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TWO DISTINCT MORPHOTYPES OF *Colleeneremia* (ANURA: PELODRYADIDAE) FROM THE TANIMBAR ISLANDS, INDONESIA: RECOGNITION OF ONE AS A NEW SPECIES

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Nabilah F. Khairunnisa[1]*, Awal Riyanto[2], A. A. Thasun Amarasinghe[2], Syahfitri Anita[2], Achmad Farajallah[1] & Amir Hamidy[2]*

[1] *Animal Bioscience, Department of Biology, Faculty of Mathematics & Natural Sciences, IPB University, Bogor, West Java 16680, Indonesia*
[2] *Laboratory of Herpetology, Museum Zoologicum Bogoriense, Research Center for Biosystematics & Evolution, National Research & Innovation Agency (BRIN; Government of Indonesia), Cibinong 16911, Indonesia*
**Corresponding author.E-mail*: *nabilah.fkh@gmail.com*; *hamidyamir@gmail.com*

Abstract

Colleeneremia capitula (Tyler, 1968) is an insular pelodryadid endemic to the Tanimbar Islands, Indonesia, originally described from a single female specimen and lacking a population-level assessment of morphological variation. We examined 44 specimens (29 males, 15 females) from Lorulun Village, Yamdena Island, integrating detailed morphometrics and mitochondrial 16S rRNA sequence data. Two sympatric and consistently diagnosable morphotypes were identified, differing in iris coloration (golden to copper-brown *vs.* uniform black), dorsal pattern, body size, and multiple size-corrected morphometric characters. Univariate analyses revealed significant differentiation in 28 of 31 measured traits, and principal component analysis recovered two largely discrete clusters in morphospace. Phylogenetic analyses of a 432 bp fragment of 16S rRNA recovered both lineages as a well-supported clade within *Colleeneremia*, with shallow but structured mitochondrial divergence (0.3–1.4%) between morphotypes. The combination of sympatric occurrence, consistent phenotypic diagnosability, and concordant mitochondrial structuring supports recognition of the black-iris morphotype as a distinct species, described here as a new species. This study reveals previously unrecognized diversity within the Tanimbar pelodryadids and underscores the potential for rapid insular divergence in Wallacean amphibians.

Keywords: integrative taxonomy, sympatric divergence, iris coloration, insular speciation, Wallacea

Introduction

Recent phylogenomic analyses of Australo-Papuan treefrogs (Anura: Pelodryadidae) have substantially revised generic boundaries within the former *Litoria* radiation. The diversification of pelodryadids across Australasia and Wallacea reflects complex biogeographic processes, including repeated dispersal and insular isolation, resulting in the recognition of several distinct genera, including *Colleeneremia* Wells & Wellington, 1985 (Donnellan *et al*. 2025). This genus comprises small to medium-sized arboreal frogs formerly assigned to the *Litoria rubella* and *L. quadrilineata* species groups and is characterized by morphological and acoustic traits distinct from its sister taxon *Pengilleyia* (Donnellan *et al*. 2025).

The Tanimbar Islands, located in southern Maluku within the Wallacean transition zone between Sundaland and Sahul, represent an oceanic archipelago that has never been connected to a continental landmass (Paulay 1994, Haryoko *et al*. 2021). Geological evidence indicates that the islands arose from interactions between the Banda Arc and the Australian Plate (Charlton *et al*. 1991, Weijola & Kraus 2023). Prolonged isolation in such systems is widely recognized as a driver of endemism and morphological divergence in vertebrates (Feng *et al*. 2017), yet the amphibian fauna of Tanimbar remains poorly documented.

The only frog formally described from the archipelago is *Colleeneremia capitula* (Tyler, 1968), originally described as *Hyla capitula* from Saumlaki based on a single gravid female collected in 1924. The original description provided limited information on variation due to the absence of comparative material. Subsequent taxonomic work confirmed its placement within the *Litoria rubella* complex sensu lato and later within *Colleeneremia* (Purser *et al*. 2025, Donnellan *et al*. 2025), recognizing it as an insular lineage endemic to the Tanimbar Islands. However, population-level morphological variation within *C. capitula* has never been evaluated.

During recent field surveys in Lorulun Village, Yamdena Island, we collected a substantial series of treefrogs assignable to *Colleeneremia*. Although these specimens generally conform to the diagnostic features of *C. capitula* as described by Tyler (1968), they exhibit striking and consistent variation in iris coloration, dorsal patterning, and body proportions. Notably, two sympatric morphotypes were observed in life: one possessing a golden to copper-brown iris typical of congeners, and another characterized by a uniform black iris. Iris coloration has been shown in multiple anuran lineages to function as a stable diagnostic trait, particularly in distinguishing sympatric species (Glaw & Vences 1997, D'Souza *et al*. 2016).

The occurrence of two diagnosable morphotypes in sympatry raises a critical taxonomic question: do these forms represent intraspecific polymorphism within *C. capitula*, or do they correspond to distinct evolutionary lineages? To address this, we conducted detailed morphometric analyses of 44 specimens and evaluated mitochondrial differentiation using the 16S rRNA gene. Here, we demonstrate that the black-iris morphotype is consistently diagnosable by multiple morphological characters and exhibits structured mitochondrial divergence, supporting its recognition as a distinct species within *Colleeneremia*. This study reveals previously unrecognized diversity within the Tanimbar pelodryadids and highlights the evolutionary potential of insular amphibian populations in Wallacea.

Materials and Methods

Field sampling and specimen preservation. Specimens were collected on 20 April 2018 from Lorulun Village (Fig. 1), Wer Tamrian District, Yamdena Island, West Southeast Maluku Regency, Indonesia. Frogs were euthanized using a buffered chlorotone solution in accordance with standard herpetological procedures. Liver tissue was excised and preserved in 95% ethanol for molecular analyses. Voucher specimens were fixed in 10% buffered formalin, subsequently transferred to 70% ethanol for long-term preservation, and deposited in the Museum Zoologicum Bogoriense (MZB), Cibinong, Indonesia.

Morphological data collection. Including the holotype of *Colleeneremia capitula*, a total of 44 specimens were examined (29 males, 15 females). Individuals were assigned to two morphotypes based on iris coloration observed in life: morphotype I (golden to copper-brown iris) and morphotype II (uniform black iris). Color terminology follows Köhler (2012).

Morphometric measurements were taken using digital calipers to the nearest 0.1 mm under a stereomicroscope. Thirty-one characters were measured following Watters *et al*. (2016) with minor modifications (Sup. Table 1). All measurements were taken on the right side of the body. Sex was determined by the presence of nuptial pads and vocal sacs in males, and by the absence of these characters and/or presence of mature ova in females. Webbing formulae follow Cisneros-Heredia & McDiarmid (2007).

Statistical analyses. All statistical analyses were conducted in R v4.2.1 (R Core Team 2020).

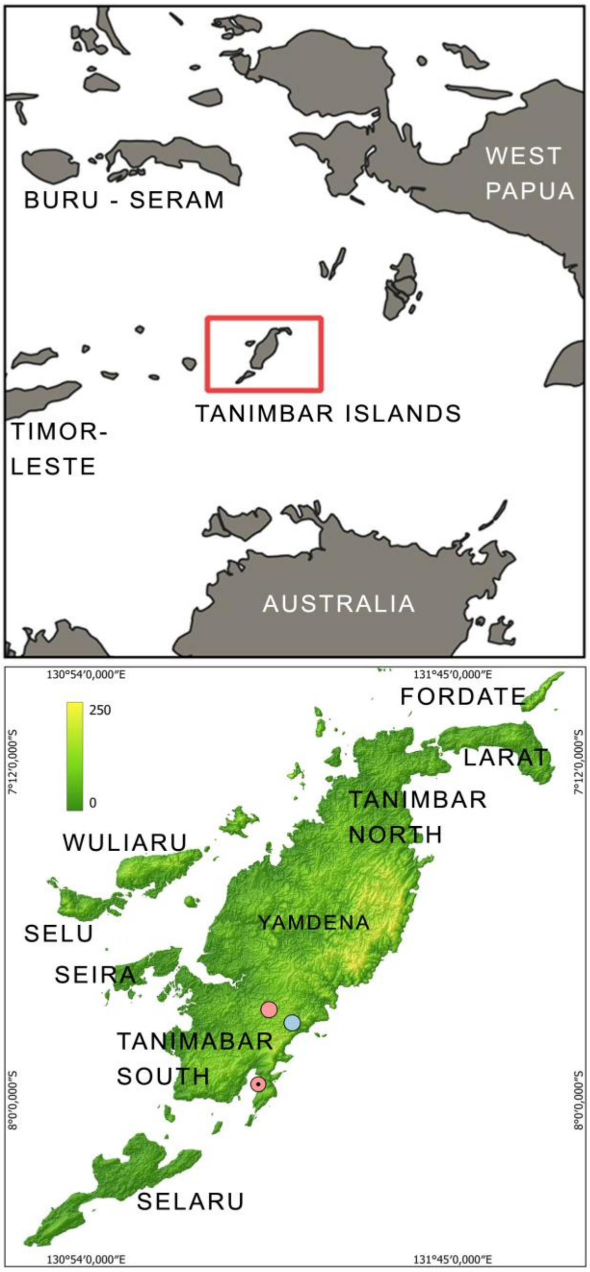

Figure 1. Maps showing the known localities of the examined specimens in this study: *Colleeneremia capitula* in pink circles (holotype locality indicated with a dot in the middle) and the new species in a blue circle.

Sexual dimorphism in morphotype I (*Colleeneremia capitula*) was assessed by comparing male and female SVLs (Welch's t-test) and by testing for multivariate differences in size-corrected traits using MANOVA (Pillai's trace). As no significant sexual dimorphism was detected, males and females were pooled for subsequent univariate and multivariate comparisons. Because only one female of morphotype II (the new species) was available, sex-specific tests for this species were not performed, but the female was retained in pooled analyses.

To reduce allometric effects, morphometric variables were size-corrected using the allometric equation:

$$X_{adj} = \log(X) - \beta[\log(SVL) - \log(SVL_{mean})]$$

where X_{adj} is the adjusted value, X is the original measurement, β is the unstandardized regression coefficient of each character against SVL, and SVL_{mean} is the overall mean snout–vent length (Thorpe 1975, 1983, Turan 1999, Lleonart *et al.* 2000). Size correction was implemented using the R package *GroupStruct* (Chan & Grismer 2022). Morphotypes were size-corrected independently and subsequently merged into a single dataset for comparative analyses.

Normality was assessed using the Shapiro–Wilk test and homogeneity of variance using Levene's test. Characters meeting parametric assumptions were compared using independent-sample t-tests; non-normal variables were analyzed using the Mann–Whitney U test.

Principal Component Analysis (PCA) was performed on size-corrected variables using the prcomp function in R. PCA was used to summarize multivariate morphometric structure and evaluate morphospace separation between morphotypes. Scatterplots of PC1 and PC2 were generated using the *Factoextra* package (Kassambara & Mundt 2020). Boxplots were produced to visualize characters showing significant differentiation.

DNA extraction, amplification, and sequencing. Genomic DNA was extracted from approximately 10 mg of liver tissue using the Qiagen DNeasy Blood & Tissue Kit following the manufacturer's protocol. DNA was eluted in 200 µl of AE buffer.

A fragment of the mitochondrial 16S rRNA gene was amplified in 25 µl PCR reactions containing 12.5 µl MyTaq HS Mix, 1 µl genomic DNA, 1 µl of each primer, and 9.5 µl ddH_2O. Primers L2606 and H3056 (Hedges 1994) were used for amplification. PCR cycling conditions consisted of an initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 30 s, 48–50°C for 30 s, and 72°C for 1 min; followed by a final extension at 72°C for 5 min. PCR products were purified and sequenced bidirectionally. Chromatograms were edited and assembled in ChromasPro (Technelysium Pty Ltd., Australia).

Phylogenetic analyses. Five newly generated sequences were analyzed together with 13 sequences retrieved from GenBank (Sup. Table 2), including representatives of *Colleeneremia* and related pelodryadid genera. Outgroup selection followed Purser *et al*. (2025) and Donnellan *et al*. (2025), using species currently assigned to *Pengilleyia* as sister taxa to *Colleeneremia*. Additional representatives from other anuran lineages were included to provide a broader phylogenetic context.

Sequences were aligned using ClustalW implemented in MEGA11 (Tamura *et al*. 2021) and visually inspected for alignment errors. The best-fit nucleotide substitution model was selected using Kakusan3 (Tanabe 2007) under the Akaike Information Criterion, resulting in a GTR+Γ model.

Phylogenetic relationships were inferred using Maximum Likelihood (ML) and Bayesian Inference (BI). ML analyses were conducted in MEGA11 with 1,000 bootstrap replicates. BI analyses were performed in MrBayes v3.2.7 (Ronquist *et al*. 2012) with two independent runs of four Markov Chain Monte Carlo (MCMC) chains for 10,000,000 generations, sampled every 1,000 generations. Convergence was assessed by examining the standard deviation of split frequencies and trace diagnostics. The first 25% of trees were discarded as burn-in.

Uncorrected p-distances were calculated in MEGA11 using pairwise deletion to quantify mitochondrial divergence among lineages.

Results

Sexual dimorphism. In morphotype I, males and females did not differ in SVL (Welch's t-test: $t = -0.85$, df = 32.7, $p = 0.403$). Multivariate analysis of size-corrected variables likewise detected no significant sex effect (MANOVA, Pillai's trace = 0.718, $F = 1.91$, $p = 0.103$). Accordingly, sexes were pooled in subsequent morphometric analyses. Sexual dimorphism in morphotype II could not be tested due to the single available female; however, the female paratype plotted within the morphospace occupied by conspecific males (Fig. 2).

Morphological differentiation. Pairwise comparisons of size-corrected morphometrics (sexes pooled) revealed significant differences between morphotype I and morphotype II for most characters (Sup. Table 3; Fig. 3). Morphotype II exhibited smaller overall body size and proportionally reduced head and limb dimensions relative to morphotype I. Multivariate analyses corroborated strong morphometric differentiation between species (MANOVA, Pillai's trace = 0.920, $F = 13.2$, $p = 2.78 \times 10^{-8}$; PERMANOVA, $R^2 = 0.481$, $F = 38.90$, $p = 0.001$). Boxplots (Fig. 3) show consistent directional shifts in body proportions, rather than overlapping stochastic variation.

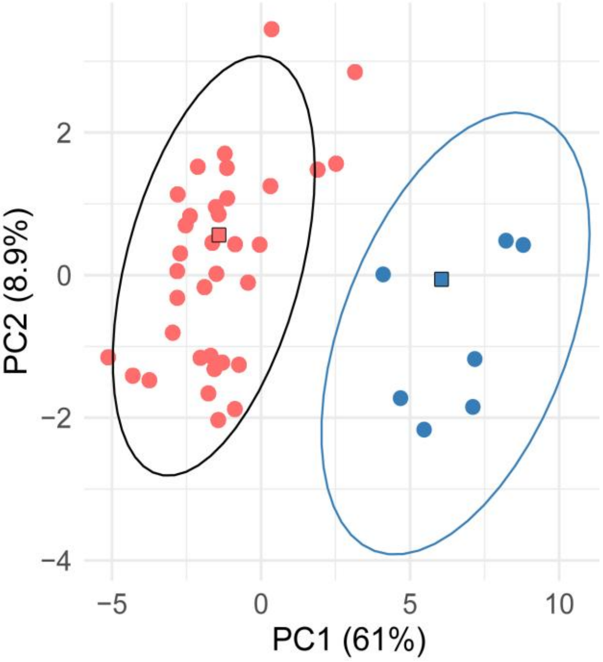

Figure 2. Principal Component Analysis (PCA) of allometry-corrected morphometric variables comparing *Colleeneremia* morphotype I (golden to copper-brown iris) and morphotype II (black iris): Pooled sex analysis (n=44). Pink dots represent morphotype I (*C. capitula*) and blue points represent morphotype II (the new species). The holotypes of *C. capitula* and the new species are represented with squares in respective colours. Ellipses indicate 95% confidence intervals for each taxon.

Principal Component Analysis of size-corrected variables (sexes pooled) explained 69.9% of total variance in the first two components (PC1 = 61.0%, PC2 = 8.9%; Sup. Table 4, Fig. 2). PC1 was strongly associated with overall body and limb dimensions (e.g., HLL, LAL, FOL, THL), indicating that interspecific differentiation is primarily driven by proportional differences in body size and limb length. PC2 was influenced mainly by head-related variables (e.g., HDD, ETD, IOD), reflecting secondary variation in cranial proportions. In morphospace, morphotype I and morphotype II form well-separated clusters along PC1 with minimal overlap, demonstrating robust multivariate differentiation independent of sex. This separation is concordant with consistent divergence in iris coloration and dorsal pattern observed in life.

Taken together, these univariate and multivariate results demonstrate that the two morphotypes are morphometrically diagnosable and structurally differentiated, rather than representing random or clinal variation within a single population.

Identity of morphotypes. Direct comparison with the holotype of *Colleeneremia capitula* (RMNH 5317) confirms that morphotype I corresponds to *C. capitula*. Because the holotype is preserved, characters such as iris coloration and dorsal pattern cannot be reliably assessed; therefore, identification is based primarily on morphometric congruence. The holotype shares with morphotype I a defined dorsolateral stripe and proportional head and limb dimensions consistent with the larger-bodied cluster recovered in multivariate analyses. In contrast, morphotype II exhibits comparatively reduced body proportions.

PCA further supports this assignment: the holotype plots within the morphospace occupied by morphotype I specimens (Fig. 2) and is clearly separated from the cluster representing the unnamed lineage. Additionally, newly collected specimens assigned to morphotype I exhibit a golden-to-copper-brown iris and a dorsal pattern characterized by a mid-dorsal band, a defined dorsolateral stripe, or irregular dark spotting. In contrast, morphotype II displays a uniformly black iris and lacks a continuous dorsolateral stripe. The concordance between morphometric placement and external diagnostic traits confirms the taxonomic identity of morphotype I as *C. capitula* and distinguishes morphotype II as the new lineage.

Molecular analyses. The aligned 16S rRNA dataset comprised 432 base pairs. Phylogenetic reconstruction using both Maximum Likelihood and Bayesian Inference recovered the Lorulun samples as a strongly supported clade within *Colleeneremia* (ML bootstrap = 99; BI posterior probability = 1.00; Fig. 4). Within this clade, individuals corresponding to morphotype I and morphotype II form two shallow but consistently structured mitochondrial lineages.

Uncorrected p-distances between morphotype I and morphotype II ranged from 0.3–1.4% (Sup. Table 5). Divergence between the Lorulun lineages and their sister taxon *C. rubella* ranged from 3.0–3.8%, whereas divergence from other congeners ranged from 7.3–9.4%.

Although mitochondrial divergence between morphotypes is relatively shallow, the presence of structured haplotypes concordant with consistent morphological diagnosability supports recognition of the black-iris lineage as an independently evolving entity within the genus.

Taxonomy

The redescription of *Colleeneremia capitula* presented herein is based on newly examined material from Yamdena Island, Tanimbar Archipelago, deposited in the Museum Zoologicum Bogoriense (MZB). These specimens correspond closely to the diagnostic features outlined in the original description of *Hyla capitula* by Tyler (1968), including small head proportions, short limbs, and characteristic webbing configuration.

Colleeneremia capitula was originally assigned to *Litoria* based on its horizontally elliptical pupil and unpigmented palpebral membrane (Duellman *et al*. 2016). It was previously considered part of the *Litoria rubella* species complex due to its small body size, short limbs, and relatively small head (Tyler 1968). However, Donnellan *et al.* (2025) classified this species within the genus *Colleeneremia,* characterized by small to medium body size.

The examined series provides the first opportunity to evaluate intraspecific morphological variation in this insular taxon, which was originally described from a single gravid female. The MZB specimens are designated as comparative voucher material to facilitate taxonomic reference and future revisionary work within *Colleeneremia*.

Colleeneremia capitula (Tyler, 1968)
(Figs. 5, 6; Sup. Table 3)

Hyla capitula Tyler 1968

Holotype. Adult gravid female, RMNH 5317 collected from Saumlaki, Tanimbar Island, Moluccas, Indonesia, collected by F. Kopstein in May 1924 (Fig. 5).

Other examined materials. *Colleeneremia capitula* collected from Yamdena Island, Lorulun Village, Sub-district Wer Tamrian, West Southeast Moluccas Regency, Maluku Province. Deposited in the Museum Zoologicum Bogoriense (MZB), collected by Amir Hamidy, Awal Riyanto, Syahfitri Anita, and Syarifudin on 20 April 2018 (Fig. 5). The measurements of 36 *C. capitula* voucher specimens (22 males and 14 females) are detailed in Sup. Table 3.

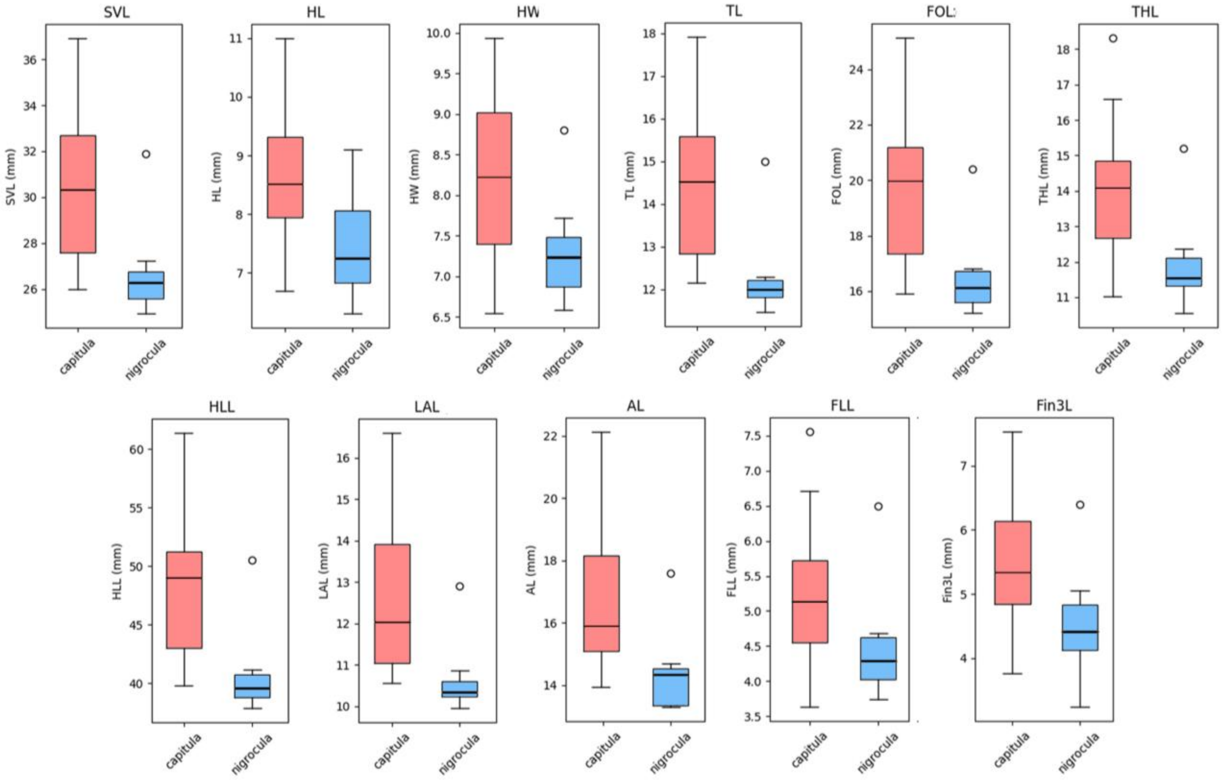

Figure 3. Boxplots of selected morphometric characters (sexes pooled) showing significant differentiation between morphotype I (pink) and morphotype II (blue). See Sup. Table 3 for full statistical results.

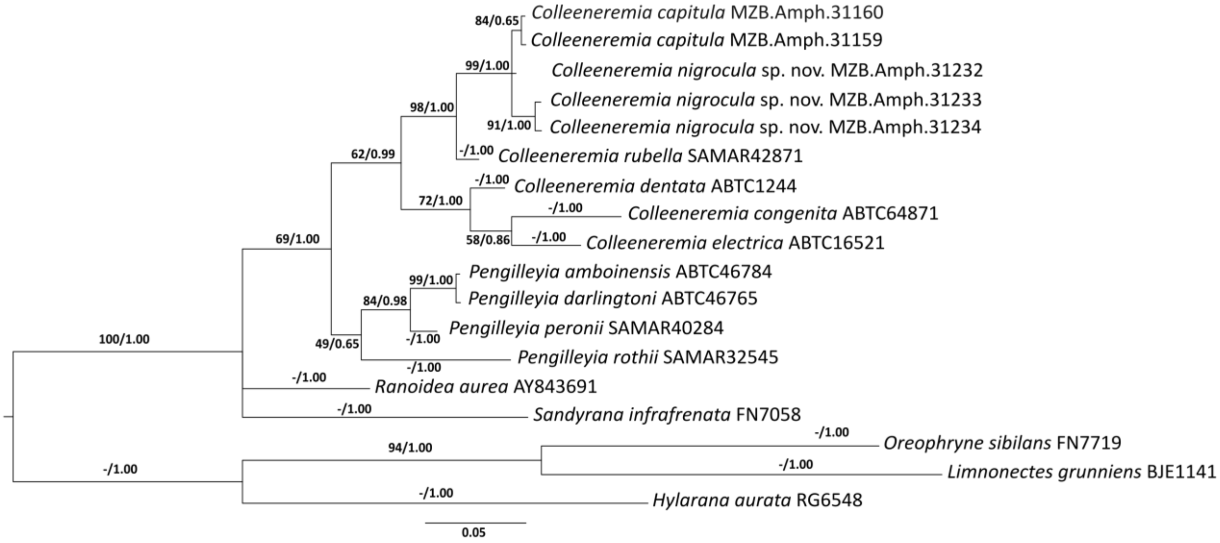

Figure 4. Bayesian Inference and Maximum Likelihood phylogeny based on 432 bp of mitochondrial 16S rRNA showing placement of the Lorulun population within *Colleeneremia* and two closely related haplotype groups corresponding to morphotype I and morphotype II.

Diagnosis. A small to moderate-sized species of *Colleeneremia* (male SVL 26.0–35.8 mm; female SVL 28.6–36.9 mm) distinguished from congeners by the following combination of characters: (1) iris golden to copper-brown in life; (2) dorsum variable but frequently with a continuous dark mid-dorsal band and well-defined dorsolateral stripe extending from nostril through eye and tympanum to groin; (3) head small and narrow (HL/SVL 0.24–0.32; HW/SVL 0.23–0.30); (4) limbs short (TL/SVL 0.44–0.51); (5) vomerine teeth present as two adjacent wedge-shaped patches; (6) fingers moderately webbed (I 2+–2- II 2—2+ III 2+–2 IV) and toes moderately webbed (I 1+–2- II 1-–2 III 0+–2- IV 2-–0+ V); (7) digital discs expanded with distinct circum-marginal grooves; (8) dorsal skin smooth; (9) ventral surfaces of thighs and belly coarsely granular; (10) dorsolateral folds and parotoid glands absent.

Redescription of the species. Moderate body size (SVL: 26.0–36.9 mm). Head small (HL/SVL: 0.24–0.32), its length and width approximately equal (HL/HW 0.93–1.16), narrow (HW/SVL: 0.23–0.30), and dorsally flattened (HDD/SVL: 0.07-0.12). Snout blunt and slightly rounded in dorsal view, rounded in profile (IND/IOD: 0.47–0.69); canthus rostralis rounded; loreal region steep, flat to slightly concave with vertical orientation. Nostrils oval, positioned on the anterolateral surface of the snout, and oriented dorsolaterally. Eye-naris distance longer than internarial distances (EN/IND: 1.31–1.74). Eyes small to moderate (ED/HL: 0.28–0.44), eye-naris distance usually exceeding eye diameter (EN/ED: 0.74–1.14). Pupil horizontal. Tympanum distinct, circular, smaller than eye (TD/ED: 0.50–0.74); supratympanic fold weakly developed. Vomerine teeth are present below the choanae as two wedge-shaped teeth adjacent to each other in the middle.

Arms moderately long (UAL+LAL/SVL: 0.45–0.62); the fingers are short, the order of finger length III > IV > II > I; well-developed discs at the tip of finger with distinct circum-marginal grooves; finger's webbing formula is I 2+ – 2- II 2- – 2+ III 2+ – 2 IV; the discs relative wider than the penultimate phalanx (Fin3DW/Fin3W: 1.27–1.99); narrow lateral fringing present, subarticular tubercles are rounded and well-defined. An oval inner metacarpal tubercle is present at the base of the palm.

Hindlimbs short (TL/SVL: 0.44–0.51); the order length of toes IV > V > III > II > I with well-developed terminal disc, slightly smaller than the finger (Toe4DW/Toe4W: 1.15–1.61); Discs bear distinct circum-marginal grooves and narrow lateral fringing; toes webbing formula is I 1+–2- II 1-–2 III 0+–2- IV 2-–0+ V; subarticular tubercles on toes are rounded and well-defined; an inner metatarsal tubercle is present and oval in shape.

The dorsal surface is smooth, with dorsolateral folds and paratoid glands absent; the ventral surfaces of the belly and thighs are coarsely granular, the gular is finely granular; limbs are smooth.

Coloration. In life (Fig. 6), dorsum variable: olive horn, drab, sayal brown, tawny olive, amber, or buff-yellow. Dark mid-dorsal band present in some specimens; band continuous, margins distinct; dorsolateral stripe extending from nostril through eye and tympanum to groin. Band faint or reduced in other individuals. Upper lip with light cream marking. Dorsum with irregular dark spots in some specimens. Yellow to white spots variably present on upper lip, around tympanum, near cloaca, along upper arm and fingers, and from knee along posterior tibia to toes. Tibia and foot sometimes with darker speckling and scattered white flecks. Venter pale cream to translucent beige. Gular region with diffuse greyish-brown suffusion in some specimens. Ventral surfaces of limbs translucent yellowish-orange; orange suffusion around cloaca, axilla, posterior thighs, palmar and plantar surfaces. Iris golden to copper-brown. Pupil horizontal, black.

In preservatives, the color tone of some preserved specimens remains the same as when they lived (slightly faded), but others have faded to a cream, greyish color.

Comparison. *Colleeneremia capitula* differs from its sympatric new species by its larger body size (males 26.0–35.8 mm *vs.* 24.9–27.2 mm, females 28.6–36.9 mm *vs.* 31.9 mm), golden to copper-brown iris (*vs.* uniform black), presence of a prominent dorsolateral stripe or defined mid-dorsal band (*vs.* irregular dorsolateral margins or blotched pattern), and proportionally longer limbs and head dimensions (see Sup. Table 3). It differs from *C. rubella*, *C. pyrina*, and *C. larisonans* by its insular distribution in the Tanimbar Islands, combination of shorter limbs, narrower head proportions, and absence of a diffuse lateral stripe typical of mainland taxa. It differs from *C. dentata*, *C. congenita*, and *C. electrica* by its smaller body size, smoother dorsum lacking bold transverse bars, and consistent golden to copper-brown iris.

Common names. Saumlaki Tree Frog (English), Katak Pohon Saumlaki (Bahasa Indonesia)

Distribution and habitat. *Colleeneremia capitula* is known to inhabit a limited area on the Tanimbar Islands, with the original description based on specimens from Saumlaki. Additional specimens were collected at Lorulun Village, Wer Tamrian, collected by voucher specimens used in this study. The species occurs in forests with dense canopy cover, associated with small, slow-flowing streams (Fig. 7)

Colleeneremia ***nigrocula*** sp. nov.

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(Figs. 8, 9; Sup. Table 3)

Holotype. Adult Male (MZB.Amph.31235) from Lorulun Village, Yamdena Island, Wer Tamrian Sub-district, West Southeast Maluku Regency, Maluku Province, Indonesia, collected by Amir Hamidy, Awal Riyanto, Syahfitri Anita, and Syarifudin on 20 April 2018 (Fig. 8).

***Paratypes* (*n*=7).** Six adult males (MZB.Amph.31177, 31188, 31199, 31232–31234) and one adult female (MZB.Amph. 31189) from Yamdena Island, Lorulun Village, Wer Tamrian Sub-district, West Southeast Maluku Regency, Maluku Province, Indonesia, collected by Amir Hamidy, Awal Riyanto, Syahfitri Anita, and Syarifudin on 20 April 2018.

Diagnosis. A small, slender species of *Colleeneremia* (male SVL 24.9–27.2 mm; female SVL 31.9 mm) distinguished from congeners by the following combination of characters: (1) iris uniformly black in life; (2) dorsum lacking a continuous mid-dorsal band or well-defined dorsolateral stripe; instead with irregular dorsolateral margins or contrasting blotches; (3) body proportions slender, with relatively reduced head and limb dimensions (see Sup. Table 3); (4) head narrow (HW/SVL 0.26–0.30); (5) limbs short (TL/SVL 0.44–0.48); (6) vomerine teeth present as two adjacent wedge-shaped patches; (7) fingers and toes moderately webbed (finger webbing I 2+–2- II 2-–2+ III 2+–2 IV; toe webbing I 1+–2- II 1-–2 III 0+–2- IV 2-–0+ V); (8) digital discs expanded with distinct circum-marginal grooves; (9) dorsal skin smooth; (10) dorsolateral folds and parotoid glands absent.

Description of holotype. Small body size (SVL: 25.8 mm); slender. The head is small (HL/SVL 0.27), the head length is almost equal to the width (HL/HW 1.1), narrow (HW/SVL 0.26), and dorsally flattened (HDD/SVL 0.09). Snout blunt, moderately pointed; rounded in dorsal and profile (IND/IOD 0.54); canthus rostralis rounded; loreal region steep, flat to slightly concave with vertical orientation. Nostrils oval, positioned on the anterolateral surface of the snout, and oriented dorsolaterally. Eye-naris distance longer than internarial distances (EN/IND 1.81). Eyes small (ED/HL 0.37), eye-naris distance usually exceeding eye diameter (EN/ED 1.12). Pupil horizontal. Tympanum distinct, circular, smaller than eye (TD/ED 0.59); supratympanic fold weakly developed. Vomerine teeth are present below the choanae as two wedge-shaped teeth adjacent to each other in the middle.

Arms moderately long (UAL+LAL/SVL 0.56); the fingers are short, the order of finger length III > IV > II > I; well-developed discs at the tip of finger with distinct circum-marginal grooves; finger's webbing formula is I 2+ – 2- II 2- – 2+ III 2+ – 2 IV; the discs relative wider than the penultimate phalanx (Fin3DW/Fin3W 1.39); narrow lateral fringing present, subarticular tubercles are rounded and well-defined. An oval inner metacarpal tubercle is present at the base of the palm.

Hindlimbs short (TL/SVL 0.46); the order length of toes IV > V > III > II > I with well-developed terminal disc, slightly smaller than the finger (Toe4DW/Toe4W 1.33); Discs bear distinct circum-marginal grooves and narrow lateral fringing; toes webbing formula is I 1+–2- II 1-–2 III 0+–2- IV 2-–0+ V; subarticular tubercles on toes are rounded and well-defined; an inner metatarsal tubercle is present and oval in shape. ***Dimension of holotype.*** SVL 25.79; HL 6.9; HW 6.59; IND 1.55; EN 2.81; ED 2.52; IOD 2.89; TD 1.48; FLL 4.07; Fin3L 4.48; TL 11.85; Toe4L 6.61.

Coloration. In life (Fig. 9), dorsum drab, burnt umber, or true cinnamon. Mid-dorsal band absent. Dorsolateral stripe absent. Dorsal pattern irregular; margins uneven; blotches present in some individuals. Pale cream dorsum with dense speckling in some specimens. Others with uniform clay to burnt umber dorsum. Irregular tawny olive blotches may occur on head, mid-dorsum, forelimbs, and hind limbs. Upper lip without distinct continuous light stripe. Dorsolateral region with irregular margins rather than defined stripe. Venter semi-translucent pale cream. Diffuse orange to yellow suffusion in groin, axilla, anterior and posterior thighs, tibia, and on palmar and plantar surfaces. Iris uniformly black. Pupil horizontal, black.

In preservative, the dorsal pattern is faded and indistinct. Some specimens have a smoke grey color on the dorsal and thigh surfaces, fading to pale cream on the dorsolateral and ventral surfaces.

Variation. The specimens are similar in body proportions but show variation in morphometrics (Sup. Table 3). The coloration of paratype varies from the palest dorsal color (cream) to the darkest (burnt umber). The pattern of the dorsal surface of the holotype differs from that of the others, which have large blotches, while the other

Plate 8

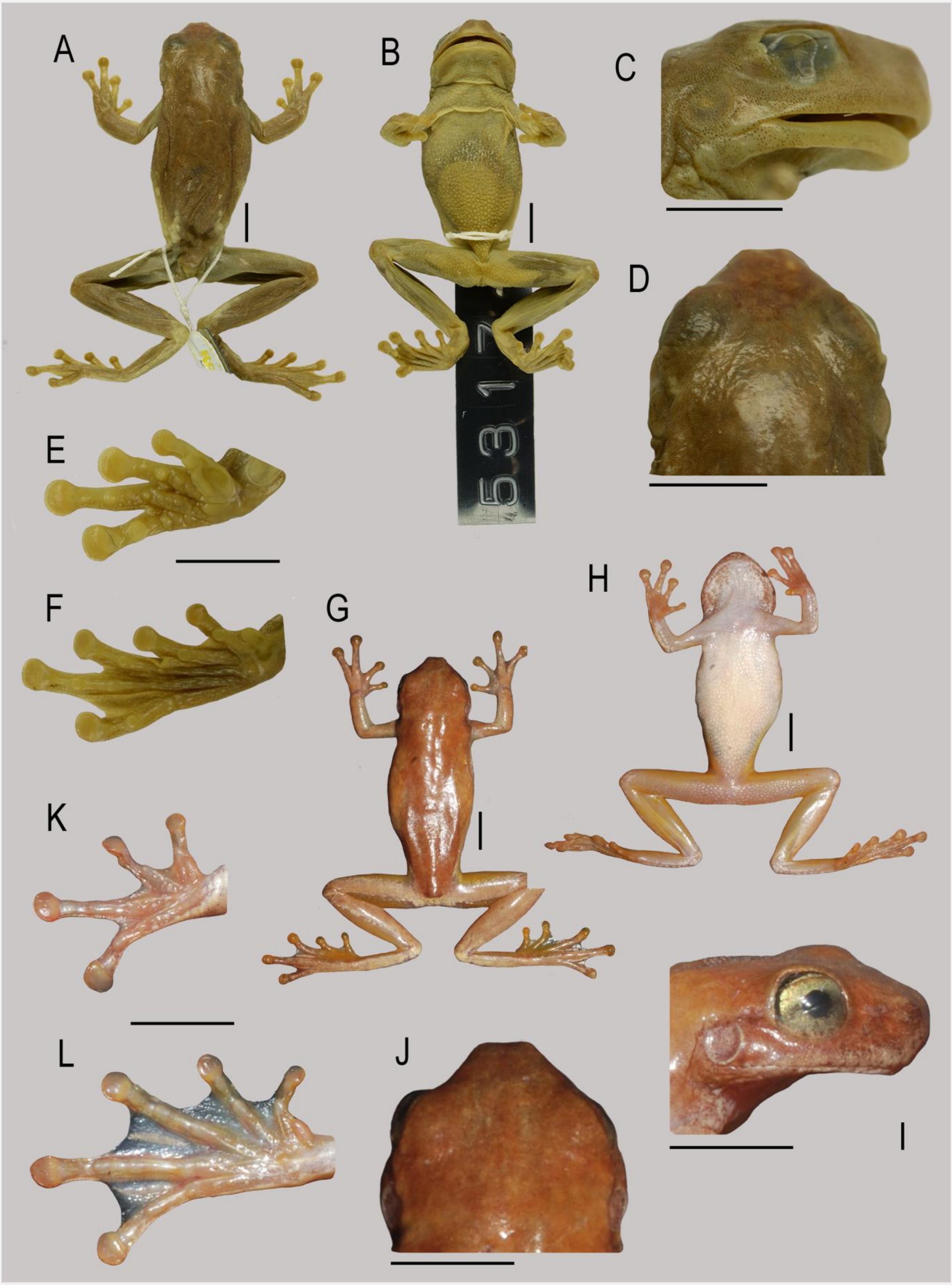

Figure 5. Holotype of *Hyla capitula* (RMNH 5317): **(A)** dorsal and **(B)** ventral views of full body, **(C)** lateral and **(D)** dorsal views of head, ventral views of **(E)** hand and **(F)** foot. © Photo: E. Dondorp & M. Veenendaal; A voucher specimen of *Colleeneremia capitula* (MZB. Amph. 31183): **(G)** dorsal and **(H)** ventral views of full body, **(I)** lateral and **(J)** dorsal views of head, ventral views of **(K)** hand and **(L)** foot (scale 5 mm) © Photo: A. Hamidy.

Plate 9

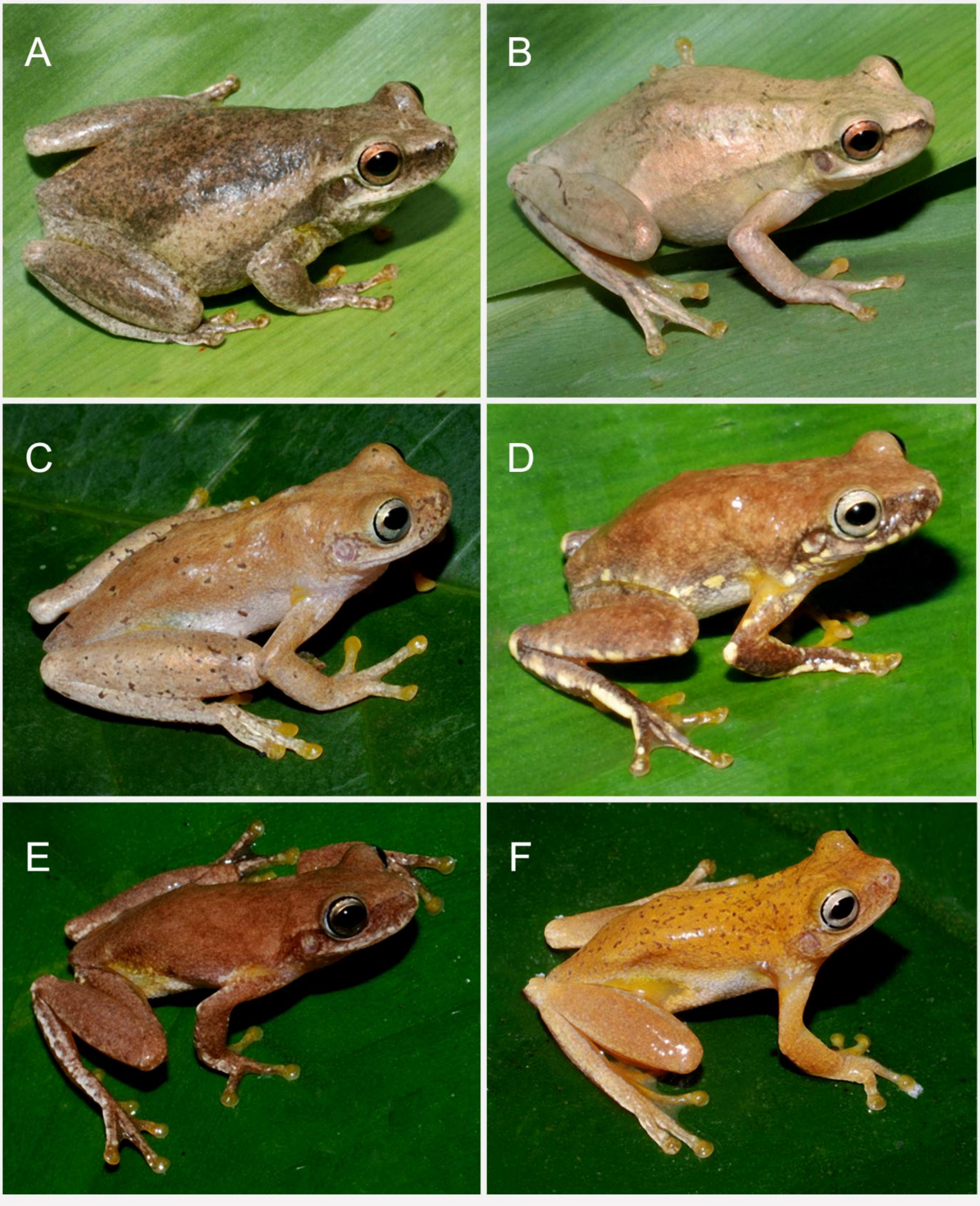

Figure 6. Adult body colour variation of *Colleeneremia capitula* (in life): **(A)** MZB.Amph. 31170 (male), **(B)** MZB. Amph. 31159 (female), **(C)** MZB.Amph. 31193 (female), **(D)** MZB.Amph. 31175 (male), **(E)** MZB.Amph. 31191 (male), and **(F)** MZB.Amph. 31166 (male) © Photo: A. Hamidy

Plate 10

Figure 7. Natural habitat of *Colleeneremia capitula* in Lorulun Village, Tanimbar Islands © Photo: T. Haryoko

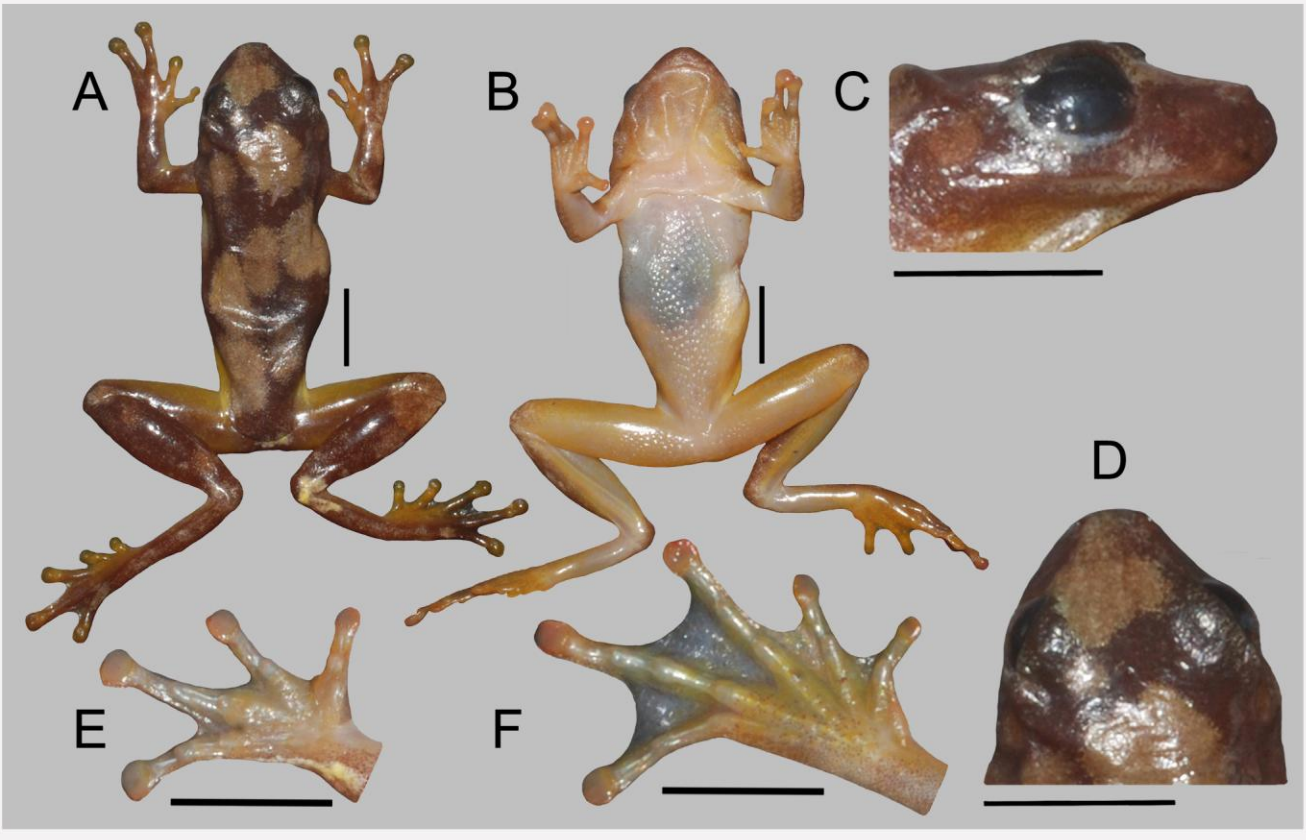

Figure 8. *Colleeneremia nigrocula* sp. nov. holotype (MZB. Amph. 31235): **(A)** dorsal and **(B)** ventral views of full body, **(C)** lateral and **(D)** dorsal views of head, ventral view of **(E)** hand and **(F)** foot (scale 5 mm) © Photo: A. Hamidy

Plate 11

Figure 9. Adult body colour variation of *Colleeneremia nigrocula* sp. nov. (in life): **(A)** MZB.Amph. 31177 (male), **(B)** MZB.Amph. 31234 (male), **(C)** MZB.Amph. 31189 (female), **(D)** MZB.Amph. 31235 (male) © Photo: A. Hamidy

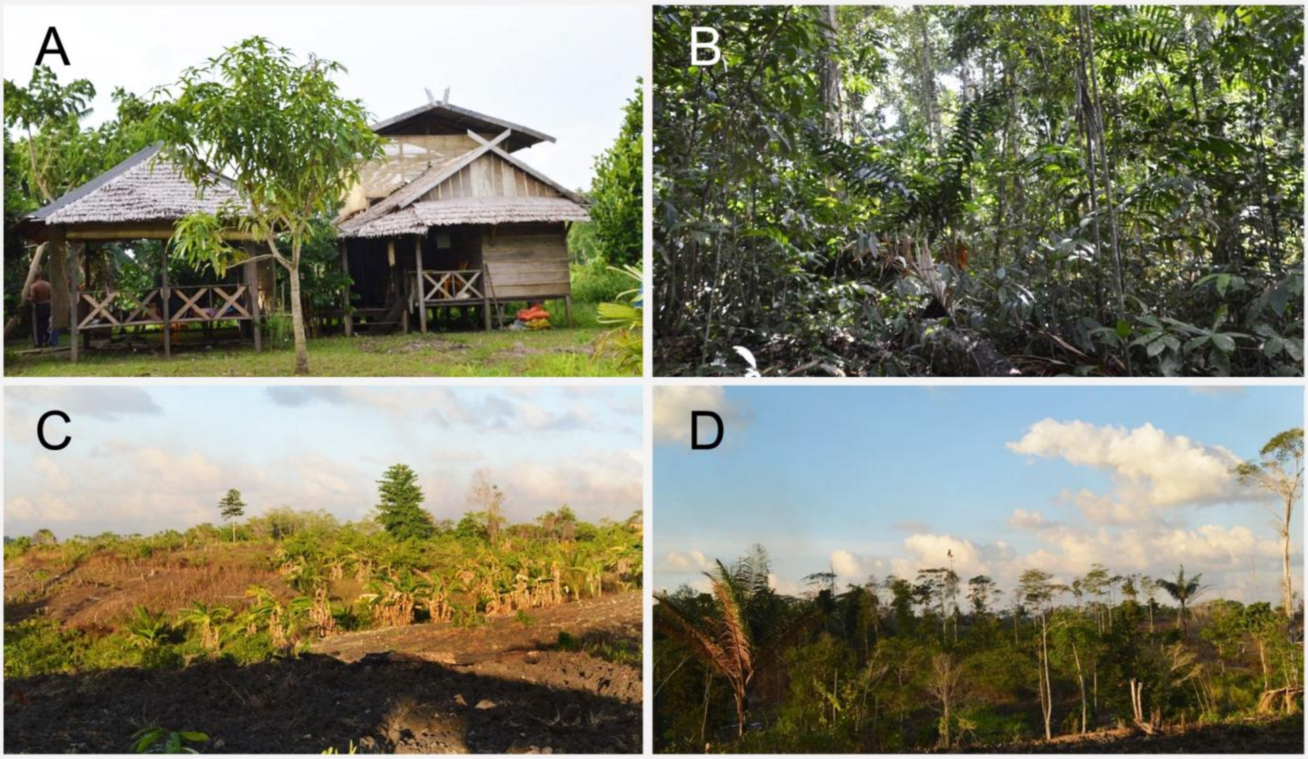

Figure 10. The habitats of *Colleeneremia nigrocula* sp. nov. in Lorulun Village, Tanimbar Island: **(A)** home garden, **(B)** secondary forest, **(C)** plantation, **(D)** open area with scattered tress. © Photo: T. Haryoko.

paratype specimens have irregular edges at the dorsolateral.

Comparison. Statistically, *Colleeneremia nigrocula* sp. nov. differs from its closest relative, *Colleeneremia capitula*, in 28 measured characteristics (see Sup. Table 3). *C. nigrocula* sp. nov. differs from *C. capitula* by its smaller body size (24.9–27.2 mm *vs.* 26.0–35.8 mm in males; 31.9 mm *vs.* 28.6–36.9 mm in females), a black iris colour (*vs.* golden to copper brown), and irregular edges at the dorsolateral, or the presence of blotches (*vs.* prominent dorsolateral or the presence of spots).

Colleeneremia nigrocula sp. nov. differ from *C. rubella, C. pyrina,* and *C. larisonans* by its smaller size (24.9–27.2 mm *vs.* 27.9–38.9 mm *vs.* 27.3–36.0 mm *vs.* 26.2–36.7 mm in males; 31.9 mm *vs.* 28.9–42.1 mm *vs.* 30.1–36.5 mm *vs.* 28.5–38.8 mm in females), the presence of irregular edges at the dorsolateral or blotches (*vs.* uniform dorsal color across head, body, and limbs), the absence of dorsolateral line (*vs.* darker lateral stripe from snout and continuous through eye and tympanum, diffuse variably to the groin, more obvious in inguinal area), and a black iris color (*vs.* golden to copper-brown, and reddish brown variation in *C. larisonans*) (Purser *et al.* 2025).

Colleeneremia nigrocula sp. nov. differs from *C. dentata, C. balatus,* and *C. quiritatus* by its smaller body size (24.9–27.2 mm *vs.* 28–42 mm *vs.* 26–44 mm *vs.* 36–43 mm in males; 31.9 mm *vs.* 29–42 mm *vs.* 33–43 mm *vs.* 34–46 mm in females), the presence of irregular edges at the dorsolateral or blotches (*vs.* the presence of a continuous, irregularly edged, dark brown dorsal band), the absence of dorsolateral line (*vs.* dorsolateral line diffusing above insertion of the arm), and a black iris color (*vs.* copper brown *vs.* copper brown to reddish-copper *vs.* copper brown to reddish-copper). *C. nigrocula* sp. nov. can differ from *C. dentata* and *C. quiritatus* by its slender body (*vs.* a robust body) (Rowley *et al.* 2021).

Colleeneremia nigrocula sp. nov. differs from *C. electrica* by its smaller body size (24.9–27.2 mm *vs.* 26–38 mm in males), the presence of irregular edges at the dorsolateral or blotches (*vs.* two brown bars across the dorsal (sometimes bold, well-defined, or vague), and the absence of dorsolateral line (*vs.* dorsolateral line from nostril to eyes, continuing to hind leg) (Ingram & Corben 1990). *Colleeneremia nigrocula* sp. nov. differs from *C. congenita* by its smaller body size (24.9–27.2 mm *vs.* 32 mm in males; 31.9 mm *vs.* <41 mm in females), the presence of irregular edges at the dorsolateral or blotches (*vs.* regular or irregular pale stripes or patches on the dorsal) (Tyler 1968).

Etymology. The specific epithet "*nigrocula*" is a Latin compound adjective formed from "*niger*" (black) and "*oculus*" (eye), given in the nominative singular feminine to agree with the generic name *Colleeneremia*. The name refers to the uniformly black iris in life, a distinct diagnostic character separating this species from its congeners. Black-eyed tree frog (in English); Katak Pohon mata-hitam (in Bahasa Indonesia).

Distribution and habitat. *Colleeneremia nigrocula* sp. nov. is currently known only from a restricted locality in the Tanimbar Islands, specifically Lorulun Village, Wer Tamrian. The species was found near the campsite at Lorulun Village in a human-influenced lowland habitat comprising secondary vegetation, plantations, and open areas with scattered trees (Fig. 10)

Discussion

The Tanimbar Islands represent an oceanic archipelago within Wallacea, positioned between Timor and New Guinea. Long-term geological isolation and complex tectonic history have promoted high levels of vertebrate endemism across multiple lineages (Charlton *et al.* 1991, Haryoko *et al.* 2021, Weijola & Kraus 2023). Insular endemism has already been documented in Tanimbar reptiles, including the pipe snake *Cylindrophis yamdena* (Amarasinghe *et al.* 2015), indicating that prolonged geographic isolation on Yamdena Island has repeatedly generated lineage-level divergence across vertebrate groups. While reptiles and birds from Tanimbar have received increasing taxonomic attention, amphibian diversity remains poorly explored. Until now, the only described anuran from the archipelago was *Colleeneremia capitula*, originally based on a single specimen.

Our examination of a substantial topotypic series reveals the presence of two morphologically diagnosable lineages occurring in sympatry at Lorulun, Yamdena Island. The newly described *C. nigrocula* sp. nov. differs consistently from *C. capitula* in body size, proportional limb and head dimensions, dorsal pattern architecture, and iris coloration. Multivariate morphometric analyses demonstrate structured separation in morphospace, and 21 of 31 size-corrected characters differ significantly between the two taxa (sexes pooled). The concordance between discrete phenotypic differentiation and structured mitochondrial haplotypes supports recognition of two independently evolving lineages within the Tanimbar pelodryadids. Sexual dimorphism was not detected in *C. capitula*, and pooling sexes did not erode species separation in multivariate space, indicating that the observed differences are not attributable to sex-related allometry.

Although mitochondrial divergence between the two species is shallow (0.3–1.4% uncorrected p-distance), low 16S divergence does not preclude species-level differentiation in anurans. Species-level paraphyly and polyphyly are common in animal mitochondrial DNA due to incomplete lineage sorting, introgression, and selective sweeps (Funk & Omland 2003, Ballard & Whitlock 2004, Toews & Brelsford 2012, Dufresnes & Litvinchuk 2022). Consequently, mitochondrial distance thresholds alone are insufficient for delimiting species, particularly in recently diverged insular lineages. Short mitochondrial fragments can underestimate lineage divergence, particularly in recently diverged or insular taxa (Chan *et al*. 2022). Comparable levels of 16S divergence have been reported between morphologically distinct species in several frog genera, including *Amolops* (Li *et al*. 2024), *Nanorana* (Liu *et al*. 2023), *Kalophrynus* (Blackburn *et al*. 2013), and *Odorrana* (Kuramoto *et al*. 2011). In such cases, species recognition is supported by consistent morphological diagnosability rather than by arbitrary mitochondrial thresholds. Moreover, mitochondrial introgression and incomplete lineage sorting are well-documented phenomena in amphibians (Dufresnes & Litvinchuk 2022), reinforcing the need to interpret mtDNA divergence cautiously.

Iris coloration represents the most conspicuous external difference between the two species. In *C. capitula*, the iris is golden to copper-brown, consistent with other congeners (Purser *et al*. 2025, Rowley *et al*. 2021). In contrast, *C. nigrocula* sp. nov. exhibits a uniformly black iris. While iris coloration can vary within some amphibian species, it is frequently stable at the species level and diagnostic in sympatric taxa (Glaw & Vences 1997, D'Souza *et al*. 2016). Black irises are uncommon in Pelodryadidae but are documented in a few species of *Nyctimystes* and related genera (Richards 2024, Zweifel 1958). In the present case, iris coloration is concordant with multiple independent morphological differences and therefore constitutes part of a broader diagnostic phenotype rather than an isolated pigment variant.

Body size and proportional differences further suggest ecological divergence. *Colleeneremia nigrocula* sp. nov. exhibits reduced body size and relatively shorter limb proportions compared to *C. capitula*. Such size reduction is consistent with patterns of insular dwarfism documented in vertebrates inhabiting small, isolated islands (Lomolino 2005). Insular environments can promote rapid morphological shifts driven by ecological specialization, altered predator regimes, or resource limitation (Losos & Ricklefs 2009). The sympatric occurrence of both species suggests that divergence is not strictly geographic but may reflect ecological partitioning within Yamdena Island.

Taken together, the available evidence supports recognition of *Colleeneremia nigrocula* sp. nov. as a distinct species endemic to the Tanimbar Islands. Future research incorporating nuclear genomic data and bioacoustic analyses will clarify the tempo and mode of divergence within this insular lineage, but current morphological and mitochondrial data demonstrate consistent diagnosability and evolutionary independence.

Suggested IUCN Conservation Status. Both *Colleeneremia capitula* and *C. nigrocula* sp. nov. are currently known only from Yamdena Island in the Tanimbar Archipelago and should be considered insular endemics. Portions of Yamdena are designated as production forest or areas allocated for potential land-use conversion, indicating vulnerability to habitat modification. However, data on population size, distributional extent, habitat specificity, and threats remain insufficient. In the absence of robust ecological data, both species are best categorized as Data Deficient (DD) under the IUCN Red List criteria pending targeted surveys and ecological assessment.

Author contributions

All the authors contributed equally.

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Research permits

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Supplemental data

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