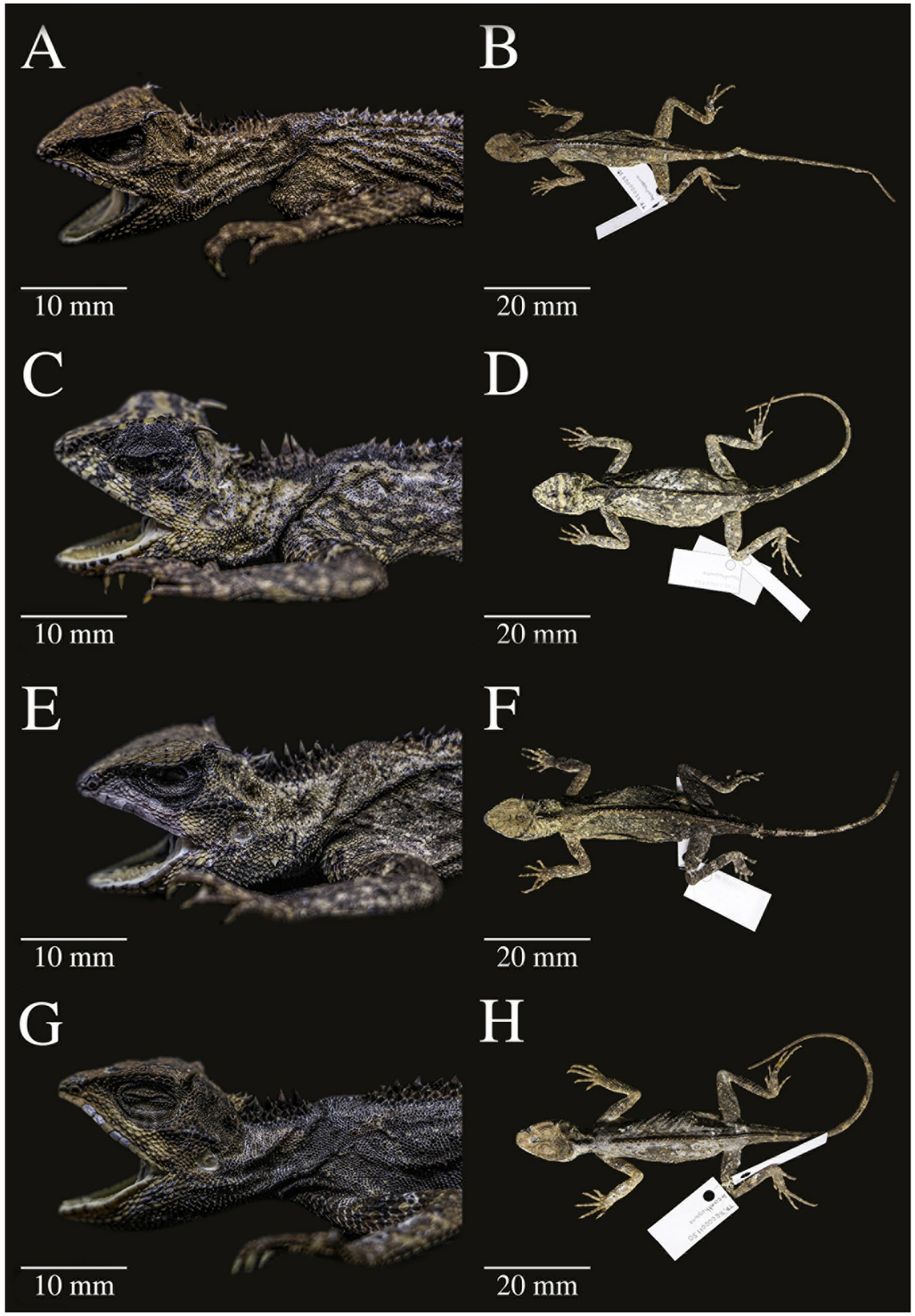

Figure 4. Paratype specimens of female *Acanthosaura meridiona* **sp. nov. (A)** Lateral view of head of QSMI1595. **(B)** Dorsal view of QSMI1595. (**C)** Lateral view of head of QSMI1596. **(D)** Dorsal view of QSMI1596. **(E)** Lateral view of head of THNHM28060. **(F)** Dorsal view of THNHM28060. **(G)** Lateral view of head of THNHM28061. **(H)** Dorsal view of THNHM28061.

Table 4. Comparison of morphometric (in mm) and meristic data for all currently recognised species of *Acanthosaura* and *Acanthosaura meridiona* **sp. nov.**, "?" = data not available.

	***A. meridiona* sp. nov.**	***A. aurantiacrista***	***A. armata***	***A. bintangensis***	***A. brachypoda***	***A. capra***	***A cardamomensis***	***A. coronata***	***A. crucigera***	***A. lepidogaster***	***A. murphyi***	***A. nataliae***	***A. phongdienensis***	***A. phuketensis***	***A. tongbinguanensis***	***A. titiwangsaensis***	***A. prasina***	***A. liui***
SVL	88.7-118.1	80.8-130.1	69.2-138.0	83.9-142.0	117	94.0-137.9	82.0-149.0	66.0-86.1	69.2-127.0	76.5-101.1	103.7-127.3	106.7-158.0	58.5-77.4	69.2-123.5	93.0-115.6	91.8-118.4	79.8-88.4	84.1-95.9
TaL	108.5-176.0	137.0-202.2	96.6-190.0	112.8-206.0	185.4	133.6-182.1	103.0-188.0	86.3-105.0	130.0-174.0	130.6-144.1	159.3-195.8	132.5-190.0	94.6-137.2	107.0-205.6	144.9-205.0	136.0-174.0	137.7-152.6	139.3-155.3
Tal/SVL	1.10-1.60	1.40-1.70	1.2-1.6	1.3-1.4	1.58	1.2-1.5	1.2-1.6	0.6-1.0	1.1-1.8	1.6-1.9	1.48-1.54	1.0-1.5	1.5-1.9	1.4-1.7	1.56-1.85	1.1-1.5	1.64-20.7	1.47-1.77
TBW	8.3-14.4	7.3-19.2	15.1-15.6	?	?	?	5.8-12.8	?	7.4-14.5	5.9-11.8	?	15.0-16.3	?	5.4-14.5	?	?	?	10.6-13.9
HL	17.0-24.1	15.6-24.2	6.6-33.7	16.9-25.4	30.3	16.3-38.9	16.3-42.2	14.4-16.3	18.7-23.6	18.9-29.7	29.1-36.8	15.0-43.6	18.6-23.8	19.7-31.4	27.5-33.2	20.0-24.3	20.7-22.4	26.7-30.0
HL/SVL	0.18-0.21	0.17-0.22	0.18	?	0.26	?	0.19-0.37	?	0.20-0.26	0.19-0.26	?	?	?	0.21-0.22	?	?	0.25-0.28	0.30-0.31
HW	14.7-22.0	14.7-19.9	15.3-23.0	17.5-23.4	20.6	16.8-27.0	16.4-27.7	13.6-17.5	16.0-22.3	13.4-20.8	20.3-24.6	20.2-27.8	13.1-15.9	14.4-22.8	18.6-23.3	17.5-23.4	14.0-16.4	18.3-22.4
HW/SVL	0.15-0.19	0.15-0.18	0.16-0.18	?	0.18	?	0.15-0.28	?	0.16-0.20	0.19-0.24	?	?	?	0.06-0.24	?	?	0.17-0.20	0.21-0.23
HD	12.4-19.6	12.5-21.7	12.2-18.9	15.0-19.2	17.2	14.8-24.3	12.6-21.7	11.9-16.8	15.7-22.5	12.0-12.5	18.5-20.6	16.9-24.9	10.4-13.6	10.9-18.6	13.9-17.4	15.7-20.2	12.3-13.3	15.1-17.3
HD/SVL	0.11-0.17	0.14-0.18	0.14-0.16	?	0.15	?	0.14-0.29	?	0.13-0.18	0.16-0.30	?	?	?	0.15-0.19	?	?	0.15	0.17-0.18
SL	9.2-12.7	6.6-12.4	6.3-16.6	7.9-11.3	12.2	7.6-16.6	8.6-18.7	6.9-8.4	8.7-12.1	9.3-10.2	10.3-15.3	12.0-19.9	?	6.8-11.0	9.2-11.0	9.7-12.5	8.8-9.4	10.3-11.3
SL/HL	0.43-0.53	0.41-0.58	0.42-0.60	?	0.40	?	0.47-0.57	?	0.38-0.50	0.42-0.66	?	?	?	0.41-0.56	?	?	0.10-0.12	0.11-0.13
ORBIT	8.5-12.4	6.8-11.8	5.4-13.3	8.4-12.6	8.3	7.6-11.6	5.8-12.7	6.9-7.5	8.9-10.8	4.7-9.1	9.9-12.3	7.2-10.9	?	6.6-11.2	7.7-11.0	9.8-13.2	6.5-8.7	8.4-8.9
ORBIT/HL	0.44-0.53	0.41-0.52	0.59-0.65	?	0.27	?	0.45-0.54	?	0.41-0.61	0.40-0.57	?	?	?	0.59-0.66	?	?	0.31-0.39	0.09-0.10
EYE	3.0-7.6	4.4-8.5	8.0-9.9	?	?	?	4.0-8.8	?	3.5-7.2	3.2-6.0	?	?	?	3.3-7.5	?	?	?	5.8-6.4
TD	2.9-4.3	2.0-4.9	2.4-5.2	2.5-3.0	3.6	3.4-5.2	2.5-5.8	1.7-2.8	2.5-3.9	2.2-3.0	3.2-5.2	3.9-7.0	1.78-2.81	3.5-4.7	3.2-4.2	2.7-4.0	2.7-5.3	2.9-3.8
TD/HD	0.14-0.27	0.15-0.30	0.19-0.28	0.16	0.21	0.21-0.23	0.20-0.27	0.14-0.17	0.14-0.21	0.18-0.24	0.17-0.28	0.23-0.28	0.17-0.22	0.22-0.33	0.21-0.24	0.17-0.20	0.22-0.43	0.03-0.04
TN	0	0	0	0	0	0	0	0	0	0-1	1	0	0	0	0	0	?	0
PS	3.4-7.0	5.5 -19.1	4.9-12.0	1.9-4.2	3.2	5.2-10.2	3.2-12.7	Absent	1.9-7.8	1.2-2.5	5.6-11.8	7.7-17.8	1.18-2.07	4.6-11.8	3.6-6.3	3.3-4.4	0.8-3.2	2.1-3.2
PS/HL	0.16-0.38	0.24-0.84	0.22-0.58	0.07-0.19	0.11	0.36	0.14-0.45	Absent	0.09-0.33	0.06-0.17	0.16-0.34	0.36-0.52	0.06-0.09	0.23-0.38	0.13-0.19	0.14-0.18	0.04-0.15	0.07-0.11
NSL	2.6-6.9	5.5-21.6	5.5-11.2	1.3-4.7	4.7	4.2-14.7	3.8-17.4	Absent	3.1-8.9	2.9-3.4	7.0-14.9	8.5-23.8	1.24-4.18	4.1-12.2	4.0-6.7	2.7-4.4	2.8-3.2	2.2-7.1
NSL/HL	0.15-0.37	0.35-0.95	0.22-0.51	0.17-0.21	0.16	0.42-0.43	0.17-0.66	Absent	0.14-0.38	0.12-0.15	0.24-0.43	0.58-0.75	0.07-0.18	0.21-0.39	0.15-0.21	0.11-0.18	0.13-0.14	0.17-0.24
NS	8-10	8	12	?	?	?	7-9	Absent	6-7	6-8	?	7	?	7-8	?	?	?	5-7
DS	1.4-3.9	2.4-8.7	4.9-11.3	1.8-2.2	1.9	3.5-6.8	2.0-14.2	Absent	2.0-5.5	0.8-3.0	2.6-10.5	6.0-17.7	0.58-1.65	2.3-8.3	2.4-4.2	1.7-2.1	?	2.7-3.7
DS/HL	0.08-0.21	0.15-0.38	0.20-0.52	0.08-0.09	0.06	0.16-0.17	0.14-0.45	Absent	0.09-0.24	0.06-0.15	0.14-0.51	0.41-0.53	0.03-0.07	0.11-0.26	0.09-0.13	0.07-0.09	?	0.10-0.12
WNC	0.8-1.6	0.6-2.9	1.0-2.2	1.6-2.1	1.6	2.3-4.1	1.8-4.2	Absent	1.3-3.4	0.9-1.5	2.9-4.8	3.0-4.8	?	1.4-2.9	1.0-1.5	1.4-1.6	?	1.1-1.3
DIAS	4.7-8.1	3.3-5.4	1.2-6.8	5.0-7.9	?	2.0-6.7	2.7-8.3	Absent	4.9-8.4	2.2-6.3	2.6-4.8	2.5-5.3	Absent	3.6-7.6	3.9-6.1	5.1-7.6	?	3.5-4.7
DIAS/SVL	0.05-0.07	0.03-0.05	0.01-0.06	0.04-0.07	?	0.05	0.03-0.09	Absent	0.04-0.08	0.02-0.08	0.02-0.04	0.03-0.04	Absent	0.05-0.08	0.03-0.07	0.05-0.07	?	0.04-0.05
DIASN	10-16	8-9	1-11	11-15	7	4-7	6-17	Absent	9-25	10-14	4-8	7-10	Absent	12-17	6-10	10-13	?	?
FOREL	40.8-56.1	36.8-54.2	33.7-56.0	33.9-61.5	?	54.2-83.8	31.7-56.8	30.2-35.3	35.6-49.8	28.2-42.8	49.8-56.6	58.4-85.0	?	22.3-42.9	34.7-43.2	38.0-51.7	?	35.8-37.0
HINDL	46.7-56.1	46.2-72.9	39.0-69.6	43.3-68.6	?	78.5-107.2	42.0-77.1	38.4-47.8	48.8-65.0	48.5-50.4	60.4-68.4	72.1-129.7	?	38.2-60.6	54.1-63.9	48.5-65.6	?	50.7-52.6
SUPRAL	11-13	10-13	10-14	12	12-13	10	11-15	12-13	10-13	10-13	12-14	10-11	9-12	10-12	11-14	12-13	9-11	10-13
INFRAL	11-13	9-11	12-15	11-12	11	12-13	10-14	11-13	10-12	9-13	12-14	11-12	10-11	10-12	10-14	11-12	9-11	10-11
VENT	60-68	63-66	51-68	51-55	63	55-66	50-67	53-58	55-63	52-61	55-65	64-71	?	57-67	52-66	47-57	59-63	52-56
FI	16-18	17-23	13-17	23	18	16-17	15-20	13-14	16-18	17-19	15-18	16-21	14-17	15-17	19-21	20-21	16-18	16-18

	A. meridiona sp. nov.	*A. aurantiacrista*	*A. armata*	*A. bintangensis*	*A. brachypoda*	*A. capra*	*A cardamomensis*	*A. coronata*	*A. crucigera*	*A. lepidogaster*	*A. murphyi*	*A. nataliae*	*A. phongdienensis*	*A. phuketensis*	*A. tongbinguanensis*	*A. titiwangsaensis*	*A. prasina*	*A. liui*
TO	22-25	25-29	19-26	26-28	24	22-24	20-26	17-19	21-26	22-23	21-23	20-27	19-23	21-24	25-28	23-27	23-26	22-25
OS	2.4-7.0	3.1-10.0	4.0-9.4	1.2-2.6	1.0	Absent	4.1-13.6	Absent	2.5-4.9	3.2-3.4	Absent	Absent	?	2.6-9.5	4.5-7.0	1.8-2.3	?	3.6-4.8
OS/HL	0.13-0.25	0.19-0.44	0.16-0.43	0.10-0.11	0.03	Absent	0.24-0.56	Absent	0.11-0.50	0.14-0.15	Absent	Absent	?	0.13-0.30	0.16-0.23	0.09-0.10	?	0.12-0.16
NSSOS	3-5	5	4-6	6-7	?	Absent	4-6	4-5	4-6	4-5	Absent	Absent	?	4-5	4-5	4-5	?	4-6
CS	12-15	10-14	11-15	14-15	?	12-14	11-16	12-15	12-15	10-14	12-14	12-13	9-13	10-14	10-14	14-15	5-6	12
RW	1.9-3.5	2.5-3.7	1.7-4.5	3.6-5.3	3.5	4.2-4.6	1.7-4.7	0.8-0.9	2.7-4.0	2.8-3.0	3.3-5.1	4.6-6.1	2.07-2.65	2.3-3.8	3.3-4.5	3.6-5.2	1.6-2.4	3.4-4.2
RH	0.8-1.8	0.9-2.1	0.9-1.8	1.7-2.0	2.3	1.8-2.3	1.1-2.2	0.5-0.8	1.3-2.0	1.4-1.5	1.2-2.0	1.8-2.9	1.00-1.32	1.1-1.7	1.0-2.0	1.4-1.8	1.0-1.5	1.4-1.9
RS	3-6	4-6	5-8	5-9	5-9	5-9	7-9	9	7-8	6-7	8-9	7	?	5-9	6-9	5	?	8-9
NS	5-9	5-6	6-10	8	9	9	7-10	7-9	7-9	7-8	7-8	5-8	?	7-8	8-9	8	?	?
NCS	10-15	11-13	10-17	10-11	?	9	9-17	8-11	9-12	7-11	13-16	10-14	?	12-13	10-13	11-12	?	?
NSCSL	9-11	6-10	6-14	7-8	?	7-8	7-12	5-6	7-11	7-12	7-10	8-11	?	8-10	7-9	9-11	?	?
NR	1	1-2	1-2	1	?	1-2	1-2	3-4	1-2	1-2	3-4	1	?	1-2	2	1-2	?	1-2
NSSLC	9-13	9-13	10-22	9-12	?	9-11	10-19	6-11	10-14	10-18	?	13-16	?	11-14	9-13	11-14	?	?
MW	0.2-1.4	1.1-2.5	0.8-2.0	1.3-1.8	2.9	1.9-2.2	0.2-2.1	0.6-1.5	1.0-1.5	1.2-1.3	1.7-2.2	2.3-2.9	0.87-1.52	0.5-1.4	1.4-1.9	1.4-2.0	1.4-1.9	1.8-2.4
MH	0.5-1.4	0.8-1.6	0.8-2.3	1.4-2.1	2.1	1.7-2.2	0.9-2.0	1.3-1.6	1.1-1.7	1.2-1.3	1.4-2.0	2.0-2.9	1.04-1.60	0.6-1.6	1.2-2.0	1.4-2.4	1.0-1.2	1.7-2.3
MW/MH	1.11-2.00	0.69-2.08	0.50-0.87	?	1.38	?	?	?	1.23-2.69	0.69-1.08	?	0.79-0.96	?	1.00-2.00	?	?	1.40-1.90	1.00-1.33
PM	4	4	3-6	4-5	4	4	4-5	4-5	4	5	?	4-5	?	4	4-5	5	?	5-6
YAS	1	1	0-1	1	1	1	0-1	0-1	1	1	0-1	1	?	0-1	1	1	?	1
ND	1	1	0-1	1	1	1	1	0	1	1	?	0	1	1	1	1	?	1
LKP	1	1	1	0	1	1	1	1	1	1	?	0	1	1	1	0	?	1
BEP	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**0**	**1**	**0-1**	**0-1**	**0-1**	**?**	**1**	**1**	**1**	**?**	**1**
ESBO	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**?**	**0**	**?**	**0**	**0**	**0**	**?**	**0**
GP	**1-2**	**1-4**	**1**	**3-4**	**0**	**3-4**	**1-4**	**0**	**1-2**	**0-1**	**4**	**3-4**	**?**	**0-2**	**1-2**	**2-4**	**?**	**2-3**
OF	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**?**	**1**	**1**	**1**	**1**	**1**	**?**	**1**

0.59 – 0.65), fewer NS (8 – 10 vs 12), greater MW/MH ratio (1.11 – 2.00 vs 0.50 – 0.87) and the presence of BEP, which is absent in *A. armata*.

Acanthosaura meridiona **sp. nov.** differs from *A. aurantiacrista* Trivalairat et al., 2020 in having smaller PS/HL ratio (0.16 – 0.25 vs 0.24 – 0.84), smaller NSL/HL (0.17 – 0.25 vs 0.35 – 0.95) and more DIASN (10 – 16 vs 8 – 9).

Acanthosaura meridiona **sp. nov.** differs from *A. bintangensis* in having more VENT (60 – 67 vs 51 – 55), fewer FI (16 – 18 vs. 23), fewer TO (22 – 25 vs. 26 – 28), greater OS/HL (0.13 – 0.25 vs. 0.10 – 0.11), fewer NSSOS (4 – 5 vs. 6 – 7), more NSCSL (9 – 11 vs. 7 – 8), LKP, fewer GP (1 – 2 vs 3 – 4), and the absence of ESBO.

Acanthosaura meridiona **sp. nov.** differs from *A. brachypoda* Ananjeva et al., 2011 in having smaller HL/SVL ratio (0.18 – 0.21 vs 0.26), greater ORBIT/HL ratio (0.44 – 0.53 vs 0.27), greater PS/HL ratio (0.16 – 0.25 vs 0.11), greater DS/HL ratio (0.08 – 0.21 vs 0.06), more DIASN (10 – 16 vs 7), fewer TO (22 – 25 vs. 24), greater OS/HL (0.13 – 0.25 vs 0.03) and more GP (1 – 2 vs 0)

Acanthosaura meridiona **sp. nov.** differs from *A. capra* in having smaller NSL/HL (0.15 – 0.37 vs 0.42 – 0.43), and the presence of occipital spines and fewer GP (1 – 2 vs 3 – 4).

Acanthosaura meridiona **sp. nov.** differs from *A. coronata* Günther, 1861 in having postorbital spines, nuchal scales, dorsal scales, diastema, and occipital spines, which is absent in *A. coronata*.

Acanthosaura meridiona **sp. nov.** differs from *A. lepidogaster* in having greater NSL/HL (0.17 – 0.25 vs 0.12 – 0.15), fewer RS (3 – 6 vs 7 – 8) and fewer PM (4 vs 5).

Acanthosaura meridiona **sp. nov.** differs from *A. murphyi* Nguyen et al., 2018 in having greater DIAS/SVL ratio (0.05 – 0.07 vs 0.02 – 0.04), more DIASN (10 – 16 vs 4 – 8), fewer RS (3 – 6 vs 8 – 9), fewer NR (1 vs 3 – 4), occipital spines, fewer GP (1 – 2 vs 4), and the absence of TN, which is present in *A. murphyi*.

Acanthosaura meridiona **sp. nov.** differs from *A. nataliae* Orlov et al., 2006 in having smaller NSL/HL ratio (0.17 – 0.25 vs 0.58 – 0.75), smaller DS/HL ratio (0.08 – 0.21 vs 0.41 – 0.53), greater DIAS/SVL ratio (0.05 – 0.07 vs 0.03 – 0.04), fewer RS (3 – 6 vs 7), greater MW/MH ratio (1.11 – 2.00 vs 0.79 – 0.96) and the presence of occipital spines, ND, LKP, and fewer GP (1 – 2 vs 3 – 4).

Acanthosaura meridiona **sp. nov.** differs from *A. phongdienensis* Nguyen et al., 2019 in having greater PS/HL ratio (0.16 – 0.38 vs 0.06 – 0.09), greater DS/HL ratio (0.08 – 0.21 vs 0.03 – 0.07), and the presence of diastema, which is absent in *A. phongdienensis*.

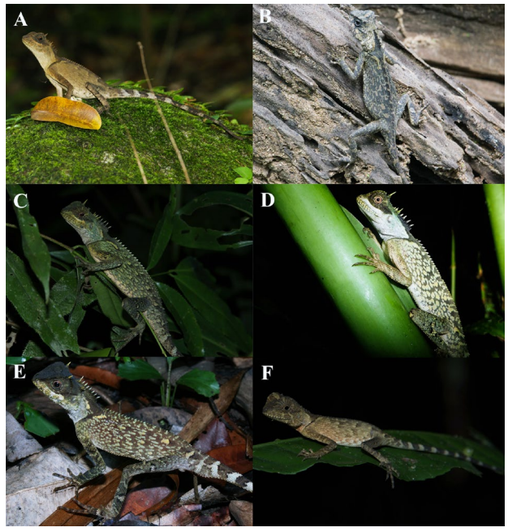

Figure 5. Colour pattern variation within *Acanthosaura meridiona* **sp. nov. (A – B)** Adult female from Wang Nam Rab Resort Na Yong District, Trang Province. **(C – D)** Adult male from Wang Hip Dam, Thung Song District, Nakhon Si Tammarat Province. **(E)** Sub adult male from Wang Hip Dam, Thung Song District, Nakhon Si Tammarat Province. **(F)** Juvenile from Wang Hip Dam, Thung Song District, Nakhon Si Tammarat Province.

Acanthosaura meridiona **sp. nov.** differs from *A. tongbiguanensis* Liu & Rao, 2019 having fewer FI (16 – 18 vs 19 – 21) and fewer NR (1 vs 2).

Acanthosaura meridiona **sp. nov.** differs from *A. titiwangsaensis* in having more VENT (60 – 67 vs 47 – 57), fewer FI (16 – 18 vs 20 – 21), greater OS/HL ratio (0.13 – 0.25 vs 0.10 – 0.11), fewer PM (4 vs 5), and the presence of LKP, which is absent in *A. titiwangsaensis*.

Acanthosaura meridiona **sp. nov.** differs from *A. prasina* Ananjeva et al., 2020 in having smaller TaL/SVL ratio (1.10 – 1.60 vs 1.64 – 2.07), smaller HL/SVL ratio (0.18 – 0.21 vs 0.25 – 0.28), greater ORBIT/HL ratio (0.44 – 0.53 vs 0.31 – 0.39), greater PS/HL ratio (0.16 – 0.25 vs 0.04 – 0.15), greater NSL/HL ratio (0.17 – 0.25 vs 0.13 – 0.14) and more CS (12 – 15 vs 5 – 6).

Acanthosaura meridiona **sp. nov.** differs from *A. liui* Liu et al., 2020 in having smaller HL/SVL ratio (0.18 – 0.21 vs 0.30 – 0.31), smaller HW/SVL ratio (0.15 – 0.19 vs 0.21 – 0.23), greater ORBIT/HL ratio (0.44 – 0.53 vs 0.09 – 0.10), greater TD/HL ratio (0.14 – 0.27 vs 0.03 – 0.04), greater PS/HL ratio (0.16 – 0.25 vs 0.07 – 0.11), more NS (8 – 10 vs 5 – 7), more VENT (60 – 67 vs 52 – 56), fewer RS (3 – 6 vs 8 – 9) and fewer PM (4 vs 5 – 6).

However, it is noted that the morphological comparative data of species in the *A. crucigera* complex, including *A. cardamomensis*, *A. crucigera*, and *A. phuketensis*, overlapped with *A. meridiona* **sp. nov.** A

Figure 6. Habitat of *Acanthosaura meridiona* **sp. nov.** Photo in Wang Nam Rab Resort Na Yong District, Trang Province, lower – southern Thailand.

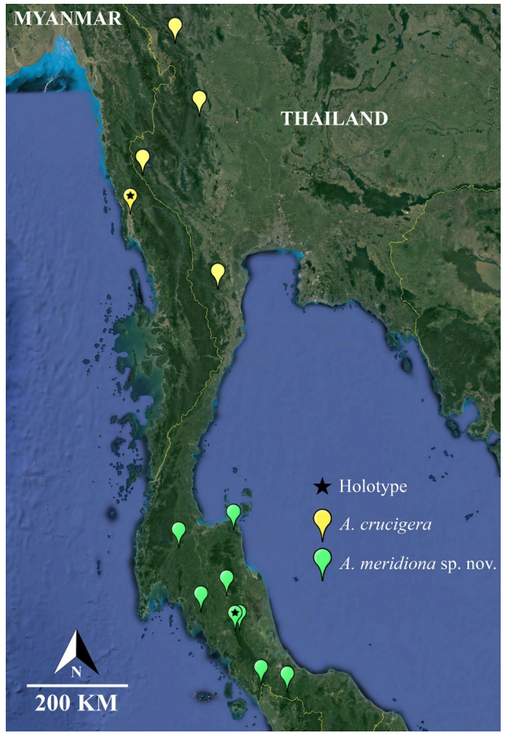

Figure 7. The distribution of *Acanthosaura meridiona* **sp. nov.** and *A. crucigera* in Thailand.

close examination of the material and comparison data by Wood et al. (2010) and Wood et al. (2015) led to separate *A. meridiona* **sp. nov.** from these similar species based on coloration, morphological characteristics, especially nuchal – dorsal scale patterns, as well as on molecular data (Fig. 8) (Table 5).

Acanthosaura meridiona **sp. nov.** differs from *A. cardamomensis* in having a much smaller maximum length of DS (7.0 vs 12.7 mm) and NSL (6.9 vs 17.4 mm), with a slight decrease in size of posterior nuchal pattern in *A. meridiona* **sp. nov.** contrast to sudden decrease in

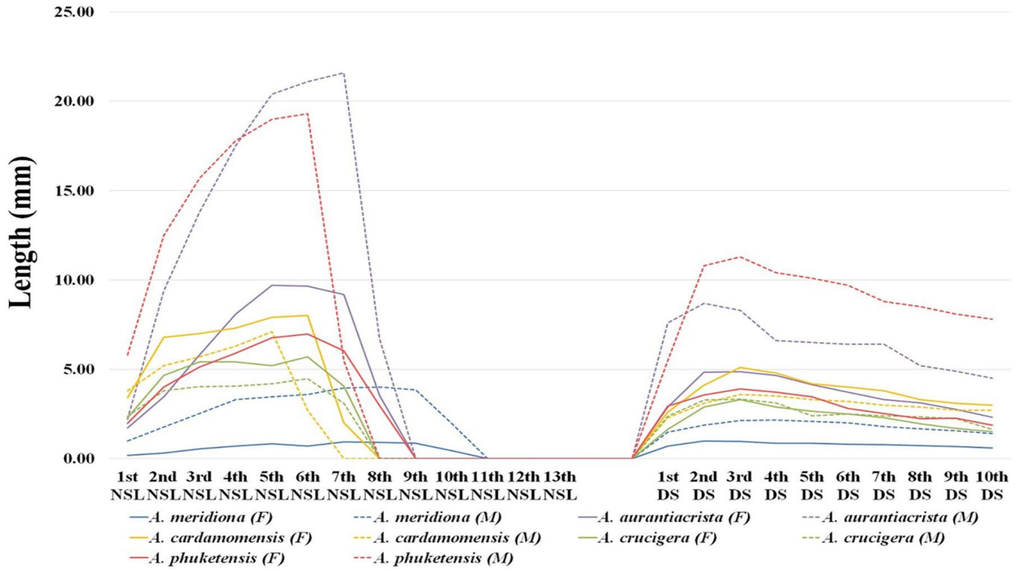

Figure 8. Average length of nuchal (NSL) and dorsal (DS) scales for five *Acanthosaura* species in the *A. crucigera* complex.

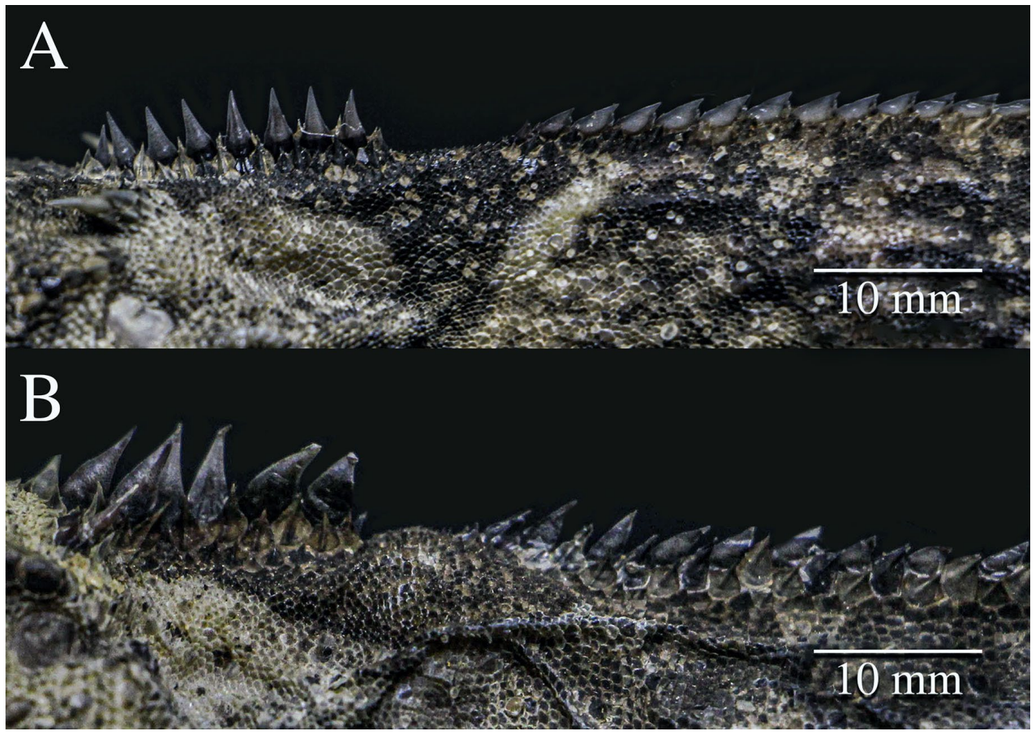

Figure 9. Morphological differentiation of nuchal scales between adult males of **(A)** *Acanthosaura meridiona* **sp. nov.** (THNHM28059) from Wang Nam Rab Resort Na Yong District, Trang Provinc, and **(B)** *Acanthosaura crucigera* (QSMI1590) from Taksin Maharat National Park, Muang District, Tak Province.

Table 5. Average and range of length (in mm) of each nuchal and first ten dorsal scales of *Acanthosaura* species in the *A. crucigera* complex for adult female (F) and male (M). "-" = no scale.

Characters	*A. meridiona* sp. nov.		*A. aurantiacrista*		*A. cardamomensis*		*A. crucigera*		*A. phuketensis*	
	F (n = 6)	M (n = 7)	F (n = 5)	M (n = 1)	F (n = 1)	M (n = 1)	F (n = 3)	M (n = 3)	F (n = 3)	M (n = 1)
1st NSL	0.19 (0.15-1.00)	1.00 (1.00)	1.72 (0.90-2.90)	2.20	3.40	3.80	2.30 (1.70-2.90)	2.37 (1.60-3.50)	1.97 (1.40-2.40)	5.80
2nd NSL	0.31 (0.20-2.30)	1.77 (0.90-3.10)	3.46 (2.10-5.00)	9.40	6.80	5.20	4.65 (3.60-5.70)	3.80 (2.20-4.60)	4.00 (2.40-5.00)	12.50
3rd NSL	0.54 (0.46-3.80)	2.51 (1.60-3.30)	5.80 (4.30-7.40)	13.80	7.00	5.70	5.40 (4.80-6.00)	4.03 (2.50-5.00)	5.13 (4.00-6.20)	15.70
4th NSL	0.69 (0.52-4.50)	3.31 (2.00-4.90)	8.10 (6.10-10.50)	17.50	7.30	6.30	5.40 (5.00-5.80)	4.07 (2.80-4.80)	5.90 (5.60-6.50)	17.80
5th NSL	0.84 (0.72-4.70)	3.47 (1.90-4.60)	9.70 (7.50-11.70)	20.40	7.90	7.10	5.20 (4.40-6.00)	4.20 (2.90-5.30)	6.77 (6.00-7.60)	19.00
6th NSL	0.70 (0.74-5.20)	3.59 (2.20-5.00)	9.64 (7.10-12.70)	21.10	8.00	2.70	5.70 (5.40-6.10)	4.47 (2.90-5.30)	6.97 (6.30-7.80)	19.30
7th NSL	0.94 (0.86-5.70)	3.95 (2.60-5.20)	9.18 (7.10-11.40)	21.60	2.00	-	4.05 (2.20-5.90)	3.10 (1.90-3.90)	6.03 (4.40-6.90)	5.50
8th NSL	0.92 (0.61-5.30)	4.01 (2.50-5.10)	3.54 (2.90-4.20)	6.70	-	-	-	-	2.97 (1.70-4.20)	-
9th NSL	0.86 (0.61-5.40)	4.01 (2.50-5.10)	-	-	-	-	-	-	-	-
10th NSL	0.45 (0.22-3.30)	1.94 (1.00-3.90)	-	-	-	-	-	-	-	-
1st DS	0.70 (0.41-2.50)	1.47 (0.80-2.70)	2.90 (1.00-5.40)	7.60	2.60	2.30	1.65 (1.40-1.90)	2.37 (1.70-2.90)	2.93 (1.50-3.70)	5.50
2nd DS	0.99 (0.96-3.10)	1.87 (1.00-3.20)	4.84 (3.60-6.50)	8.70	4.10	3.10	2.90 (2.10-3.70)	3.27 (2.60-4.40)	2.93 (1.50-3.70)	10.80
3rd DS	0.96 (0.77-3.10)	2.14 (1.00-3.50)	4.88 (3.70-5.80)	8.30	5.10	3.60	3.30 (2.70-3.90)	3.33 (2.60-4.60)	3.90 (3.30-4.50)	11.30
4th DS	0.86 (0.73-2.70)	2.17 (1.40-3.20)	4.66 (3.80-5.60)	6.60	4.80	3.50	2.90 (2.40-3.40)	3.13 (1.90-4.40)	3.73 (2.90-4.50)	10.40
5th DS	0.85 (0.71-2.40)	2.07 (1.40-2.70)	4.14 (2.60-5.40)	6.50	4.20	3.30	2.65 (2.20-3.10)	2.40 (1.90-2.90)	3.47 (2.60-4.20)	10.10
6th DS	0.81 (0.71-2.20)	2.01 (1.30-2.70)	3.72 (2.80-5.00)	6.40	4.00	3.20	2.50 (2.10-2.90)	2.50 (1.90-3.10)	2.80 (2.10-3.30)	9.70
7th DS	0.77 (0.62-2.20)	1.80 (1.30-2.60)	3.30 (2.50-4.60)	6.40	3.80	3.00	2.30 (2.00-2.60)	2.40 (1.80-3.00))	2.53 (1.90-2.90)	8.80
8th DS	0.74 (0.59-2.20)	1.67 (1.20-2.40)	3.12 (2.20-4.30)	5.20	3.30	2.90	1.95 (1.70-2.20)	2.35 (1.60-3.10)	2.53 (1.90-2.90)	8.50
9th DS	0.68 (0.52-2.10)	1.56 (1.20-2.20)	2.76 (1.80-3.60)	4.90	3.10	2.70	1.70 (1.40-2.00)	2.25 (1.70-2.80)	2.27 (2.10-2.50)	8.10
10th DS	0.59 (0.45-1.90)	1.41 (1.10-2.10)	2.32 (1.40-3.00)	4.50	3.00	2.70	1.50 (1.10-1.90)	1.65 (0.80-2.50)	1.87 (1.60-2.00)	7.80

A. cardamomensis, smaller maximum length of PS (7.0 vs 12.7 mm) and OS (7.0 – 13.6 mm, and OS/HL ratio 0.13 – 0.25 vs 0.24 – 0.56), less – developed GP (1 – 2 vs 1 – 4), and the presence of BEP extend posteriorly to reach the occipital spine, which reach to the nuchal crest in *A. cardamomensis*.

Acanthosaura meridiona **sp. nov.** is most closely related to *A. crucigera*. The most important character separating these two species is the nuchal scales. *A. meridiona* **sp. nov.** has 8 – 10 short semi – conical scales that are different from those of *A. crucigera*, which exhibits 6 – 8 short triangular lanceolate scales (Fig. 9). *A. meridiona* **sp. nov.** can also be identified from *A. crucigera*. in presenting more VENT (60 – 68 vs 55 – 63), fewer RS (3 – 6 vs 7 – 9), more NCS (10 – 15 vs 9 – 12), and smaller MH (0.5 – 1.4 vs 1.1 – 1.7).

Acanthosaura meridiona **sp. nov.** differs from *A. phuketensis* in having a much smaller maximum length of DS (7.0 vs 8.3 mm) and NSL (6.9 vs 12.2 mm), with a sudden decrease in *A. phuketensis*, smaller ORBIT/HL ratio (0.44 – 0.53 vs 0.59 – 0.66), smaller TD/HD (0.14 – 0.22 vs 0.22 – 0.33), smaller maximum length of PS (7.0 vs 11.8 mm) and OS (7.0 vs 9.5), and longer FOREL (40.8 – 56.1 vs 22.3 – 42.9 mm).

DISCUSSION

Historically, *Acanthosaura crucigera* was reported to have a wide distribution and various conserved morphological characteristics. However, after the 20th century, cryptic species in the crucigera complex have been increasingly described and designated as new members in the genus *Acanthosaura*, such as *A. cardamomensis* from eastern Thailand and Cambodia, *A. phuketensis* from south-western Thailand, and *A. bintangensis* and *A. titiwangsaensis* from Peninsular Malaysia. *Acanthosaura meridiona* **sp. nov.** is separated from the true *A. crucigera* population from the western region of Thailand and

Southern Myanmar and *A. cardamomensis* by the Kra Isthmus based on the combination of morphological comparisons, based on nuchal scales, and molecular data (Boulenger, 1885; Orlov et al., 2006; Stuart et al,. 2006; Ananjeva et al., 2008; Wood et al., 2009, 2010; Pauwels et al., 2015). Distinct characteristics have also been reported for other currently recognised species of *Acanthosaura* in the southern region, such as *A. armata* and *A. phuketensis*, which present smaller nuchal and dorsal spines. And also different from *A. bintangensis* and *A. titiwangsaensis* from Peninsular Malaysia by present light knee patch. Therefore, our research team suggested that the previously reported specimens and distribution range of *A.* cf. *crucigera* south of the Kra Isthmus should be redescribed as *A. meridiona* **sp. nov.** as a specific name related to southern Thailand.

Furthermore, *Acanthosaura meridiona* **sp. nov.** usually inhabits forests near streams and waterfalls in areas that are conserved, including Khao Pu-Khao Ya National Park; Yong Waterfall National Park; Hat Khanom – Mu Koh Thale Tai National Park; Khao Sok National Park; and the Khao Bantad Wildlife Sanctuary. However, several headwater areas are currently experiencing the effects of forest degradation for dam creation, such as those of the Wang Hip River and Yong Waterfall National Park, which are important habitats for rare endemic nearly aquatic reptiles from southern Thailand, such as *Bronchocela cristatella* (Kuhl, 1820), *B. rayaensis* Grismer et al, 2015, Varanus dumerilii Schlegel, 1839, and *V. rudicollis* (Gray, 1845), including *A. meridiona* **sp. nov.** (Lauprasert & Thirakhupt, 2001; Grismer et al., 2015, 2016). To protect this *Acanthosaura* species and all others rare endemic species that inhabit nearby streams or waterfalls, we implore the government to inhibit forest degradation and dam creation in evergreen rainforests in the southern region of Thailand.

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REFERENCES

Ananjeva, N.B., Orlov, N.L. & Kalyabina – Hauf, S.A. (2008). Species of *Acanthosaura* Gray, 1831 (Agamidae: Sauria, Reptile) of Vietnam: results of molecular and morphological study. *Biology Bulletin* 35(2), 178 – 186. DOI: 10.1134/s106235900802012x.

Ananjeva, N.B., Orlov, N.L., Nguyen, T.T. & Ryabov, S.A. (2011). A new species of *Acanthosaura* (Agamidae, Sauria) from northwest Vietnam. *Russian Journal of Herpetology* 18(3), 195 – 202.

Ananjeva, N., Ermakov, O., Nguyen, S., Nguyen, T., Murphy, R., Lukonina, S. & Orlov, N. (2020). A New Species of *Acanthosaura* Gray, 1831 (Squamata: Agamidae) from Central Highlands, Vietnam. *Russian Journal of Herpetology* 27, 217 – 230. DOI: 10.30906/1026 -2296-2020-27-4-217-230.

Blanford, W.T. (1879). Notes on a collection of reptiles made by Major O. B. St. John, R.E., at Ajmere in Rajputana. Journal of the Asiatic Society of Bengal, Vol. 48, 130 p.

Bobrov, V.V. (2015). Lizards (Reptilia, Sauria) of Vietnam in the collection of the Zoological Museum of Moscow State University. Communication 2. Genus *Acanthosaura* (family Agamidae). *Modern Herpetology* 15(3/4), 146 – 152.

Boulenger, G.A. (1885). *Catalogue of the lizards in the British Museum (Natural History). Second edition. Volume I.* Geckonidae, Eublepharidae, Uroplatidae, Pygopodidae, Agamidae. British Museum (Natural History), London, 299 – 304 p.

Boulenger, G.A. (1912). *A vertebrate fauna of the Malay Peninsular from the Isthmus of Kra to Singapore including the adjacent islands*. Reptilia and Batrachia. Taylor and Francis, London, xiv, 294 p.

Chan – ard, T., Grossmann, W., Gumprecht, A. & Schulz K.- D. (1999). *Amphibians and reptiles of Peninsular Malaysia and Thailand. An illustrated checklist. Amphibien und reptilien der Halbinsel Malaysia und Thailands. Eine illustrierte Checkliste*. Bushmaster Publication, Wuerselen, 240 p.

Cuvier, G.J.L.N.F.D. (1829). *Le Regne Animal Distribué, d'apres son Organisation, pur servir de base à l'Histoire naturelle des Animaux et d'introduction à l'Anatomie Comparé.* Nouvelle Edition [second edition]. Vol. 2. Les Reptiles. Déterville, Paris, i – xvi, 1 – 406 p.

Das, I. (2010). *A Field Guide to the Reptile of Thailand & South – East Asia*. New Holland, London. Felsenstein J. 2004. Inferring Phylogenies. Sinauer Associates, Sunderland, Massachusetts.

Gray, J.E. (1831). *A Synopsis of the Species of the Class Reptilia*. Bredell, South Africa: Herprint 1996.

Gray, J.E. (1845). *Catalogue of the Specimens of Lizards in the Collection of the British Museum*. Trustees of die British Museum/Edward Newman, London: xxvii + 289 p.

Gamble, T., Bauer, A.M., Greenbuam, E. & Jackman, T.R. (2008). Evidence for Gondwanan vicariance in an ancient clade of gecko lizard. *Journal of Biogeographyi* 35, 88 – 104.

Grismer, L.L., Youmans, T.M., Wood, P.L. Jr., Ponce, A., Johnson, R., Wright, S.B. & Norsham, S.Y. (2006). Checklist of the herpetofauna of Pulau Langkawi with taxonomic comments. H*amadryad* 29, 15 – 32.

Grismer, L.L., Neng, T., Chav, T., Wood, P.L. Jr., Oaks, J.R., Holden, J., Grismer, J.L., Szutz, T.R. & Youmans, T.M. (2008). Additional amphibians and reptiles from the Phnom Samkos Wildlife Sanctuary in the northwestern Cardamom Mountains, Cambodia, with comments on their taxonomy and the discovery of three new species. *The Raffles Bulletin of Zoology* 56, 161 – 175.

Grismer, L.L. (2011). Lizards of Peninsular Malaysia, Singapore and their adjacent archipelagos. Edition Chimaira, Frankfurtam Main, 728 p.

Grismer, L.L., Wood, P.L. Jr., Lee, C., Quah, E., Anuar, S., Ngadi, E. & Sites, J.W. Jr. (2015). An integrative taxonomic review of the agamid genus *Bronchocela* (Kuhl, 1820) from Peninsular Malaysia with descriptions of new montane and insular endemics. *Zootaxa,* 3948. 1 – 23. DOI: 10.11646/zootaxa.3948.1.1.

Grismer, L.L., Wood, P.L. Jr., Aowphol, A., Cota, M., Murdoch, M., Puntriano, C. & Grismer, M. (2016). Taxonomy, phylogeny, and distribution of *Bronchocela rayaensis* (Squamata: Agamidae) on the Thai-Malay Peninsula. *Zootaxa* 4092(3), 414 – 420. DOI: 10.11646/zootaxa.4092.3.6.

Günther, A.C.L.G. (1861). Second list of Siamese reptiles. *Proceedings of the Zoological Society of London* 1861(8), 135 – 140.

Hardwicke, T. & Gray, J.E. (1827). A synopsis of the species of saurian reptiles, collected by Major – General Hardwicke. *Zoological Journal* 3, 213 – 229.

Hillis, D.M. & Bull, J.J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42, 182 – 192. DOI: 10.1093/sysbio/42.2.182

Jackman, T.R., Bauer, A.M., Greenbaum, E., Glaw, F. & Vences, M. (2008). Molecular phylogenetic relationships among species of the Malagasy – Comoran gecko genus *Paroedura* (Squamata: Gekkonidae). *Molecular Phylogenetics and Evolution* 46, 74 – 81.

Kuhl, H. (1820). *Beiträge zur Zoologie und vergleichenden Anatomie*. Hermannsche Buchhandlung, Frankfurt, 152 p.

Lauprasert, K. & Thirakhupt, K. (2001). Species Diversity, Distribution and Proposed Status of Monitor Lizards (Family Varanidae) in Southern Thailand. *The Natural History Journal of Chulalongkorn University* 1(1), 39 – 46.

Liu, S. & Rao, D. (2019). A new species of the genus *Acanthosaura* from Yunnan, China (Squamata, Agamidae). *ZooKeys* 888, 105 – 132. DOI: 10.3897/zookeys.959.54601.

Liu, S., HOU, M., Mo, M. & Rao, D. (2020). A new species of the genus *Acanthosaura* (Squamata, Agamidae) from Yunnan, China, with comments on its conservation status. *ZooKeys* 959, 113 – 135. DOI: 10.3897/zookeys.959.54601.

Manthey, U. (2008). Agamid Lizards of Southern Asia – Agamen des südlichen Asien – Draconinae 1. Terralog Vol. 7a. Edition Chimira, Frankfurt am main, 160 p.

Macey, J.R., Larson, A., Ananjeva, N.B., Fang, Z. & Papenfuss, T.J. (1997). Two novel gene orders and the role of ligh – strand replication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution*, 14, 91 – 104.

Macey, J.R., Schulte, J.A., Larson, A., Ananjeva, N.B., Wang, Y., Pethiyagoda, R., Rastegar – Pouyani, N. & Papenfuss, T.J. (2000). Evaluating trans – tethys migration: An example using acrodont lizard phylogenetics. *Systematic Biology* 49(2), 233 – 256.

Nguyen, L.T., Do, D.T., Hoang, H.V., Nguyen, T.T., McCormack, T.E.M., Nguyen, T.Q., Orlov, N.L., Nguyen, V.D.H. & Nguyen, S.N. (2018). A new species of the genus *Acanthosaura* Gray, 1831 (Reptilia: Agamidae) from Central Vietnam. *Russian Journal of Herpetology* 25(4), 259–274. DOI: 10.30906/1026 – 2296 – 2018 – 25 – 4 – 259 – 274.

Nguyen, S.N., Jin, J.Q., Dinh, B.V., Nguyen, L.T., Zhou, W.W., Che, J., Murphy, R.W. & Zhang, Y.P. (2019). A new species of *Acanthosaura* Gray 1831 (Reptilia: Agamidae) from Central Vietnam. *Zootaxa* 4612(4), 555 – 565. DOI: 10.11646/zootaxa.4612.4.7

Nylander, J.A. (2004). MrModeltest. Version 2. Program distributed by the author. 2. Evolutionary Biology Centre, Uppsala University, Uppsala.

Okajima, Y. & Kumazawa, Y. (2010). Mitochondrial genomes of acrodont lizards: timing of gene rearrangements and phylogenetic and biogeographic implications. *BMC Evolutionary Biology* 10, 141

Orlov, N.L., Nguyen, Q.T. & Nguye, V.S. (2006). A new Acanthosaura allied to *A. capra* Günther, 1861 (Agamidae, Sauria) from Central Vietnam and Southern Laos. *Russian Journal of Herpetology* 13(1), 61 – 76.

Pauwels, O.S.G., Laohawat, O. – A., Naaktae, W., Puangjit, C., Wisutharom, T., Chimsunchart, C. & David, P. (2002). Reptile and amphibian diversity in Phang – Nga Province, southern Thailand. *Natural History Journal of Chulalongkon University* 2(1), 25 – 30.

Pauwels, O.S.G., David, P., Chimsunchart, C. & Thirakhupt, K. (2003). Reptiles of Phetchaburi Province, Western Thailand: a list of species, with natural history notes, and a discussion on the biogeography at the Isthmus of Kra. *The Natural History Journal of Chulalongkorn University* 3(1), 233 – 53.

Pauwels, O.S.G. & Iskandar, D.T. (2010). Book review. Agamid Lizards of Southern Asia – Agamen des südlichen Asien – Draconinae 2, Leiolepidinae, by Ulrich Manthey. *Herpetological Review* 41(4), 523 – 525.

Pauwels, O.S.G., Sumontha, M., Kunya, K., Nitikul, A., Samphanthamit, P., Wood, P.L. Jr. & Grismer, L.L. (2015). *Acanthosaura phuketensis* (Squamata: Agamidae), a new long – horned tree agamid from southwestern Thailand. *Zootaxa* 4020(3), 473 – 494.

Ronquist, F. & Huelsenbeck, J.P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed model. *Bioinformatics* 19, 1572 – 1574.

Schlegel, H. (1839). Abbildungen neuer oder unvollstandig bekannter Amphibien, nach der Natur oder dem Laben entworfen. Dusseldorf.

Stuart, B.L., Sok, K. & Neang, T. (2006). A collection of amphibians and reptiles from hilly eastern Cambodia. *The Raffles Bulletin of Zoology* 54, 129 – 155.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30, 2725 – 2729. DOI: 10.1093/molbev/mst197.

Taylor, E.H. (1963). *The Lizard of Thailand*. Kansus University Science Bulletin 46(5), 687 – 1077.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting. Position – specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22, 4673 – 80. DOI: 10.1093/nar/22.22.4673.

Trivalairat, P., Kunya, K., Chanhome, L., Sumontha, M., Vasaruchapong, T., Chomngam, N. & Chiangkul, K. (2020). *Acanthosaura aurantiacrista* (Squamata: Agamidae), a new long horn lizard from northern Thailand. *Biodiversity Data Journal* 8, e48587. DOI: 10.3897/BDJ.8.e48587.

Wood, P.L. Jr., Grismer, J.L., Grismer, L.L., Northayati, A., Chan, K. – O. & Bauer, A.M. (2009). Two new montane species of *Acanthosaura* Gray, 1831 (Squamata: Agamidae) from Peninsular Malaysia. *Zootaxa* 2012, 28 – 46.

Wood, P.L. Jr., Grismer, L.L., Grismer, J.L., Neang, T., Chav, T. & Holden, J. (2010). A new cryptic species of *Acanthosaura* Gray, 1831 (Squamata: Agamidae) from Thailand and Cambodia. *Zootaxa* 2488, 22 – 38.

Yu, X.L., Du, Y., Yao, Y.T., Lin, C.X. & Lin, L.H. (2015). The complete

mitochondrial genome of *Acanthosaura lepidogaster* (Squamata: Agamidae). *Mitochondrial DNA* 28(2), 182 – 184.

Zug, G.R., Brown, H.K., Schult, J.A. II & Vindum, J.V. (2006). Systematics of the garden lizards, Calotes versicolor group (Reptilia, Squamata, Agamidae), in Myanmar: central dry zone populations. *Proceedings of the California Academy of Sciences* 57(2), 35 – 68.

Appendix 1. List of comparative material examined.

Acanthosaura armata: THNHM15209, Hala – Bala, Narathiwat Province; THNHM18884, Sungai Kolok District, Narathiwat Province

Acanthosaura aurantiacrista: THNHM28064, Mae Sariang District, Mae Hong Son Province; THNHM28521, 28522, 28523, 28524, QSMI1446, 1447, Omkoi District, Chiang Mai Province.

Acanthosaura cardamomensis: THNHM15597, 20169, Koh Kut, Trat Province, THNHM24711, 24712, 24715, Khao Yai National Park, Nakhon Ratchasrima Province.

Acanthosaura crucigera: QSMI1590, 1591, 1592, 1593, THNHM28507, 28508, Taksin Maharat National Park, Muang District, Tak Province; THNHM22658 Thong Pha Phum District, Kanchanaburi Province; THNHM18594, Huai Kha Khaeng, Lan Sak District, Uthai Thani Province.

Acanthosaura lepidogaster: THNHM08736, 08777, Phu Luang District, Loei Province; THNHM19619, Phu Kieo District, Chaiyaphum Province; THNHM20537, Ban Sun Phae Kae, Chiang Dao District, Chiang Mai Province; THNHM20647, Roi Praputabath, Umphang District, Tak Province; THNHM10080, Huai Na Tee, Pua District, Nan Province; THNHM16569, 16570, 16571, Doi Khun Tan National Park, Lam Phun Province.

Acanthosaura meridiona **sp. nov.**: QSMI 1594, 1595 & 1596 and THNHM 28059, 28060, 28061 & 28062, Wang Nam Rab Resort, Na Yong District, Trang Province; THNHM 12687 & 12688, Kanom Waterfall, Lan Saka, Nakhon Si Thammarat Province; THNHM 13449, Krabi Province; ; THNHM 19793, Tak Ta Khum, Ban Ta Khum, Surat Thani Province; THNHM 23843 & 23844, Khao Bantad Wildlife Sanctuary, Trang & Phattalung Province

Acanthosaura nataliae: THNHM13454, 13455, Xe Sap National Biodiversity Conservation Area, Samoy District, Saravane Province, Laos.

Acanthosaura phuketensis: THNHM08865, Ton Sai Waterfall, Thalang District, Phuket Province; THNHM22663, Khao Sok, Ban Ta Khun, Surat Thani Province.

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Websites:

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