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A systematic review of the *Pulchrana picturata* complex, with the description of a new species from Peninsular Malaysia, Sumatra, and southern Thailand

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Abstract. A genomic study by Chan and colleagues demonstrated that *Pulchrana picturata* from Peninsular Malaysia and Sumatra (and southern Thailand by implication) are distinct from the name-bearing lineage from Borneo. In this study, we present additional morphological and bioacoustic data to support the recognition of the new species, which we describe herein. The new species, *P. sundabarat*, can be distinguished from its sister species, *P. picturata*, by having a more conspicuous and defined dorsolateral stripe, brighter coloured dorsal markings, smaller male body size, and marked differences in various aspects of the male advertisement call. We also provide an updated synthesis on the current knowledge of the *P. picturata* group with regard to taxonomy, distribution, evolutionary relationships, and ecology.

Key words. taxonomy, *Pulchrana signata*, morphology, bioacoustics, advertisement call

INTRODUCTION

The systematics of the *Pulchrana signata-picturata* complex has been in a protracted state of confusion due to the overlapping distributions and phenotypic similarities among constituent species. *Pulchrana picturata* and *P. signata* were previously thought to occur in southern Thailand (south of the Isthmus of Kra), Peninsular Malaysia (PM), the Indonesian island of Sumatra, and on Borneo (Taylor, 1962; Brown & Guttman, 2002). These two species were initially considered to be conspecific (Inger, 1966, 1984; Inger & Stuebing, 1990; Manthey & Grossmann, 1997; Chan-ard et al., 1999), but *P. picturata* was removed subsequently from synonymy by Inger & Tan (1996) without accompanying data. More contemporary studies utilising genetic data corroborated the distinction of these species and provided new insights on their distribution ranges (Brown & Siler, 2014; Chan et al., 2020). As currently understood, *P. signata* is endemic to Borneo, where it is sympatric with *P. picturata* (Chan et al., 2020); the latter is also distributed throughout southern Thailand (south of the Isthmus of Kra), PM, and Sumatra (Nutphund, 2001; Frost, 2020). In PM, *P. picturata* is sympatric with two other morphologically similar species, *P. banjarana* (Chan et al., 2019) and *P. centropeninsularis* (Chan et al., 2014). In PM, *P. picturata* is sympatric with two other morphologically similar species, *P. banjarana* (Chan et al., 2019) and *P. centropeninsularis* (Chan et al., 2014), while in Sumatra, it is putatively sympatric with *P. fantastica* (Arifin et al., 2018).

Throughout its range, *Pulchrana picturata* is associated with lowland to hill dipterocarp forests where the species occurs along small to moderate sized streams (Chan et al., 2019). Frogs are generally characterised by having a dark brown to black dorsum, scattered with orange to red spots (Inger, 1966; Inger et al., 2017). Despite overall morphological similarities, high levels of colour pattern variation exist among populations (Brown & Guttman, 2002; Fig. 1), leading researchers to suggest that *P. picturata* could consist of multiple species (Zainudin et al., 2009; Brown & Siler, 2014; Haas et al., 2018). Chan et al. (2020) tested this hypothesis using robust genomic datasets and conclusively demonstrated that conspecific populations from PM and Sumatra were genetically distinct from the name-bearing *P. picturata* lineage from Borneo. Consequently, true *P. picturata* was restricted to Borneo, leaving populations from PM, Sumatra, and southern Thailand without a name. This study provides novel morphological and bioacoustic data that allow us to clarify the diagnosis of the PM and Sumatra lineage with respect to *P. picturata*, and facilitate the recognition of the new species. We also provide an updated synthesis of the current knowledge of the *P. picturata* group with regard to taxonomy, distribution, evolutionary relationships, and ecology.

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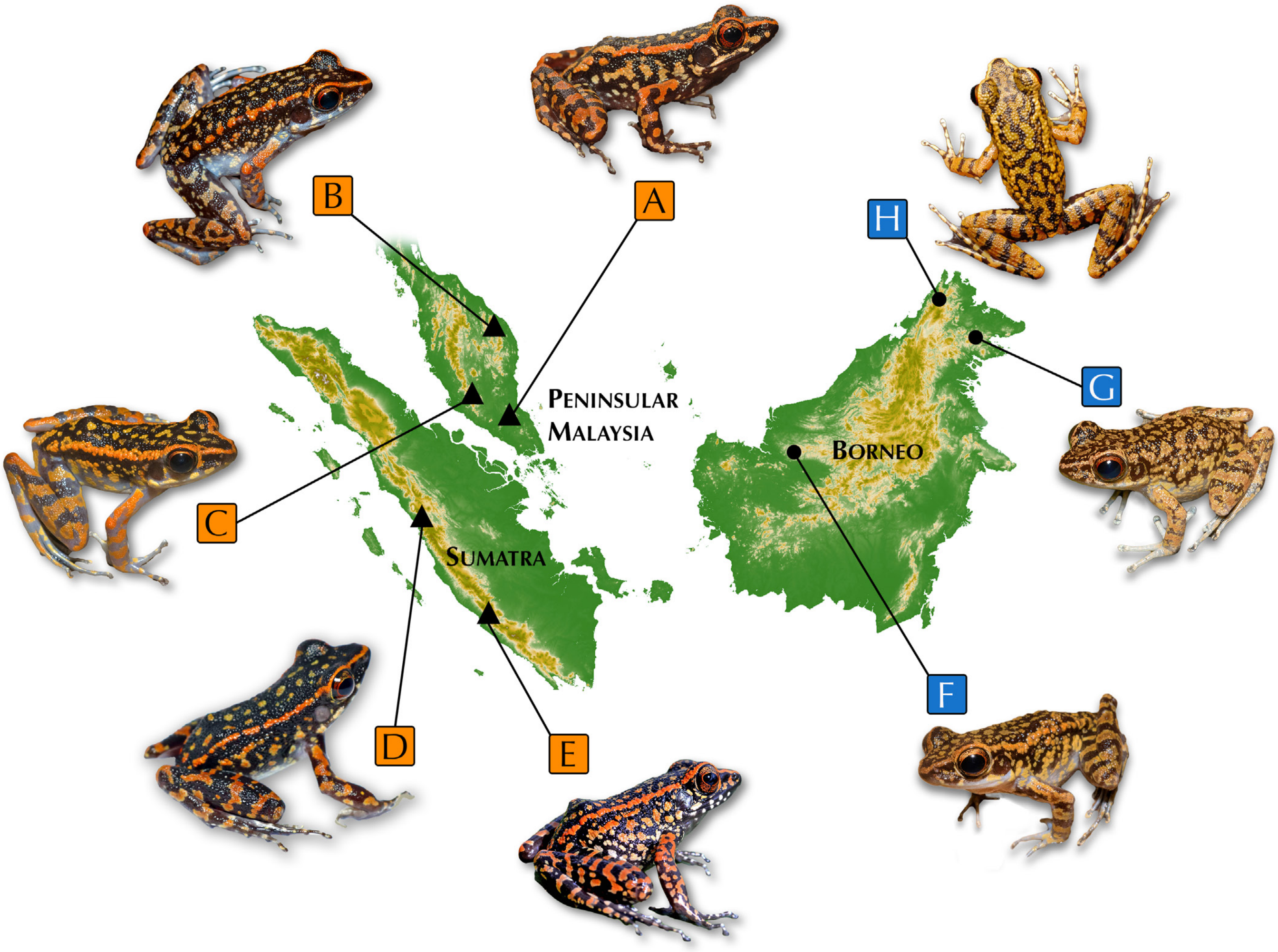

Fig. 1. Colour-pattern variation of the new species (A–E) and *Pulchrana picturata* (F–H) across Peninsular Malaysia, Sumatra, and Borneo. A = Endau-Rompin, Johor; B = Sekayu, Terengganu; C = Lentang, Pahang; D = Padang, Sumatra; E = Bengkulu, Sumatra; F = Lanjak Entimau, Sarawak; G = Danum Valley, Lahad Datu, Sabah; H = Kinabalu (type locality for *P. picturata*). Photo credits: Ihsan Insani (D); Umilaela Arifin (E), Alexander Haas (G, H).

METHODS

Sampling. We examined a total of 197 adult specimens (159 males; 38 females) of *Pulchrana picturata* from several populations in PM and Borneo. Specimens were obtained from the museum holdings of Lee Kong Chian Natural History Museum, Singapore (LKCNHM) and the Field Museum of Natural History, Chicago, USA (FMNH). Type material is deposited at LKCNHM and the University of Kansas Biodiversity Institute (KU; paratypes deposited at KU were not included in analyses). The list of examined specimens and their associated metadata are presented in Supplementary Material (Table S1).

Morphometric analysis. The following measurements were measured with a Mitutoyo digital caliper (to the nearest 0.01 mm): snout-vent-length (SVL), cloaca to tip of snout; snout length (SNL), anterior margin of orbit to tip of snout; head length (HL), posterior margin of jaw angle to tip of snout; head width (HW), widest distance of head measured posterior to jaw; interorbital diameter (IOD), smallest distance between medial margins of palpebrae; tympanum diameter (TYD), measured vertically, including the rim; forearm length (FAL), outer margin of inflected elbow to posterior margin of outer metacarpal tubercle; femur length (FML), cloaca to outer margin of inflected knee; tibia length (TBL), outer margin of inflected knee to outer margin of inflected ankle. To eliminate bias resulting from ontogenetic and interpopulational variation, each character (except SVL) was scaled to the same size by adjusting their shape using the following allometric equation: $X_{adj} = \log(X) - \beta[\log(SVL) - \log(SVL_{mean})]$, where X_{adj} = adjusted value; X = measured value; β = unstandardised regression coefficient for each population; SVL = measured snout-vent length; SVL_{mean} = overall average SVL of all samples (Thorpe, 1983; Lleonart et al., 2000). Because this equation adjusts for intraspecific variation (as opposed to interspecies), adjustments were applied to PM and Borneo populations separately and all downstream analyses were performed on the adjusted values. Male and female measurements were analysed separately to further eliminate bias caused by sexual dimorphism. Raw measurement data are presented in Supplementary Material (Table S1).

A *t*-test was conducted to determine whether the means of morphological characters differed significantly between

PM and Bornean populations. We then used principal components analysis (PCA) to find the best low-dimensional representation of variation in the data to determine whether morphological variation could form detectable group structure that aligns with our hypothesised species boundaries. To further characterise group differences, we performed a discriminant analysis of principal components (DAPC) that minimises within-group and maximises between-group variation (Jombart et al., 2010). All morphological analyses were performed and visualised in R (R Core Team, 2014).

Bioacoustic analysis. Advertisement call recordings from PM were provided by Mohd Abdul Muin, while recordings from Borneo were obtained from the 'Frog Voices of Borneo Soundcloud Database' (https://soundcloud.com/frogvoicesofborneo). In addition to comparisons between the new species and *Pulchrana picturata*, we also examined calls from *P. banjarana* (sympatric with the new species) and *P. signata* (sympatric with *P. picturata*) to assess how calls differed between sympatric species. Calls of the new species and *P. banjarana* were recorded at Gunung Korbu, Perak (04°39.415′N, 101°16.333′E; 796 m a.s.l.), while calls of *P. picturata* and *P. signata* were recorded from Ulu Temburong National Park, Brunei (Frog Voices of Borneo Soundcloud Database). Calls were recorded at a sampling rate of 44.1 kHz. Unfortunately, the ambient temperatures at the time of the recording were not available. The recordings were cleaned of background noise using Audacity version 2.3.3 (Audacity, GNU General Public License). We generated oscillograms and spectrograms using the R package of SEEWAVE (Sueur et al., 2008). Parameters were estimated for one call each from PM and Borneo. Details of these multi-note calls are provided in the results. Call parameters including mean dominant frequency, mean call duration, and call rise and fall times were measured using Raven© Pro 1.5 with the Hanning window type and a DFT (discrete Fourier transform) window size of 256 points and 50% overlap with 44.1 Hz sampling rate (Center for Conservation Bioacoustics, 2014).

RESULTS

Morphometrics. The *t*-test at $\alpha = 0.05$ revealed that males from PM and Borneo were significantly different for all characters, except FAL (Fig. 2; females were not assessed due to insufficient numbers of specimens from PM). For males, the PCA showed some separation between PM and Borneo along PC1, which accounted for 39.4% of the variance (Fig. 3A), while the DAPC analysis demonstrated that the separation was significant (Fig. 3B). In females, the PCA did not show clear separation between PM and Borneo (Fig. 3C), but the DAPC showed significant separation (Fig. 3D).

Bioacoustics. The call of Bornean *Pulchrana picturata* is a frequency modulated call with uniform notes, as per the classification of Emmrich et al. (2020), where notes are structurally similar to each other, but the dominant frequency changes over the call duration; the envelope of the waveform tapers in amplitude gradually from the antepenultimate note. The PM call, in contrast, is a non-frequency modulated call with uniform notes, where the call comprises of several similarly structured notes and the dominant frequency does not change over the call duration, and the waveform envelope declines immediately after the second note, following a declining trend till the end of the call. Characteristics of the call are as follows: Borneo: call duration, 0.65 s; mean note duration, 0.04 s (mean = 0.04, range = 0.02–0.07, SD = 0.01, n = 10; Fig. 4); and with a dominant frequency of 0.2928 kHz. PM: call duration, 0.57 s; mean note duration, 0.04 s (mean = 0.05, range = 0.05–0.05, SD = 0.006, n = 6; Fig. 4); and with the dominant frequencies being modulated between 0.2239, 0.2412, and 0.2584 kHz.

The call of *P. banjarana* from PM is a frequency modulated call with uniform notes, with call duration, 0.32 s; mean note duration, 0.05 s (mean = 0.05, range = 0.03–0.08, SD = 0.02, n = 4; Fig. 4); and with two dominant frequencies, rising from 0.3273 to 0.3445 kHz. *Pulchrana signata* from Borneo has a non-frequency modulated call with uniform notes and call duration of 2.4 s; mean note duration, 0.13 s (mean = 0.13, range = 0.09–0.15, SD = 0.01, n = 10; Fig. 4); and with a dominant frequency of 0.2756 kHz.

In addition to the clear evolutionary lineage independence demonstrated in Chan et al. (2020), our results show that PM populations also exhibit marked morphological and bioacoustic differences compared to the name-bearing lineage of *Pulchran picturata* from Borneo. These multiple lines of evidence provide compelling support for the recognition of the PM + Sumatra lineage (which by implication, includes populations from southern Thailand, south of the Isthmus of Kra) as a new species, which we describe below.

TAXONOMY

Pulchrana sundabarat, new species

Western Sunda Spotted Stream Frog

(Fig. 5)

Holotype. ZRC 1.10388, adult male, collected by Leong Tzi Ming and Tan Heok Hui from Bunker Trail, Panti Forest Reserve, Johor, Malaysia, on 17 January 2003.

Paratypes. ZRC 1.10226–27 (adult males), same collection information as holotype; ZRC 1.9752–53, 1.10771 (adult males) collected by LZM from the base of Gunung Berlumut, Johor, Malaysia on 22 May 2002; ZRC 1.10538–39 (adult males) and ZRC 1.10540 (adult female), collected by Lim C. C. from Lakum Forest Reserve, Pahang, Malaysia on 18 May 2003; ZRC 1.10789, adult female, collected by Lim Boo Liat from Ulu Muda Forest Reserve, Kedah, Malaysia on 8 April 2003.

Other paratypes. KU 335661–70 (adult males), collected 2002–2003 by J. Sukumaran from Sungai Tupah, Gunung Jerai, Kedah, Malaysia (5.7489 E, 100.4428 N; 525–550 m elevation).

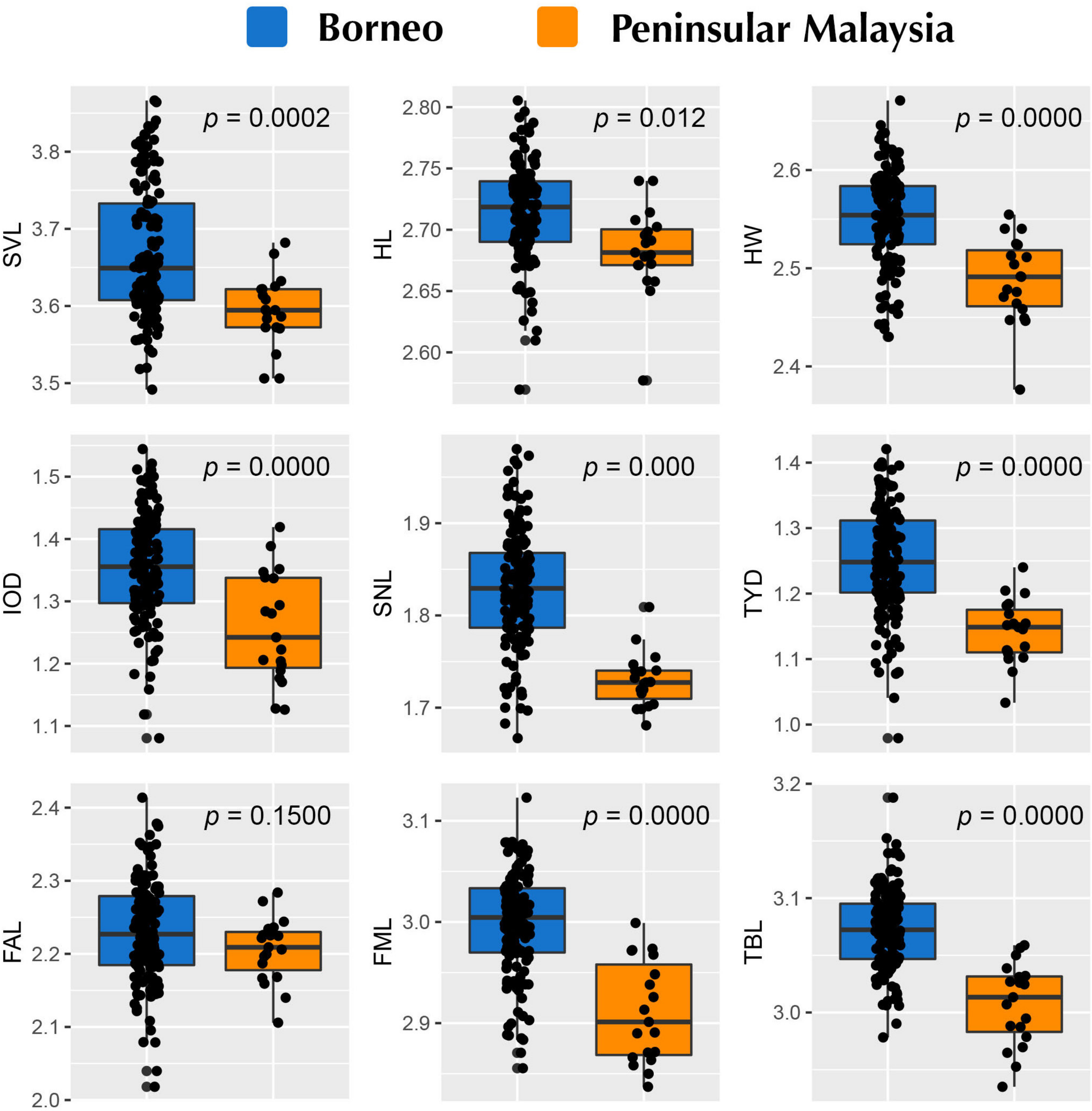

Fig. 2. Boxplots and *p*-values of the *t*-test comparing adjusted measurements of various populations across Borneo and Peninsular Malaysia.

Description of holotype. Adult male; medium-sized (SVL 37.4 mm); head longer than wide; snout pointed in dorsal profile, rounded in lateral profile, projecting beyond lower jaw; nostrils located laterally, close to tip of snout; lores slanting, slightly concave; canthus rostralis absent; tympanum circular, relatively large, smaller than eye diameter; vomerine teeth indistinct; left dentigerous process of vomer oval and protruding, right low and ridgelike; shape of tongue spatulated, tapering to a point posteriorly; choanae oval.

Forelimbs slender; length of fingers II < I = IV < III; no webbing on fingers; fingertips slightly expanded, flat, bearing horizontal supradigital notch above the ultimate-penultimate phalangeal articulation; circummarginal groove present; subarticular tubercles distinct, raised, numbering one under fingers I and II, two beneath fingers III and IV; one supernumerary tubercle present at base of each digit; inner (thenar) metacarpal tubercle large, elongated; outer metacarpal tubercle irregularly shaped, smaller than inner, in contact with smaller palmar tubercle (Fig. 5C).

Hindlimbs long, slender; toetips expanded, flat, slightly pointed, horizontal supradigital notch above the ultimate-penultimate phalangeal articulation indistinct; toe webbing formula: I 2 – 3 II 1 – 3 III 1 – 3.5 IV 3.5 – 1 V; subarticular tubercles distinct, raised, numbering one on toes I and II, two on toes III and V, three on toe IV; supernumerary tubercles present, randomly scattered across digits and webbing; inner metatarsal tubercle large, elongated; outer metatarsal tubercle small, rounded (Fig. 5D).

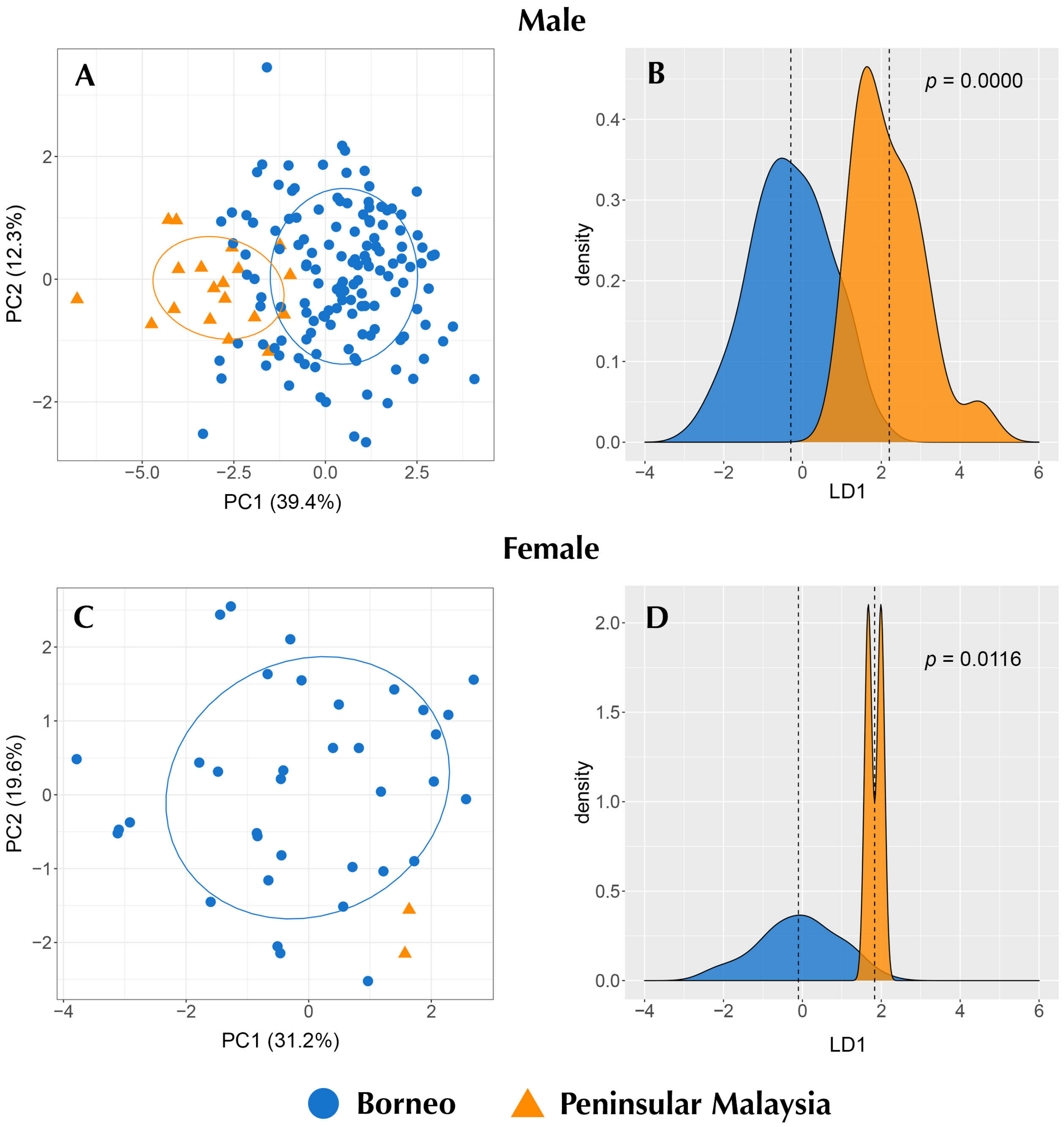

Fig. 3. PCA (A, C) and DAPC plots (B, D) of males and females. Populations from Peninsular Malaysia are represented by triangles, whereas Bornean populations are represented by circles. PC = principal component, followed by the proportion of variance in parentheses; LD = linear discriminant.

Skin on dorsum smooth, indistinctly glandular; glandules on flanks and cloacal region more prominent; supratympanic and dorsolateral folds absent; rictal tubercle present but indistinct; brachial (humeral) gland small and indistinct on the left, larger on the right; tarsal fold absent; small tubercles irregularly scattered on dorsal and ventral side of foot including web; nuptial pad distinct, consisting of two lobes that are connected on the left thumb but separated on the right, located venteromedially on the first metacarpal and dorsomedially on the proximal phalanx of the first finger; paired subgular vocal sac present; vocal sac aperture oval, located on both sides of the buccal floor.

In preservative, dorsal base colour dark brown; continuous white stripe encircles tip of snout, canthus, and outer margins of palpebrae; semi-continuous white dorsolateral stripe from back of eyes to groin; top of snout without markings; top and side of head, dorsum, flanks, and posterodorsal side of thighs scattered with round spots; spots coalesce to form irregular bars on dorsal side of front and hindlimbs; venter

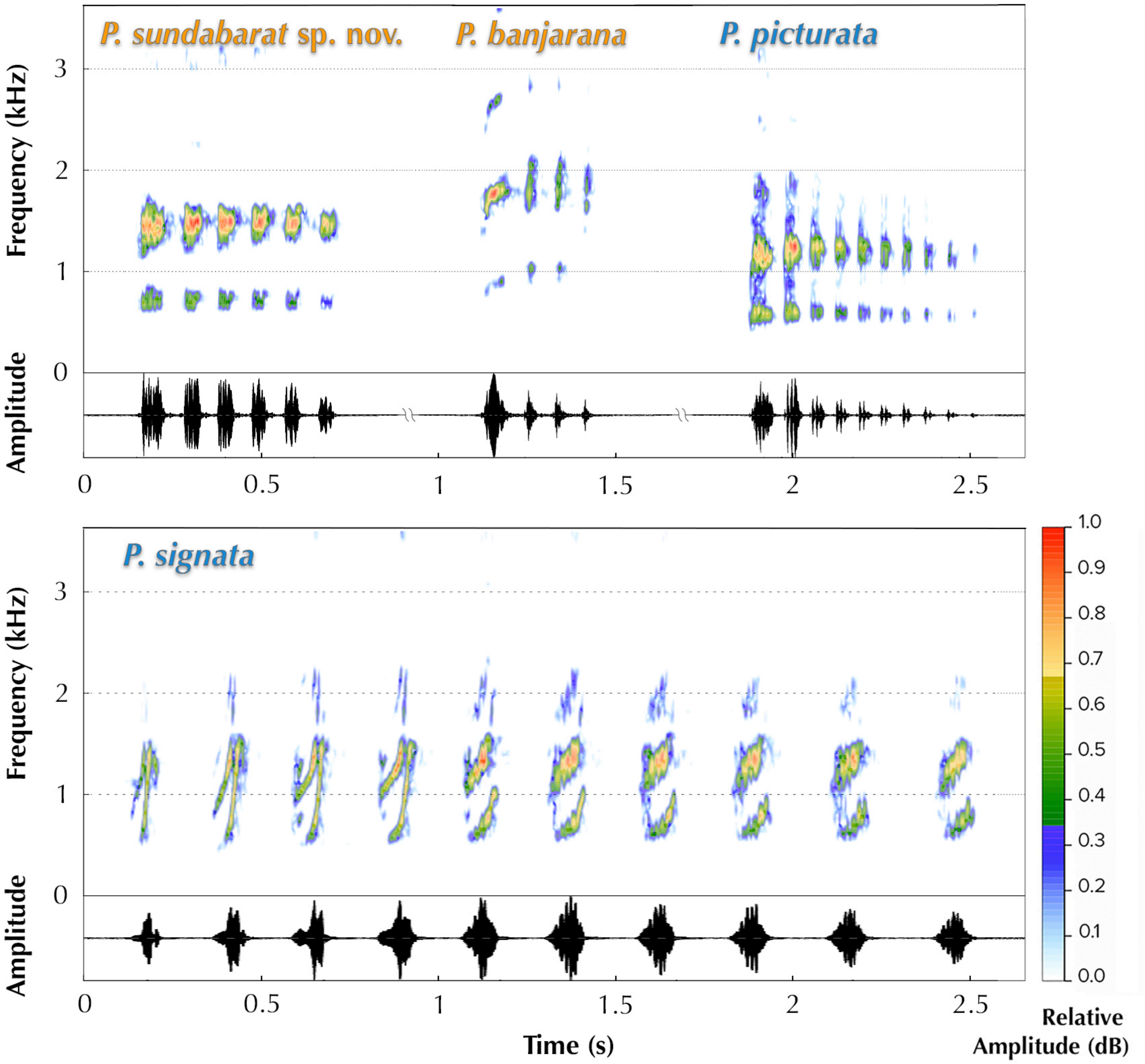

Fig. 4. Audio spectrograms and corresponding oscillograms for the sympatric species pairs *Pulchrana sundabarat*, new species, and *P. banjarana* in Peninsular Malaysia; *P. signata* and *P. picturata* in Borneo. Upper call envelopes of *P. sundabarat*, new species, and *P. picturata* graphically represented as purple lines.

cream with dense dark brown stippling that is sparser on the belly, underside of thighs, brachium, and hands; stippling most dense on underside of feet; webbing dark brown; small, sparsely spaced white spots on throat and chest; spots become more sparse and indistinct on belly; tubercles on underside of hands and feet opague; nuptial pad cream. Holotype measurements (in mm): SVL = 37.40, HL = 14.78, HW = 12.19, SNL = 5.90, IOD = 3.40, TYD = 3.05, FAL = 9.40, FML = 19.14, TBL = 20.25.

Diagnosis. *Pulchrana sundabarat*, new species, can be differentiated from all other congeners by the following combination of characters: adult males relatively small (SVL < 40 mm); dorsum smooth with low, indistinct glandules; in life, base colour of dorsal surfaces brown to black, well-defined orange to red dorsolateral stripe (continuous or partially broken), dorsum and flanks covered with orange spots, irregularly shaped orange bars on front and hindlimbs, dorsal pigmentation fades towards flanks and venter, ventral surfaces with or without spots; vomerine teeth and paired subgular vocal sac present.

Comparison. *Pulchrana sundabarat*, new species, differs from the name-bearing *P. picturata* lineage from Borneo (Ranau [including Mount Kinabalu], Tawau, and Lahad Datu districts) by having a more conspicuous and defined dorsolateral stripe (vs. poorly defined, interrupted, or dorsolateral stripes indistinct), bright orange to red dorsolateral stripe and dorsal spots (vs. brown to pale orange spots), and smaller body size in males (Figs. 2, 3). Certain genetically admixed populations of *P. picturata* from central and western Borneo may possess colour

Fig. 5. Dorsal (A), ventral (B), left hand (C), and left foot (D) of *Pulchrana sundabarat*, new species, holotype.

pattern characteristics that are similar to *P. sundabarat* which precludes their distinction using colour pattern (see Discussion). *Pulchrana sundabarat*, new species, differs from *P. signata* by having more variable, jagged, interrupted, and/or less continuous dorsolateral stripes (vs. smooth and continuous) and brighter. Compared to *P. banjarana* and *P. fantastica*, *P. sundabarat* has smooth skin on the dorsum and flanks (vs. bearing apical glandules and peripheral subglandules. *Pulchrana sundabarat*, new species, differs from *P. centropeninsularis*, *P. fantastica, and P. siberu* by having more jagged and interrupted dorsolateral stripes (vs. smooth and continuous) and denser dorsal spots (vs. sparse spots or immaculate dorsum).

Variation. The paratypes vary in the size and density of dorsal and ventral spotting, continuity of the dorsolateral stripe, and tubercles on the foot webbing. Some specimens lack vomerine teeth and a dentigerous process. When present, the state of the dentigerous process varies from being protruding and oval-shaped, to low and ridge-like. As in the holotype, the same individual may exhibit a different state on each side of the vomer. The size of the humeral gland and degree of separation between nuptial pad lobes also varies within (as in the holotype) and between individuals.

Distribution. *Pulchrana sundabarat*, new species, is known from southern Thailand (south of the Isthmus of Kra), Peninsular Malaysia, and Sumatra.

Natural History. *Pulchrana sundabarat*, new species, is associated with small to moderately sized forest streams/rivers in lowland and hill dipterocarp forests up to ~850 m a.s.l. (Berry, 1975; Chan et al., 2009, 2010, 2019; Nurulhuda et al., 2014; Sumarli et al., 2015) [reported therein as *Rana*/*Hylarana*/*Pulchrana picturata* or *signata*]. Males begin calling singly at dusk from river banks, rocks, low vegetation, or hidden among thick debris within, along, or not far from the water's edge.

Etymology. The specific epithet is constructed from the words *Sunda*, in reference to the region of Sundaland where the new species resides; and the Malay word *barat* (= west), in reference to the distribution range of the new species that is restricted to western Sundaland.

DISCUSSION

The distinction of *Pulchrana signata*. *Pulchrana picturata* exhibits high levels of colour pattern variation, particularly with regard to the condition of the dorsolateral stripe (Inger, 1966; Brown & Guttman, 2002; Leong & Lim, 2003; Haas et al., 2018). This character has been primarily used to distinguish *P. picturata* from *P. signata*, with which it has been previously confused (Inger, 1966; Brown & Guttman, 2002; Zainudin et al., 2009; Zainudin & Sazali, 2012). In a comprehensive study that included detailed morphological comparisons among species in the *P. signata* complex, Brown & Guttman (2002) demonstrated that the condition of the dorsolateral stripe in *P. signata* was continuous/unbroken in the majority of specimens examined (64.8%), while others were partially broken (35.2%; N = 145). In contrast, the majority of *P. picturata* specimens had spotted rows (77.7%), while others had broken (11.4%), or no dorsolateral stripes (10.9%; N = 184). *Pulchrana sundabarat*, new species, and *P. signata* have more continuous dorsolateral stripes compared to *P. picturata*. However, the stripe in *P. sundabarat* is generally more rugose (vs. smooth in *P. signata*). Juvenile *P. signata* may have brighter orange to reddish dorsal markings that are similar to *P. sundabarat*, new species, but the vibrancy of these colours tend to fade in adults (Inger et al., 2017; pers. obs.).

Despite some similarities in adult and even larval morphology (Haas et al., 2020), robust molecular data have shown that *P. signata* is genetically distinct from *P. picturata* and *P. sundabarat*, new species. *Pulchrana signata* is phylogenetically more closely related to a clade of Philippine *Pulchrana* including *P. moellendorffi*, *P. mangyanum*, *P. grandocula*, *P. guttmani*, and *P. similis* than it is to *P. picturata* or *P. sundabarat*, new species (Brown & Siler, 2014; Chan et al., 2014; Brown, 2015). Additionally, novel results from our bioacoustic analysis show that the male advertisement call of *P. signata* is markedly different from the call of *P. picturata* and *P. sundabarat*, new species (Fig. 4). Despite the lack of clear diagnostic morphological characters in adults and larvae, incontrovertible evidence from bioacoustic and genomic data unambiguously demonstrate that *P. signata* is distinct from *P. picturata* and *P. sundabarat*, new species. Additionally, data indicate that *P. signata* prefers heath forests and coastal peat swamps (Inger et al., 2017), as opposed to *P. picturata* and *P. sundabarat* which occur in lowland and hill dipterocarp forests (Chan et al., 2009, 2010, 2019; Ahmad et al., 2010; Sumarli et al., 2015). However, habitat preference data are preliminary (at least for *P. signata*) and hence, more studies will be required to determine whether these species exhibit distinct ecological separation. To avoid future misidentifications, we suggest that male advertisement calls and genetic data be used to diagnose *P. signata* from *P. sundabarat* and *P. picturata*, as opposed to morphological characters that are highly variable due to genetic admixture (see below).

Colour pattern variation. Although population-level dorsolateral stripe variation in *P. picturata* is evident throughout Borneo (Brown & Guttman, 2002), it does not correspond to undiagnosed or "cryptic" species, but rather may be due to high levels of genetic admixture between *P. picturata* and *P. sundabarat*, new species (Chan et al., 2020). The study by Chan et al. (2020) showed that populations from northern (Ranau District) and eastern Sabah (Tawau and Lahad Datu Districts), including the topotypic population from Mount Kinabalu are genetically pure (not admixed) and represented the true evolutionary lineage of *P. picturata*. Our examination of colour pattern variation revealed that the true *P. picturata* lineage can also be differentiated from genetically admixed populations in other parts of Borneo (Chan et al., 2020). Frogs from Ranau, Tawau, and Lahad Datu Districts lack a well-defined dorsolateral stripe and have more muted colours comprising brown or pale-orange spots. In contrast, frogs from other parts of Borneo may possess a more defined dorsolateral stripe and orange to red spots, which are characters shared by *P. sundabarat*, new species. The patterns of morphological variation in *P. picturata* and *P. sundabarat* are congruent with patterns of genetic admixture showed in Chan et al. (2020), suggesting that the more continuous dorsolateral stripe and brighter colours observed in admixed *P. picturata* populations across central and western Borneo could be morphological traits inherited from *P. sundabarat*, new species, through past hybridisation events. Chan et al. (2020) further suggested that hybridisation was likely facilitated by landbridge connections between Sumatra and western Borneo via the Karimata Strait. Although certain Bornean populations of *P. picturata* share genetic and morphological affinities with *P. sundabarat*, new species, we consider all Bornean populations to be conspecific (= *P. picturata*) under the framework of the Unified Concept of Species (de Queiroz, 2005) due to their ability to exchange genes with one another and their evolutionary distinctness compared to *P. sundabarat* (Chan et al., 2020).

Distribution, natural history, and evolutionary relationships of *Pulchrana sundabarat*, new species. Using ~12,000 genomic markers (~5.4 million basepairs), Chan et al. (2020) demonstrated the conspecificity between populations of *Pulchrana sundabarat*, new species, from Peninsular Malaysia and Sumatra. Although specimens from southern Thailand were not examined, Thai populations are presumed to be conspecific with *P. sundabarat* based on their geographic distribution, which is continuous with PM populations genotyped by Chan et al. (2020). *Pulchrana sundabarat*, new species, is usually associated with lowland forests (Chan et al., 2009, 2010, 2019; Nurulhuda et al., 2014; Sumarli et al., 2015), but may occur in hill forests up to 850 m a.s.l. At higher elevations, *P. sundabarat* can be sympatric with another morphologically similar species, *P. banjarana*. However, *P. banjarana* is only found at higher elevations (> 800 m) and in smaller and slower-moving streams or seepages (Leong & Lim, 2003; Grismer et al., 2010; Ahmad et al., 2011), whereas *P. sundabarat* occurs along larger and swifter streams or rivers (Grismer et al., 2011; Chan et al., 2019). Additionally, *P. banjarana* can be morphologically differentiated from *P. sundabarat* by its unique granular texture of the dorsum and flanks, which bear apical granules and peripheral sub-glandules (Leong & Lim, 2003). Our bioacoustic analysis also showed that the advertisement calls of *P. banjarana* and *P. sundabarat*, new species, are quite different (Fig. 4). Although the temperature was not documented, these calls were recorded from the same locality and time, hence, we do not expect call attributes to be skewed by negligible differences in ambient temperature.

In the lowland forest of central Peninsular Malaysia, *P. sundabarat*, new species, co-occurs with *P. centropeninsularis* (Chan et al., 2014; Nurulhuda et al., 2014), and in Sumatra, *P. sundabarat* could potentially be sympatric with *P. siberu* (Chan et al., 2014) and *P. fantastica* (Arifin et al., 2018. However, while *P. sundabarat* is associated with moderately large and swifter flowing lowland forest streams (Grismer et al., 2011; Chan et al., 2019), *P. centropeninsularis* and *P. siberu* occur in lowland swampy areas, whereas *P. fantastica* occurs at higher elevations (450–1,065 m a.s.l.) along small streams (Arifin et al., 2018). Although *P. sundabarat*, new species, is sympatric with *P. banjarana* and *P. centropeninsularis* (and potentially *P. siberu* and *P. fantastica*), all co-occurring species pairs exhibit clear niche segregation: *P. sundabarat* occurs along larger and swifter streams; *P. banjarana* and *P. fantastica* prefer small streams with trickling seepages at higher elevations; while *P. centropeninsularis* and *P. siberu* inhabit swampy areas. Interestingly, all sympatric species pairs are not each other's closest relatives and belong to separate clades (Brown & Siler, 2014; Chan et al., 2014). A congruent pattern of niche segregation between phenotypically similar sympatric species pairs is observed in Borneo and the Philippine island of Mindanao. In Borneo, *P. signata* occurs in coastal forests and peat swamps, compared to *P. picturata* that inhabits more inland dipterocarp forests. In the Philippines, *P. grandocula* is a lowland species, whereas *P. guttmani* is associated with high-elevation swampy habitats above 1,550 m a.s.l. (Brown, 2015). This suggests that the topographical and environmental variability that exists on large Sundaic landmasses are key for the accumulation of phenotypically similar and sympatric species pairs, which adapt to distinct habitats.

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SUPPLEMENTARY MATERIAL

Table S1. List of specimens examined and their associated metadata and raw measurements. Data available at https://doi.org/10.6084/m9.figshare.13371269

Bioacoustic recordings are available at https:/doi.org/10.6084/m9.figshare.13371257