Phylogeny, Diversity, and Distribution of *Micryletta* (Anura: Microhylidae) in Myanmar

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The microhylid genus *Micryletta*, often called Paddy Frogs, is a taxonomically modest group of frogs currently comprised of eight species distributed across India, China, and Southeast Asia. None of the recent investigations into this group have explicitly focused on the diversity of these frogs in Myanmar, a critically undersampled region previously thought to contain only one species of *Micryletta*, *M. inornata*. Recent field expeditions to Myanmar conducted by the authors have resulted in the discovery of numerous populations of *Micryletta* in the northern and southern portions of the country in Kachin State and Tanintharyi Region, respectively. In this study, we investigate the status of these populations, their taxonomic identities, and broadly assess the diversity of this enigmatic group of frogs in Myanmar. Using comparative morphological and molecular data, we find the diversity of *Micryletta* in Myanmar has been poorly appreciated, which we demonstrate with the discovery of *M. aishani*, a species previously only known from neighboring India, and *M. lineata*, a species originally described from Peninsular Thailand. Our investigation into the taxonomic status of *M. lineata* demonstrates the validity in the recognition of this species, which is supported with evidence derived from morphology, geography, and molecular data. We additionally provide a detailed re-description of this species based on novel specimens from southern Myanmar. We also document *M. inornata sensu stricto* in Myanmar, sympatric with *M. lineata*, bringing the total to three species of *Micryletta* occurring in Myanmar.

*Micryletta*, members of the remarkably speciose family Microhylidae (spanning five continents and totaling 694 species [Frost, 2020]), are a poorly known group of terrestrial frogs distributed throughout northeastern India, southern China and Taiwan, and much of Southeast Asia into Sundaland. *Micryletta* has had a turbulent taxonomic history. Originally, *M. inornata*, *M. erythrhopoda*, and *M. steinegeri* were described as members of the closely related genus *Microhyla*, but they were subsequently allocated to the more taxonomically modest genus *Micryletta* on the basis of morphological characters such as tympanum visibility, head shape, and non-expanded digit tips (Dubois, 1987; Bain and Nguyen, 2004).

The recent discoveries of *M. nigromaculata* (Poyarkov et al., 2018), *M. alishani* (Das et al., 2019), *M. sumatrana* (Munir et al., 2020), and *M. dissimilans* (Suwannapoom et al., 2020) mark the first taxonomic additions to the diversity of *Micryletta* in over 20 years. Until recently, the absence of topotypic material (Deli, Sumatra, Indonesia) for the widespread taxon and type species of the genus, *M. inornata*, made taxonomic revision and research within the group difficult since the phylogenetic position of “true” *M. inornata* relative to other populations was uncertain. Given limited taxonomic investigation, populations across mainland Southeast Asia have remained classified as *M. inornata*, despite underestimated diversity (Poyarkov et al., 2018; Alhadi et al., 2019; Das et al., 2019; Munir et al., 2020). Recently, Alhadi et al. (2019) used mitochondrial sequence data (16S rRNA) to determine the phylogenetic position of topotypic Sumatran populations of *M. inornata* relative to other Asian *Micryletta*, which were recovered as sister to a larger clade composed of *M. erythrhopoda*, *M. steinegeri*, and multiple other clades containing candidate species previously considered to belong to *M. inornata*. Together, these were all sister to the Vietnamese *M. nigromaculata* (Alhadi et al., 2019). In determining the phylogenetic position of *M. inornata sensu stricto*, it has become obvious that many of the uninvestigated populations of *Micryletta* across Southeast Asia are not conspecific with *M. inornata sensu stricto*.

Despite this important insight into the taxonomy of *M. inornata*, our cumulative understanding of the diversity and distribution of the genus *Micryletta* remains poor, but nowhere more so than in Myanmar. In Myanmar, only one species of *Micryletta* is thus far known, *M. inornata sensu lato* (Mulcahy et al., 2018). This species has been found in the country’s most southern subdivision, the Tanintharyi Region, which abuts neighboring Thailand to the east and the Andaman Sea to the west (Fig. 1). The southernmost edge of the Taninthary Region sits just north of a well-known biogeographic filter, the Isthmus of Kra, which denotes the contemporary over-land transition from mainland Southeast Asia to the Sunda Shelf (Sundaland). Recent surveys in the Taninthary Region (e.g., Mulcahy et al., 2018) yielded numerous country records, new species (Connette et al., 2017; Mulcahy et al., 2017; Zug et al., 2017; Lee et al., 2019), and many regional faunal records, denoting this area as...
containing a hyperdiverse herpetofaunal community. As noted by Mulcahy et al. (2018), *M. inornata sensu lato* had been discovered during the California Academy of Sciences (CAS) and the Smithsonian Institution’s National Museum of Natural History (NMNH) Myanmar Herpetology Survey (MHS) in Dawei and Kawthaung, which are in the northern and southern portions of the Tanintharyi Region, respectively. Approximately 190 km southeast of Kawthaung, in Peninsular Thailand (Nakhon Si Thammarat Province), Taylor (1962) described a new subspecies, *M. inornata lineata*, arguing its distinctiveness from *M. inornata inornata* owing to sets of longitudinally organized markings across the dorsum forming broken and/or continuous lines. Notably, a recent guide to the amphibians and reptiles of the Tanintharyi Region of Myanmar (Zug and Mulcahy, 2020) has recognized this taxon at the species level, but this guide provided little data to substantiate this conclusion. Hence, there has been little direct research on this taxon since its original description, although some researchers have designated populations of *Micryletta* east of Dawei in adjacent Thailand at the subspecies level as *M. i. lineata* (Sumontha et al., 2017).

During recent surveys conducted by us in the northern province of Kachin State (GOUW) and the southern province of Tanintharyi Region (DGM), we discovered novel populations of microhylid frogs matching the diagnosis of *Micryletta* (Dubois, 1987). We leverage these specimens to investigate diversity of this genus in Myanmar, which we supplement with additional natural history museum specimens and previously published sequence data from populations across mainland Southeast Asia and Sundaland. Using morphological and molecular data, we examine the status of *M. inornata* in Myanmar, and investigate intra- and interspecific variation within this group throughout Southeast Asia.

**MATERIALS AND METHODS**

We collected specimens in the field by hand during biodiversity surveys led by CAS, Fauna and Flora International (FFI), and Smithsonian scientists in Tanintharyi Region and Kachin State, Myanmar. We euthanized specimens following Smithsonian Institutional Animal Care and Use Committee (IACUC) protocol (2014-02). We extracted liver samples from euthanized animals and stored them in either 95% ethanol or DMSO/EDTA buffer (as modified by Mulcahy et al., 2016). We preserved whole voucher specimens in 10% formalin, and subsequently transferred them to 70% ethanol. We collected and exported specimens and tissue samples to CAS or the NMNH with permission and a Memorandum of Understanding (MOU) arranged between FFI, CAS, the Forest Department of Myanmar, and the Smithsonian. We deposited tissue samples in either the CAS Herpetology Collection or the Smithsonian Biorepository.

We performed extractions of genomic DNA from specimens on an AutoGen prep 965 (2011 AutoGen, Inc.) using standard phenol manufacturer protocols or using Qiagen DNeasy extraction kits (Qiagen Inc.). We eluted genomic DNA in 100 μl of re-suspension buffer. We conducted polymerase chain reactions (PCRs) for the mitochondrial (mtDNA) loci cytochrome-oxidase I (COI; COI-ReptBCF and COI-ReptBCR; Castaño and de Queiroz [2011]) and 16S rRNA (16S; 16Sar and 16Sbr; Palumbi et al. [1991]). We performed PCRs in 10 μl reactions, following protocols ‘3.6 PCR Methods: Amplification’ and ‘3.8 PCR Purifications: EXOSAP-IT’ in Weigt et al. (2012) with annealing tempera-
tures of 48°C for COI and 54°C for 16S. We performed sequence reactions with both forward and reverse PCR primers using BigDye® Terminator v3.1 Cycle Sequencing Kits in 0.25 × 10 μl reactions and then ran them on an Automated ABI3730 Sequencer (2011 Life Technologies). We then edited raw trace files in Geneious v10.2.4 (Biomatters Ltd.), aligned and edited complementary strands, and then inspected for translation. We manually edited and aligned all novel sequences with GenBank and Barcode of Life Database (BOLD) sequences in Geneious using the MUSCLE v7.308 (Edgar, 2004) and MAFFT v7.450 (Katoh and Standley, 2013) plug-ins with default settings and subsequently inspected COI for appropriate translation. We deposited novel mtDNA sequences in GenBank (Supplementary Table 1; see Data Accessibility; GenBank accession numbers for new sequences in GenBank (Supplementary Table 1; see Data Accessibility) were TIM2e_Mt609035–Mt609036; Mt609037–Mt609045, MW042900–MW042904: COI: Mt608365–Mt608375, Mt608377–Mt608384; Micryletta inornata sensu stricto [16S: Mt609033–Mt609034; COI: Mt608363–Mt608364]; Micryletta aishani [16S: MW035599–MW035603]).

We used IQ-TREE v2.0 (Minh et al., 2020) to generate maximum likelihood (ML) gene trees for the COI and 16S datasets, deploying the Ultrafast Bootstrap (1,000 replicates; Minh et al., 2013) and ModelFinder options (Kalyaanamoorthy et al., 2017). We did not use the invariant sites (+I) parameter if a gamma (+G) parameter was already present within a selected model (because the I parameter overfits a G rate category). The best-fit nucleotide substitution models as determined by ModelFinder for the 16S and COI datasets were TIM2e+G4 and TPM2+F+G4, respectively. We considered Ultrafast Bootstrap values equal to or greater than 95 to be robust. We used multiple preexisting COI and 16S sequences of closely related Southeast Asian microhylids (e.g., Glyphoglossus, Kalophrynus, Microhyla, Mysticellus) as outgroups based on previously generated phylogenetic estimations of Microhylidae (Garg and Biju, 2019; Streicher et al., 2020). We did not concatenate the two loci since only the USNM specimens had data for both loci, thus the resulting matrix would have had an amount of missing data inappropriate for phylogenetic inference. We calculated Tamura-Nei genetic distances in MEGA7 (Tamura and Nei, 1993; Kumar et al., 2016).

We quantified morphometric diversity in populations of Micryletta across mainland Southeast Asia using vouchered museum specimens (Supplementary Table 2; see Data Accessibility). Museum abbreviations concerning whole voucher specimens follow Sabaj (2020). Literature sources that were also used in this study include Taylor (1962), Tarkhnishvili (1994), Poyarkov et al. (2018), Alhadi et al. (2019), and Das et al. (2019).

For morphological analysis, all body measurements were measured using dial calipers to the nearest 0.1 mm underneath a standard dissecting microscope. All bilateral measurements were recorded from the right side and collected by only one of us (GRZ) to establish consistency among measurements. Morphological character definitions and abbreviations follow as: head length (HeadL; straight-line, horizontal distance from tip of snout to posterior corner of jaws); head width—posterior (HeadWP; straight-line, transverse distance from left to right edges of corner of jaws); snout–eye length (SnEye; distance from snout-tip to anterior edge of orbit); nares–eye distance (NarEye; distance between naris and anterior edge of orbit; eye-diameter (EyeD); mid-horizontal distance from anterior to posterior edge of exposed eyeball); interorbital distance (IntOrb; straight-line, transverse distance from left to right inner edges of orbit at middle of eyes); internarial distance (IntNar; distance between left and right nares); snout–vent length (SVL; distance from tip of snout to vent); trunk length (TrunkL; straight-line, horizontal distance from axilla rear of forelimb insert to inguinal front of hindlimb insert); forearm length (ForamL; straight-line distance from elbow to wrist); hand length (HandL; distance from base of the palm [proximal edge of medial outer palmar tubercle] to tip of third finger); third-finger length (3rdFingL; straight-line distance from junction of second and third fingers); thigh length (ThighL; straight-line distance from vent to knee); crus length (CrusL; straight-line distance from knee to ankle); tarsus length (TarsL; straight-line distance from ankle joint to heel); foot length (FootL; straight-line distance from heel to tip of 4th toe); hindlimb length (HindL; sum of ThighL, CrusL, TarsL, and FootL); and fourth-toe length (4thToel; straight-line distance from junction of third and fourth toes to tip of digit). Subarticular tubercle formulae follow Savage (1975).

We determined sex and maturity of individuals via dissection and gonadal inspection. We included only mature individuals in morphological data analysis (n = 117), with males (n = 71) and females (n = 46) analyzed separately owing to observed sexual dimorphism in body size (Mann-Whitney U-test, P < 0.001). Specimens were assigned a priori to statistical analysis to operational taxonomic units (OTUs; either M. aishani, M. inornata sensu stricto, M. cf. inornata, or M. lineata) using evidence derived primarily from morphology and molecular data when available. A large number of specimens in our morphological dataset (Supplementary Table 2; see Data Accessibility) were not able to be binned into currently recognized species, and thus were consequently left as M. cf. inornata (n = 29 females; n = 43 males). We log transformed all continuous morphological data (18 variables) and then subsequently size-corrected by regressing all characters against SVL to normalize the data and remove allometric skew using custom R scripts. We conducted principal component analyses (PCA) on the remaining residual data for both sexes separately using the pcomp function in R (R Core Team, 2013).

RESULTS

The 16S and COI alignments (Supplementary Data; see Data Accessibility) were 472 bp (7 outgroup, 71 ingroup) and 657 bp in length (7 outgroup, 41 ingroup), respectively. The 16S dataset included all eight currently recognized species of Micryletta (M. aishani, M. dissimulans, M. erythropoda, M. inornata, M. lineata, M. nigromaculata, M. steinegeri, and M. sumatrana), while the COI dataset only included four of the eight (M. erythropoda, M. inornata, M. lineata, and M. steinegeri). Because the two mtDNA loci sampled were discordant in both species and individual samples included (owing to a lack of multi-locus data available for Micryletta), we were unable to directly draw comparisons between the two trees.

As with prior investigations of the phylogeny of Micryletta (see Poyarkov et al., 2018; Alhadi et al., 2019; Das et al., 2019), the deeper nodes within both phylogenies suggest a poorly supported reconstruction of the phylogeny of Micry-
The 16S phylogenetic reconstruction (Fig. 2A) recovered the monophyly of Micryletta as near well supported (UFBoot = 91), whereas this was poorly supported in the COI reconstruction (Fig. 2B; UFBoot = 54). In both the 16S and COI phylogenies, sympatric Tanintharyi populations of M. inornata and M. lineata were placed into two, distantly related clades: 1) two specimens (USNM 587625, 587901) were placed in a clade with topotypic M. inornata sensu stricto, in the COI reconstruction sister to all other Paddy Frogs (UFBoot = 91), and in the 16S reconstruction sister to M. aishani, M. cf. inornata, M. erythropoda, and M. lineata (UFBoot = 72); and 2) all remaining Tanintharyi specimens were placed in a separate clade nested deeper in the tree sister to M. erythropoda with moderate to high support (UFBoot = 80; UFBoot = 98).

In the 16S reconstruction, the Kachin State specimens formed a well-supported monophyletic group with the nearby Indian specimen of M. aishani (UFBoot = 99). This clade was recovered with poor support (UFBoot = 66) as sister to two larger clades, one composed of M. steinegeri and M. cf. inornata, and one composed of M. lineata and M. erythropoda. In both the COI and 16S phylogenies, M. erythropoda and M. lineata were well supported as each other's closest relatives (UFBoot = 98, UFBoot = 100, respectively), although M. lineata was paraphyletic with respect to M. erythropoda in the 16S phylogeny.

In the 16S reconstruction, the Vietnamese species M. nigromaculata (Poyarkov et al., 2018) was recovered as sister (UFBoot = 74) to all other Micryletta, with the exception of the two the newly described species from Sumatra and Thailand, M. sumatrana (Munir et al., 2020) and M. dissimulans (Suwannapoom et al., 2020), which were reconstructed as sister taxa (UFBoot = 87). The COI reconstruction placed M. steinegeri, a poorly characterized species described from Taiwan, as sister to populations of M. cf. inornata from mainland central Southeast Asia (UFBoot = 88), a result concordant with the 16S reconstruction, although this tree
exhibited more complicated interspersion of *M. steinegeri* among a clade composed of *M. steinegeri* and *M. cf. inornata*.

At the COI locus, *M. inornata sensu stricto* exhibited a minimum Tamura-Nei sequence divergence of 16.5% (*M. lineata*), and maximally 18.3% (*M. steinegeri*) from all sampled OTUs of *Micryletta* (Table 1; Supplementary Table 3; see Data Accessibility), whereas genetic distances among taxa respective to *M. inornata sensu stricto* for 16S was comparatively lower but still substantial, ranging from 5.9% (*M. aishani*) to 8.8% (*M. erythropoda*; Table 2; Supplementary Table 4; see Data Accessibility). At the 16S locus, Tanintharyi specimens of *M. inornata sensu stricto* were 2.0% divergent from Sumatran *M. inornata sensu stricto* (note that no Sumatran COI sequences exist to our knowledge; thus, we are unable to conduct this comparison for the protein-coding locus).

*Micryletta lineata* exhibited both insubstantial and substantial divergences from congeners, most minimally divergent from the Vietnamese *M. erythropoda* (2.8% for 16S; 4.8% for COI), and most maximally divergent from *M. inornata sensu stricto* (7.5% for 16S) and *M. steinegeri* (17.1% for COI).

The PCAs yielded little intergroup separation, with general intermixing of individuals of different OTUs. In the bivariate ordination of females from Tanintharyi Region, Myanmar.

**Table 1.** Pairwise mean Tamura-Nei genetic distances (Tamura and Nei, 1993) for COI among operational taxonomic units (OTUs) of *Micryletta* sampled. Bolded values represent mean intra-group genetic distances.

| OTU                        | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  |
|----------------------------|----|----|----|----|----|----|----|----|----|
| 1. *M. inornata sensu stricto* | 0  |    |    |    |    |    |    |    |    |
| 2. *M. lineata*             | 16.5 | 0.3 |    |    |    |    |    |    |    |
| 3. *M. erythropoda*         | 17.6 | 4.8 | 0.6 |    |    |    |    |    |    |
| 4. *M. steinegeri*          | 18.3 | 17.1 | 16.9 | 0.7 |    |    |    |    |    |
| 5. *M. cf. inornata*        | 17.6 | 14.4 | 14.5 | 9.3 | 4  |    |    |    |    |

**Diagnosis.**—*Micryletta lineata* is a diminutive member of the genus *Micryletta* with adult size SVL 22.1–23.5 mm in males (*n* = 17), 21.6–28.3 mm in females (*n* = 13); snout slightly truncate in dorsal view, obverse in lateral view, head length 28–34% SVL; head width 95–114% head length; hindlimb length 145–203% SVL. Skin, dorsal trunk, and hindlimbs moderately shagreen; dorsally snout, canthus, and forelimb smooth; hand with numerous metacarpal tubercles, foot with modest oblong inner metatarsal tubercles and no outer metatarsal tubercles; no webbing between fingers and toes; digit tips rounded, not expanded; dorsal coloration variable, usually light brown, grayish brown or rufous brown ground color and dorsally three or four rows of scattered dark-brown marks (usually elongate) from middle of head to sacrum, often forming three or four longitudinal rows; laterally dark-brown stripe from snout through eye to midtrunk or inguinal region; dorsally fore- and hindlimbs lightly patterned with small irregular dark marks except unicolor upper arm; venter beige with light-brown mottingle along throat. This diagnosis and following description are based on 17 males and 13 females from Tanintharyi Region, Myanmar.

**Description.**—Body measurements summarized (in mm) in Tables 5 and 6. Proportions (%) for the sampled males (MM) and females (FF) are: HeadL/SVL 30–34 (32±1) MM, 28–34 (31±2) FF; HeadWP/HeadL 96–110 (101±4) MM, 95–114 (102±5) FF; SnEye/HeadL 34–45 (39±3) MM, 29–44 (40±4) FF; TrunkL/SVL 36–49 (41±3) MM, 37–52 (44±5) FF; ForamL/SVL 24–29 (26±2) MM, 23–28 (26±1) FF; ForamL/ThighL 51–64 (58±4) MM, 52–65 (58±4) FF; 3rdFingL/HandL 62–86 (72±6) MM, 62–74 (69±4) FF; HindL/SVL 150–203 (172±14) MM, 145–180 (165±8) FF; CrusL/SVL 42–52 (47±2) MM, 43–50 (46±2) FF; CrusL/ThighL 96–111 MM (105±4), 97–110 (102±4) FF; ThighL/SVL 40–50 (44±3) MM, 41–48 (45±2) FF; FootL/HindL 23–32 (29±2) MM, 29–33 (30±1) FF; FootL/SVL 45–58 (50±3) MM, 46–52 (49±2) FF; 4thToeL/FootL 58–68 (62±3) MM, 54–68 (62±4) FF; EyeD/ SnEye 83–124 (95±11) MM, 81–138 (95±14) FF; IntNar/ SnEye 55–80 (70±6) MM, 63–81 (69±5) FF; NarEye/SnEye 48–80 (59±7) MM, 45–67 (56±5) FF.

Body oblong with truncate snout; limbs slender with somewhat elongate digits; forefoot digit lengths 3 > 2 > 4 > 1, hindfoot 4 > 3 > 5 > 2 > 1. Head as broad, or broader than long; canthus rostralis rounded; naris closer to snout than eye and slightly protuberant; tympanum visible, horizontal diameter about two-thirds that of eye. Dorsal skin surface as

**Table 2.** Pairwise mean Tamura-Nei genetic distances (Tamura and Nei, 1993) for 16S among operational taxonomic units (OTUs) of *Micryletta* sampled. Bolded values represent mean intra-OTU sequence divergence.

| OTU                        | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  |
|----------------------------|----|----|----|----|----|----|----|----|----|
| 1. *M. cf. inornata*       | 2.3 |    |    |    |    |    |    |    |    |
| 2. *M. steinegeri*         | 2.5 | NA |    |    |    |    |    |    |    |
| 3. *M. aishani*            | 4.3 | 4.4 | 0.1 |    |    |    |    |    |    |
| 4. *M. lineata*            | 5.2 | 5.4 | 4  | 0.3 |    |    |    |    |    |
| 5. *M. erythropoda*        | 6.6 | 6.6 | 5.6 | 2.8 | 0  |    |    |    |    |
| 6. *M. inornata sensu stricto* | 7.6 | 6.4 | 5.9 | 7.5 | 8.8 | 1.3 |    |    |    |
| 7. *M. nigromaculata*      | 7.5 | 6.5 | 5.8 | 6.8 | 9.5 | 7.7 | 0.8 |    |    |
| 8. *M. sumatrana*          | 6  | 4.9 | 4.9 | 6.6 | 8.7 | 9.7 | 7.5 | 4.7 | NA |
| 9. *M. dissimulans*        | 6.8 | 5.9 | 5.4 | 7.4 | 9  | 7  | 6.1 | 3.9 | 0  |
described above, ventrally smoother with large flat abutting tubercles most evident on chest and abdomen. Dorsal ground color as above; dark-brown markings variable in density from few to many and most somewhat elongate rather than round; bilateral dorsolateral series/rows usually present, middorsal ones from moderate to near absent. Ventrally light cream background overlain by diffuse dark speckling on chin and throat or more frequently black sublabial border; trunk either unicolor or diffuse dark variegation.

**Distribution.**—Presently, *Micryletta lineata* is restricted to the Isthmus of Kra region in southern Myanmar (Tanintharyi Region) and adjacent western Thailand, reaching its apparent northern latitudinal limit at nearly 15°N. *Micryletta lineata* likely has a larger distribution and possibly extends north into the Salween Basin of Myanmar and south into northern Peninsular Malaysia, although these areas have yet to be thoroughly investigated. Specimens of *Micryletta* from this area must be examined closely (optimally integrating both morphology, genetics, and bioacoustics) owing to demonstration of sympatry of *M. inornata sensu stricto* and *M. lineata*.

**Natural history.**—*Micryletta lineata* is a diurnal terrestrial frog usually found among leaf litter and often near forest streams. Tadpole morphology, diet, advertisement calls, and various other crucial facets of the ecology and biology of *M. lineata* have yet to be characterized.

**Comparison to other Myanmar Micryletta.**—Our sample of Tanintharyi *M. inornata* is small (single adult female [Fig. 6] and single immature female). Morphometrically, all its

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**Fig. 3.** Box plots of snout–vent lengths (SVL) for *Micryletta* sampled in the morphological dataset—mature males (A) and females (B). (C, D, respectively males and females) Principal components analysis of males and females for 17 continuous morphological characters examined. Colors correspond in both plots, with *M. aishani* represented by green circles, *M. cf. inornata* represented by orange triangles, *M. inornata sensu stricto* as the blue triangle in the females plot, and *M. lineata* represented by the purple squares.
Table 3. Loadings of top five principal components among male Micryletta examined using log transformed and subsequently SVL-corrected residual data.

|          | PC1     | PC2     | PC3     | PC4     | PC5     |
|----------|---------|---------|---------|---------|---------|
| Standard deviation | 2.136   | 1.728   | 1.296   | 1.195   | 1.09    |
| Proportion of variance | 0.268   | 0.176   | 0.099   | 0.084   | 0.07    |
| Cumulative proportion | 0.268   | 0.444   | 0.543   | 0.627   | 0.697   |
| Eigenvalue | 4.563   | 2.984   | 1.68    | 1.428   | 1.188   |
| HeadL | 0.108   | 0.351   | 0.367   | 0.278   | 0.0134  |
| HeadWP | 0.116   | 0.413   | 0.236   | 0.023   | 0.205   |
| NarEye | 0.043   | 0.083   | 0.141   | 0.298   | 0.72    |
| NarEye | 0.001   | 0.425   | 0.093   | 0.251   | 0.007   |
| IntNar | 0.129   | 0.445   | 0.049   | 0.061   | 0.138   |
| IntOrb | 0.155   | 0.358   | 0.2     | 0.141   | 0.167   |
| HandL | 0.043   | 0.101   | 0.453   | 0.485   | 0.04    |
| ForarmL | 0.235   | 0.13    | 0.345   | 0.112   | 0.153   |
| HandL | 0.243   | 0.373   | 0.406   | 0.313   | 0.012   |
| 3rdFingL | 0.255   | 0.083   | 0.378   | 0.388   | 0.01    |
| 4thToeL | 0.391   | 0.133   | 0.137   | 0.117   | 0.138   |
| TarsL | 0.354   | 0.011   | 0.159   | 0.101   | 0.128   |
| FootL | 0.358   | 0.22    | 0.026   | 0.059   | 0.149   |
| HindL | 0.361   | 0.184   | 0.218   | 0.02    | 0.042   |
| 3rdFingL | 0.343   | 0.18    | 0.084   | 0.06    | 0.094   |

Table 4. Loadings of top five principal components among female Micryletta examined using log transformed and subsequently SVL-corrected residual data.

|          | PC1     | PC2     | PC3     | PC4     | PC5     |
|----------|---------|---------|---------|---------|---------|
| Standard deviation | 3.310   | 2.153   | 1.345   | 1.185   | 1.032   |
| Proportion of variance | 0.314   | 0.136   | 0.106   | 0.083   | 0.063   |
| Cumulative proportion | 0.314   | 0.450   | 0.557   | 0.639   | 0.702   |
| Eigenvalue | 5.335   | 2.319   | 1.809   | 1.404   | 1.064   |
| HeadL | 0.108   | 0.351   | 0.367   | 0.278   | 0.0134  |
| HeadWP | 0.024   | 0.335   | 0.174   | 0.178   | 0.102   |
| SnEye | 0.008   | 0.533   | 0.150   | 0.052   | 0.142   |
| Nareye | 0.111   | 0.361   | 0.268   | 0.124   | 0.237   |
| IntOrb | 0.008   | 0.008   | 0.324   | 0.296   | 0.127   |
| IntNar | 0.008   | 0.493   | 0.157   | 0.062   | 0.219   |
| TrunkL | 0.171   | 0.057   | 0.408   | 0.267   | 0.315   |
| ForarmL | 0.263   | 0.034   | 0.266   | 0.241   | 0.363   |
| HandL | 0.212   | 0.053   | 0.105   | 0.512   | 0.430   |
| 3rdFingL | 0.255   | 0.035   | 0.049   | 0.536   | 0.069   |
| 4thToeL | 0.328   | 0.117   | 0.203   | 0.054   | 0.241   |
| CrussL | 0.336   | 0.177   | 0.140   | 0.203   | 0.059   |
| TarsL | 0.312   | 0.107   | 0.030   | 0.332   | 0.376   |
| FootL | 0.368   | 0.165   | 0.076   | 0.094   | 0.101   |
| HindL | 0.381   | 0.137   | 0.167   | 0.188   | 0.111   |
| 3rdFingL | 0.225   | 0.005   | 0.152   | 0.053   | 0.018   |

Measurements lie within the range of the sample of *M. lineata* (Table 5). Its distinguishing attributes are a combination of coloration features: dorsal nape with a single median narrow dark nape bar vs. few scattered small dark spots or smudges; upper lip uniformly white vs. mottled or narrow white stripe with dark ventral border; side of trunk with broad dark, irregular-edged stripe vs. narrow, smooth-edged stripe of series of spots and bars as continuation of postocular stripe; ventrally chin unicolor vs. dark bordered lower lip; and unicolor chest vs. diffuse reticulate chest pattern.

We have 14 specimens representing northern Myanmar *M. aishani* (Tables 5, 6). Morphometrically, *M. aishani* and *M. lineata* share most traits, although the former has a larger head in both length and width even though they share similar SVLs. It is important to note that both sexes of the Kachin *M. aishani* are significantly smaller than the topotypic *M. aishani* with no overlap of body lengths (Das et al., 2019: table 3). Furthermore, coloration differs between Kachin *M. aishani* and Tanintharyi *M. lineata*: dorsally snout usually with faint marks or reticulation vs. often unicolor; lateral trunk stripe continuous for third or more of length vs. series of spots and bars; dorsal surface of femur light and dark variegated vs. distinctly longitudinally striped, median light stripe bordered anteriorly and posterior by dark color; upper lip with longitudinal white stripe irregular vs. mottled or narrow white stripe with dark ventral border; dark postorbital strip crosses tympanum vs. dark postorbital strip not on tympanum.

**Conservation status**.—Specific threats imposing substantial risk to populations of *M. lineata* in southern Myanmar are largely unknown, but presumably include those endangering other anurans in Southeast Asia—primarily rapid rates of deforestation (Rowley et al., 2010). In the Taninthary Region, Connette et al. (2017) reported extensive deforestation of critical lowland wet evergreen forest which hosts a wide array of endemic or seriously threatened species (e.g., *Cyrtodactylus*, *Panthera*, *Elephas*). Indeed, between 2002 to 2014, the Taninthary Region lost 185,952 ha of forest (Bhagwat et al., 2017), largely owing to expanding oil palm plantations (which, in Myanmar, are specific to the Taninthary Region). Despite the increasing loss of forest throughout southern Myanmar, the Taninthary Region, along with Kachin State in the north, remain the country’s major strongholds for remaining intact forest tracts (Bhagwat et al., 2017).

Presently, the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2020) contains classifications for three Paddy Frog species: *M. inornata* (Least Concern), *M. steinegeri* (Vulnerable), and *M. erythropoda* (Data Deficient [DD]). In Myanmar, *M. lineata* occurs at an approximate extent of occurrence (EOO) of 21,054 km², whereas its distribution in adjacent Thailand is largely uncharacterized. Given the uncertainty surrounding the distribution of this species, and a lack of population density estimates in this region, we recommend classifying *M. lineata* as DD under the IUCN Red List, although substantial concerns exist regarding accelerating rates of deforestation of lowland evergreen wet forests in the Taninthary Region.

**Discussion**

The discovery of *M. lineata* in Myanmar is not unexpected, as *M. lineata* was originally described in adjacent Thailand. The discovery of *M. inornata sensu stricto* in southern Myanmar is perhaps more surprising given that it is otherwise known from approximately 700 km south in Sumatra. Molecular characterization of the Malay Peninsula populations will be informative in elucidating a more fine-scale distribution for *M. inornata sensu stricto*. The Kachin State record of *M. aishani*, originally described from the Indian states of...
Manipur, Assam, and Tripura, extends the known range of this species by nearly 250 km.

*Micryletta lineata* was originally described as a subspecies of *M. inornata* (Taylor, 1962), although our data now clearly show that this species is more closely related to the southern Vietnamese populations currently under the name *M. erythropoda* than *M. inornata* sensu stricto. Thus, the question must be raised: is the minimal divergence observed between *M. erythropoda* and *M. lineata* an artifact of geographic distance, resulting in a pattern of isolation-by-distance (IBD), or do these two taxa represent independently evolving lineages? If the former, following International Commission on Zoological Nomenclature (ICZN) rules, Vietnamese populations of *M. erythropoda* (Tarkhnishvili, 1994) are a junior synonym of *M. lineata* (Taylor, 1962). To test this, focused sampling along the Gulf of Thailand from the Tanintharyi Region to southern Vietnam are required to assess the distinctiveness of these taxa.

*Micryletta erythropoda* has been traditionally diagnosed from other congeners by its irregularly spotted pattern, presence of an outer metatarsal tubercle, and brick-reddish dorsum (Tarkhnishvili, 1994; Poyarkov et al., 2018; Das et al., 2019). Yet, based on our analysis of novel specimens of *M. lineata*, this species can also have an irregularly spotted pattern similar to that described in *M. erythropoda*, contrary to the color descriptions of Taylor (1962). Several of the specimens we examined across Thailand and Myanmar shared these characteristics, suggesting that members of *Micryletta* have color patterns with higher intraspecific variation than previously appreciated. Consequently, this should be taken into account in future species descriptions.

In this study, we detected strong sexual dimorphism, but we did not note any obvious sexual dichromatism in the

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**Fig. 4.** *Micryletta lineata* (USNM 587907) from Tanintharyi Region, Myanmar. Scale for each perspective given in parenthesis. (A) Ventral view (10 mm); (B) dorsal view (10 mm); (C) lateral view (1 mm); (D) volar view of right hand (1 mm); (E) plantar view of left foot (1 mm). Note that gold dots are heads of pins that were used to hold the specimen in place.
specimens and populations examined. Consequently, although we have not thoroughly examined the coloration of these populations in life, we urge caution in using coloration to differentiate species of *Micryletta* along the Gulf of Thailand and Malay Peninsula. For now, we take a conservative approach and refrain from placing *M. erythropoda* in synonymy with *M. lineata* owing to a lack of adequate sampling in southern Southeast Asia between Myanmar and Vietnam.

The status of populations of *Micryletta* in Cambodia, Thailand, and Peninsular Malaysia all remain unresolved. In Peninsular Malaysia, populations of *Micryletta* have been moderately documented (e.g., Wood et al., 2008; Onn et al., 2010), although poorly investigated. Surveys conducted by Zakaria et al. (2014) report high densities of *Micryletta* in Pahang, Peninsular Malaysia, where the 413 specimens of *M. inornata* detected represented the most commonly encountered anurans. *Micryletta* have been detected (sparsely) throughout Thailand across a moderately wide geographic span, but there has been no direct investigation into the phylogenetic affinities of these populations, although our

![Image](image-url)

**Fig. 5.** *Micryletta lineata* (USNM 587923) from the Tanintharyi Region, Myanmar. Dorsal views of in-life (A) and subsequent to preservation (B). Scale bar is 10 mm.

| Character  | *M. aishani* (*n* = 3) | *M. inornata sensu stricto* (USNM 587901) | *M. lineata* (*n* = 13) |
|------------|------------------------|------------------------------------------|-------------------------|
| HeadL      | 6.7–7.2 (6.9±0.3)      | 6.9                                      | 6.8–8.5 (7.7±0.4)       |
| HeadWP     | 6.8–7 (6.9±0.1)        | 7.1                                      | 6.8–8.8 (7.8±0.6)       |
| SnEye      | 2.8–3 (2.9±0.1)        | 2.9                                      | 2.1–3.4 (3.1±0.3)       |
| NarEye     | 1.6–1.8 (1.7±0.1)      | 1.5                                      | 1.4–1.9 (1.7±0.2)       |
| EyeD       | 2.4–2.5 (2.4±0.1)      | 2.6                                      | 2.6–3.1 (2.9±0.2)       |
| IntOrb     | 5.2–5.5 (5.4±0.2)      | 5.3                                      | 5.4–6.7 (6.2±0.4)       |
| IntNar     | 1.9–2.1 (2±0.1)        | 2.1                                      | 1.7–2.3 (2.1±0.2)       |
| SVL        | 22.2–23 (22.5±0.4)     | 22.1                                     | 21.6–28.3 (25±2.2)      |
| TrunkL     | 10–12.4 (11.1±1.2)     | 9.6                                      | 8.1–14.4 (11.1±2.1)     |
| ForarmL    | 5.6–5.7 (5.6±0.1)      | 5.2                                      | 5.7–7.5 (6.5±0.5)       |
| HandL      | 6–6.5 (6.3±0.3)        | 4.9                                      | 5.4–7.5 (6.8±0.6)       |
| 3rdFingL   | 4.1–4.3 (4.2±0.1)      | 3.4                                      | 4–5.3 (4.7±0.4)         |
| ThighL     | 10.8–11.1 (10.9±0.2)   | 9.3                                      | 9.8–12.8 (11.2±1)       |
| CrusL      | 10.9–11.2 (11.1±0.2)   | 9.4                                      | 9.8–12.4 (11.4±0.8)     |
| TarsL      | 6–6.7 (6.4±0.4)        | 5.5                                      | 5.7–7.5 (6.8±0.6)       |
| FootL      | 11.6–12.2 (11.8±0.3)   | 9.6                                      | 10.4–13.7 (12.4±1.1)    |
| HindL      | 39.7–40.9 (40.2±0.6)   | 33.8                                     | 35.4–45.9 (41.2±3.7)    |
| 4thToeL    | 7–7.6 (7.3±0.3)        | 6                                        | 6.5–8.5 (7.7±0.7)       |
analyses demonstrate that multiple species occur in this region.

The discovery of *M. inornata sensu stricto* north of the Isthmus of Kra adds this species to the small list of anuran fauna that cross this biogeographic barrier, which demarcates substantial faunal and floristic turnover between mainland Southeast Asia and Sundaland (Hughes et al., 2003). Indeed, other frogs (e.g., *Microhyla mukhlesuri*, *Microhyla berdmorei*, *Occidozyga lima*, *Duttaphrynus melanostictus*) have distributions spanning Sumatra and mainland Southeast Asia, although many of these taxa may in reality represent geographically isolated cryptic species complexes comprised of numerous, geographically isolated species (e.g., Wogan et al., 2016).

Repeated cycles of seawater transgressions and regressions throughout Sundaland during the Neogene likely aided in reducing or ceasing gene flow and consequently promoting widespread vicariance-driven diversification scenarios north and south of ancient seaways dividing the Thai-Malay Peninsula (Inger and Voris, 2001; Hughes et al., 2003; de Bruyn et al., 2005). Indeed, this transitional zoogeographical pattern of divergence is concordant for a variety of

### Table 6. Summary statistics for male *Micryletta* examined in the morphological dataset. Format follows minimum–maximum (mean ± standard deviation). All measurements given in mm.

| Character     | *M. aishani* (*n* = 11) | *M. lineata* (*n* = 17) |
|---------------|--------------------------|--------------------------|
| HeadL         | 5.7–6.4 (6.1 ± 0.2)      | 6.4–7.6 (7.1 ± 0.4)      |
| HeadWP        | 5.4–6.4 (6.0 ± 0.3)      | 6.2–7.7 (7.1 ± 0.4)      |
| SnEye         | 2.4–3 (2.7 ± 0.2)        | 2.4–3.2 (2.7 ± 0.2)      |
| NarEye        | 1.4–1.7 (1.5 ± 0.1)      | 1.3–2 (1.6 ± 0.2)        |
| EyeD          | 2–2.6 (2.3 ± 0.2)        | 2–3.1 (2.6 ± 0.3)        |
| IntOrb        | 4.7–5.6 (5.0 ± 0.2)      | 5–6.5 (5.6 ± 0.4)        |
| IntNar        | 1.6–2 (1.8 ± 0.1)        | 1.5–2.2 (1.9 ± 0.2)      |
| SVL           | 18.8–21.8 (20.3 ± 0.9)   | 19.1–23.6 (21.6 ± 1.4)   |
| TrunkL        | 7.4–9.6 (8.7 ± 0.7)      | 7.2–10.6 (8.8 ± 1.1)     |
| ForarmL       | 4.3–5.8 (5.2 ± 0.4)      | 5–6.1 (5.6 ± 0.3)        |
| HandL         | 4.7–6.5 (5.5 ± 0.4)      | 4.8–7.2 (5.7 ± 0.6)      |
| 3rdFingL      | 3.1–4.6 (3.8 ± 0.4)      | 3.6–5.1 (4.1 ± 0.4)      |
| ThighL        | 8.7–10.6 (9.6 ± 0.6)     | 8.2–11.2 (9.6 ± 0.8)     |
| CrusL         | 8.7–10.2 (9.5 ± 0.5)     | 9.1–11 (10.1 ± 0.6)      |
| TarsL         | 5.3–6.3 (5.7 ± 0.3)      | 5.2–6.6 (6.0 ± 0.4)      |
| FootL         | 9.6–11.6 (10.6 ± 0.8)    | 8.6–12.1 (10.8 ± 1)      |
| HindL         | 32.3–38.2 (35.4 ± 1.9)   | 33.5–40.7 (37.1 ± 2)     |
| 4thToeL       | 5.9–7.2 (6.4 ± 0.5)      | 5.3–7.9 (6.7 ± 0.7)      |

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**Fig. 6.** *Micryletta inornata sensu stricto* (USNM 587901) from Tanintharyi Region, Myanmar. Scale for each perspective given in parentheses. (A) Ventral view (10 mm); (B) dorsal view (10 mm); (C) lateral view (1 mm); (D) volar view of right hand (1 mm); (E) plantar view of left foot (1 mm). Note that gold dots are heads of pins that were used to hold the specimen in place.
faunal and floristic groups (Parnell, 2013), demonstrated recently in a study of the widespread anuran genus *Megophrys* (Liu et al., 2017). Reconciling the biogeographic history of *Micryletta* is difficult, as patterns in diversity and distributions across Southeast Asia into Sundaland remain poorly characterized. Unraveling the influence of the Isthmus of Kra on distributions of *Micryletta* requires improved population sampling in Peninsular Malaysia, Thailand, Cambodia, and Vietnam. Furthermore, the inclusion of novel genome-scale data, in combination with a growing foundation of mtDNA sequence data, will be important to more confidently reconstruct the evolutionary and biogeographic history of *Micryletta*.

In this study, we provide a clearer picture of the diversity of *Micryletta* in Myanmar using evidence derived from molecular and morphological data of specimens collected in the extreme southern and northern portions of the country. We note that our sampling and sequencing of multiple individuals per locality enabled us to detect the presence of two species in the Tanintharyi Region, where only two specimens of the 16 specimens collected in the Smithsonian surveys were identified to be *M. inornata sensu stricto*. In Myanmar, sampling efforts for anurans between Kachin State and the Tanintharyi Region have not yielded any additional populations of *Micryletta*. Because a number of sites in Bago, Ayeyarwady, Rakhine, Magway, and Mandalay have been examined repeatedly in different seasons over multiple years by team members from the MHS, *Micryletta* appear to be absent (although this cannot be said with certainty). If one thing is evident, it is that our understanding of the phylogeny, diversity, and distributions of *Micryletta* is still in its infancy.

**DATA ACCESSIBILITY**

All trees, alignments, pairwise distance matrices, and morphological data included in this study are available at https://www.ichthyologyandherpetology.org/mysurvey/Myanmar-Micryletta. R code is available on GitHub (https://github.com/AryehMiller/Myanmar-Micryletta). Unless otherwise indicated in the figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source in accordance with the Creative Commons Attribution CC BY License.

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