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Taxonomic Relationships of *Ansonia anotis* Inger, Tan, and Yambun, 2001 and *Pedostibes maculatus* (Mocquard, 1890), with a Description of a New Genus (Amphibia, Bufonidae)

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Examination of types and recently collected specimens revealed that *Ansonia anotis* Inger, Tan, and Yambun, 2001 and *Pedostibes maculatus* (Mocquard, 1890), both described from Kinabalu, Sabah, Malaysia, are hardly differentiated morphologically. Analyses of a total of 2,427 bp of the 12S rRNA, tRNA^{val}, and 16S mitochondrial rRNA genes revealed that the two species are very close genetically. Thus *A. anotis* is regarded as conspecific and is synonymized with *P. maculatus*. Genetically, this species proved to form a lineage distinct from other bufonids from Southeast Asia, including species of *Ansonia* and *Pedostibes*. Because the species has also some unique morphological traits different from known bufonid genera, we propose to establish a new genus for *Nectophryne maculata* Mocquard, 1890.

Key words: Malaysia, molecular phylogeny, new genus, Sabah, synonymy

INTRODUCTION

Southeast Asia is one of the centers of amphibian diversification (Inger, 1999), and this holds for the toad family Bufonidae. On the island of Borneo, as many as six bufonid genera (*Bufo* Laurenti, 1768, *Ansonia* Stoliczka, 1870, *Leptophryne* Fitzinger, 1843, *Pedostibes* Günther, 1876, *Pelophryne* Barbour, 1938, and *Pseudobufo* Tschudi, 1838) have been recorded (Inger, 1966; Inger and Tan, 1996; Malkmus *et al.*, 2002), and the number is even greater if we admit Frost *et al.*'s (2006) proposal to split *Bufo* into several distinct genera. This might promote uncovering the paraphyletic nature of the genus *Bufo* (e.g., Graybeal and Cannatella, 1995), but applying unfamiliar names for many taxa, without substantially providing their diagnostic characters, could result in further taxonomic confusion.

Some other Bornean genera are taxonomically more conservative than *Bufo* and occupy specific ecological niches (Inger, 1958). Of these, *Ansonia* is characterized by small to medium adult body size and a unique larva with a large oral sucker adapted for life in torrents. Both *Pelophryne* and *Pedostibes* are adapted for arboreal life,

but *Pelophryne* is small in body size, and lays small numbers of large yolky eggs, while *Pedostibes* is moderate to large sized, and its eggs are numerous and small. Recent molecular studies indicate close relationships of *Pedostibes* with *Bufo asper* Gravenhorst, 1829 and *B. juxtasper* Inger, 1960 (Frost *et al.*, 2006; our own observations, see Results).

Inger *et al.* (2001) described *Ansonia anotis* Inger, Tan, and Yambun, 2001 from Sayap, in the Kinabalu National Park of Sabah, Malaysian part of northern Borneo (Fig. 1). The species is characterized mainly by lack of a tympanum, large spatulate finger disks, and the unique morphology of the putative larva. However, because only several specimens have been obtained since its description, details of the species are unclear. Recently, on the joint expedition of UMS (University Malaysia Sabah) and JICA (Japan International Cooperation Agency) to the Crocker Range National Park (Fig. 1), Sabah, in 2002, four toads were collected. These specimens were very similar to *Pedostibes maculatus* (Mocquard, 1890), originally described as *Nectophryne*, and were identified as that species (Kueh *et al.*, 2004). Like *A. anotis*, *P. maculatus* is characterized by lack of the tympanum and large finger disks (Boulenger, 1918; Inger, 1966), and these species could not be clearly distinguished from one another by any morphological traits described in the literature.

We compared the two species morphologically using available specimens, including three syntypes of *N. maculata*,

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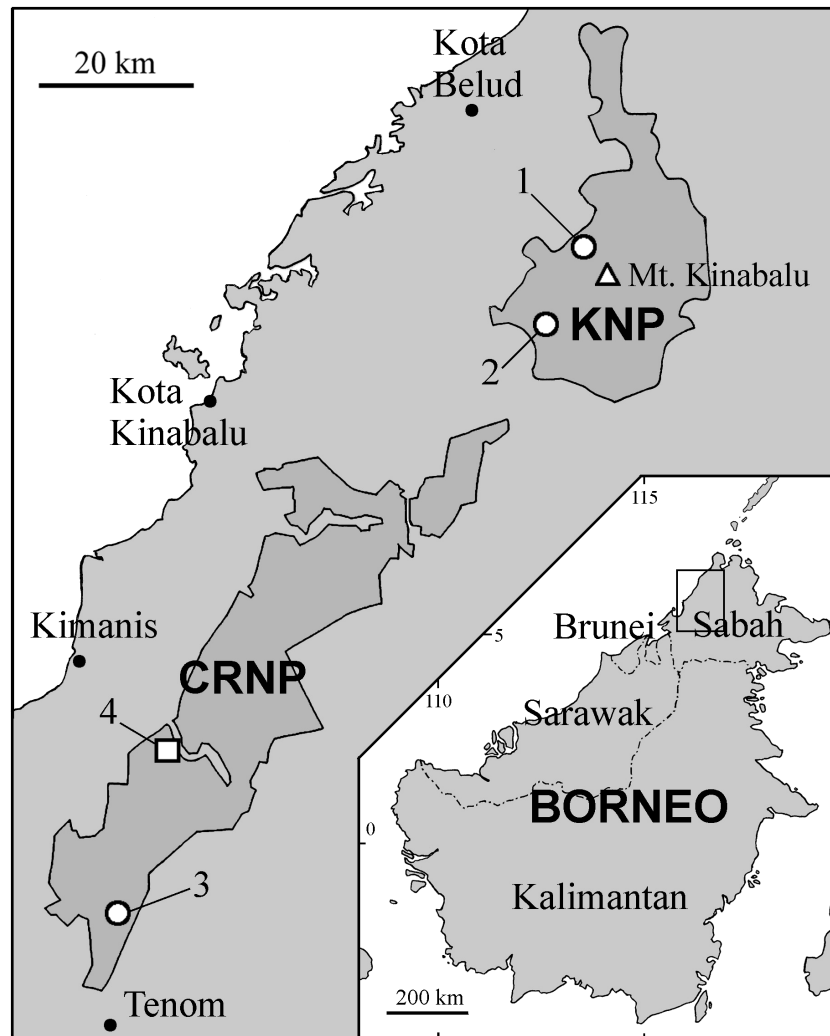


Fig. 1. Map of Borneo showing the localities where *Ansonia anotis* (open circle) and *Pedostibes maculatus* (open square) were recorded. 1, Sayap; 2, Kiau; 3, Sungei Purulon; 4, Trail 5 of Ulu Kimanis. Hatched areas show the Kinabalu and the Crocker Range National Parks (KNP and CRNP, respectively).

stored in the collection of Museum National d'Histoire Naturelle, Paris (MNHN), to determine their taxonomic relationships. Further, in order to clarify their phylogenetic relationships among Southeast Asian bufonids, we analyzed sequences of the 12S and 16S mitochondrial rRNA genes of the two species, as well as of representatives of related bufonids for comparisons.

MATERIALS AND METHODS

For morphological comparisons, we studied three specimens of *Ansonia anotis* (Sabah Parks, Kota Kinabalu [SP] 01762 (holotype) from Sungei (River) Wario, Sayap, Mt. Kinabalu, Sabah; SP 26033 and 26043 from Riou Hutu Simpan, Kiau, Mt. Kinabalu, Sabah), three syntypes of *N. maculata* from Kinabalu (MNHN 1899-266–268; syntypes), and two of four specimens of *P. maculatus* (Graduate School of Human and Environmental Studies, Kyoto University [KUHE] 38505 and 38506 from Ulu Kimanis of the Crocker National Park [Trail 5 of UMS 2002 expedition to the Crocker Range], Sabah).

Six body measurements were taken, following Matsui (1984): 1) snout-vent length (SVL), 2) head width (HW), 3) lower arm length (LAL), 4) thigh length (THIGH), 5) tibia length (TL), and 6) foot

length (FL). All measurements were made to the nearest 0.1 mm with dial calipers. Dimensions were converted to a percentage ratio (R) in relation to SVL for comparisons, although small sample sizes examined prohibited statistical comparisons. We made slight dissections to examine tympanic structure and gonads, and prepared radiographs to examine gross osteology.

For genetic comparisons, we studied one specimen each of *A. anotis* and *P. maculatus* from among the specimens used for morphological comparisons. For comparisons, we used the following species (Table 1): *A. malayana* Inger, 1960; *A. hanitschi* Inger, 1960; *A. longidigita* Inger, 1960; *A. fuliginea* (Mocquard, 1890); *Pedostibes hosii* (Boulenger, 1892); *P. rugosus* Inger, 1958; *Pelophryne misera* (Mocquard, 1890); *P. brevipes* (Peters, 1867); *Leptophryne borbonica* (Tschudi, 1838); *Bufo juxtasper* (= *Phrynooidis juxtaspera* in Frost *et al.*, 2006); *B. melanostictus* Schneider, 1799 (= *Duttaphrynus melanostictus* in Frost *et al.*, 2006); *B. divergens* Peters, 1871 (= *Ingerophrynus divergens* in Frost *et al.*, 2006); *Didynamipus sjostedti* Andersson, 1903 from GenBank (AY325991); and *Atelopus flavescens* Duméril and Bibron, 1841 from GenBank (DQ283259). We chose *Dendrobates auratus* (Girard, 1855) from GenBank (AY326030), a member of the supposed bufonid sister family, Dendrobatidae Cope, 1865 (Frost *et al.*, 2006), as outgroup (Table 1).

Table 1. Samples used for DNA analyses and GenBank accession numbers. *BORNEENSIS=Institute for Tropical Biology and Conservation, University Malaysia Sabah; KUHE=Graduate School of Human and Environmental Studies, Kyoto University; SP=Sabah Parks.

Species	Voucher*	Locality	Accession number
<i>Ansonia anotis</i>	SP 26033	Kiau, Mt. Kinabalu, Sabah	AB331708
<i>Ansonia fuliginea</i>	KUHE 17537	Mt. Kinabalu, Sabah	AB331709
<i>Ansonia hanitschi</i>	BORNEENSIS 22640	Silau Silau, Mt. Kinabalu, Sabah	AB331710
<i>Ansonia longidigita</i>	BORNEENSIS 12463	Mahua, Crocker Range, Sabah	AB331711
<i>Ansonia malayana</i>	KUHE 15472	Larut, Peninsular Malaysia	AB331712
<i>Bufo juxtasper</i>	KUHE 12363	Bareo, Sarawak	AB331713
<i>Bufo melanostictus</i>	KUHE 10524	Marudi, Sarawak	AB331714
<i>Bufo divergens</i>	BORNEENSIS 09169	Tawau Hills NP, Sabah	AB331715
<i>Leptophryne borbonica</i>	BORNEENSIS 08127	Ulu Kimanis, Crocker Range, Sabah	AB331716
<i>Pedostibes hosii</i>	BORNEENSIS 22088	Tawau Hills NP, Sabah	AB331717
<i>Pedostibes maculatus</i>	BORNEENSIS 08425	Ulu Kimanis, Crocker Range, Sabah	AB331718
<i>Pedostibes rugosus</i>	SP 21556	Mahua, Crocker Range, Sabah	AB331719
<i>Pelophryne brevipes</i>	KUHE 35585	Peninsular Malaysia	AB331720
<i>Pelophryne misera</i>	KUHE 37191	Mt. Kinabalu, Sabah	AB331721
<i>Didynamipus sjostedti</i>	–	Cameroon	AY325991
<i>Atelopus flavescens</i>	–	French Guiana	DQ283259
<i>Dendrobates auratus</i>	–	–	AY326030

We extracted DNA from small amounts of frozen or ethanol-preserved tissues using standard phenol-chloroform extraction procedures (Hillis *et al.*, 1996). We conducted amplifications by the polymerase chain reaction (PCR) with the primers Thr Lrn (AAAR-CATKGGTCTTGTAARCC) modified from Shaffer and Mcknight (1996) and Hedges 16H1 from Hedges and Maxson (1993) to obtain 2.4 kb of the 12S and 16S rRNA genes and the intervening tRNA gene for valine. We sequenced the amplified fragments in an automated DNA sequencer (ABI PRISM 3100) using the same primers and eight additional primers (sequences of primers available on request from the senior author), and following the manufacturer's instructions. Sequences newly obtained will be deposited in GenBank (accession numbers AB331708–331721).

We obtained and eye-checked sequence data for each sample using ABI PRISM Sequencing Analysis Software (V3.6.2), and performed alignments of data from all samples by clustal option of the BioEdit software (Hall, 1999). After testing consistency among the genes by the use of incongruence length difference (ILD) tests with 1,000 randomized partitions (Farris *et al.*, 1994) and confirming no significant heterogeneity, we combined the 12S, tRNA^{val}, and 16S sequences into a single data set of 2,427 bp. We constructed neighbor-joining (NJ) phylogenies using Kimura two-parameter (K2p) distances (Kimura, 1980) and maximum-parsimony (MP) phylogenies using heuristic searches with TBR branch swapping implemented in PAUP*4.0b10 (Swofford, 2002). We tested robustness of the NJ and MP tree topologies by bootstrap analyses (Felsenstein, 1985),

with 1,000 replicates (Hedges, 1992). We regarded tree topologies with bootstrap values (BS) $\geq 70\%$ as sufficiently resolved (Huelsenbeck and Hillis, 1993).

We also performed Bayesian analyses using the Markov chain Monte Carlo technique (MCMC) implemented in MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001). Hierarchical likelihood ratio test (hLRTs) using the program MODELTEST 3.06 (Posada and Crandall, 1998) selected the GTR+I+G model as the DNA substitution model that best fit our data. We initiated four independent analyses with a random starting tree that ran for 2.0 million generations. Because we found the log likelihood scores to stabilize after 50,000 generations, we conservatively discarded the first 1.0 million generations from each run as "burn-in". We sampled one of every 100 generations from the remaining 1.0 million generations (10,001 trees across all four independent analyses) to calculate posterior probabilities (BPP) for each branch in the Bayesian tree, and considered BPP $\geq 95\%$ as significant support (Larget and Simon, 1999; Huelsenbeck *et al.*, 2001).

RESULTS

Morphology

Specimens of *A. anotis* agreed well with the diagnosis given in the original description of Inger *et al.* (2001): "lacking a tympanum; snout projecting beyond lower jaw; first finger not reaching swollen tip of second; tips of outer fingers

Table 2. Morphometric comparisons of specimens examined (SVL in mm and ratios [R] in % of other characters to SVL). See text for museum and character abbreviations.

Species	Sp. Number	Sex	SVL	RHW	RLAL	RTHIGH	RTL	RFL
<i>Ansonia anotis</i>	SP 01762 (holotype)	F	52.6	29.3	59.5	51.9	54.6	40.9
<i>Ansonia anotis</i>	SP 26033	M	35.5	29.0	60.3	53.8	60.6	43.7
<i>Ansonia anotis</i>	SP 26043	M	39.0	29.0	61.8	51.8	59.2	47.2
<i>Nectophryne maculata</i>	NMHNP 1899-266 (syntype)	FY?	45.4	28.6	60.1	52.4	56.6	44.1
<i>Nectophryne maculata</i>	NMHNP 1899-267 (syntype)	F	51.5	28.5	60.8	50.9	54.8	43.7
<i>Nectophryne maculata</i>	NMHNP 1899-268 (syntype)	?	37.5	28.0	59.2	53.3	60.0	43.7
<i>Pedostibes maculatus</i>	BORNEENSIS 08425	?	39.7	28.2	–	–	60.5	–
<i>Pedostibes maculatus</i>	BORNEENSIS 08426	?	31.9	30.1	–	–	62.4	–
<i>Pedostibes maculatus</i>	KUHE 38505	MY?	30.4	27.6	60.5	56.6	62.2	45.7
<i>Pedostibes maculatus</i>	KUHE 38506	F	48.6	27.4	59.5	53.5	57.4	45.3

expanded into distinct, spatulate discs; web extending beyond distal subarticular tubercles of third and fifth toes".

Similarly, by examining the types of *N. maculata*, we confirmed Mocquard's (1890) original description except for color, which has faded seriously: "body slender, limbs very long; head short, snout truncate, length equal to eye; canthus rostralis angulated, vertical; nostril near tip of snout;

interorbital as wide as upper eyelid; tympanum not distinct; limbs very thin, toes two-thirds webbed; fingers webbed only at base, flattened, tips wide and truncate, much stronger than toes; subarticular tubercles slightly visible, metatarsal tubercles very distinct, outer more protruded; tibio-tarsal articulation passes tip of snout; body covered by rough granulations, both dorsally and ventrally".

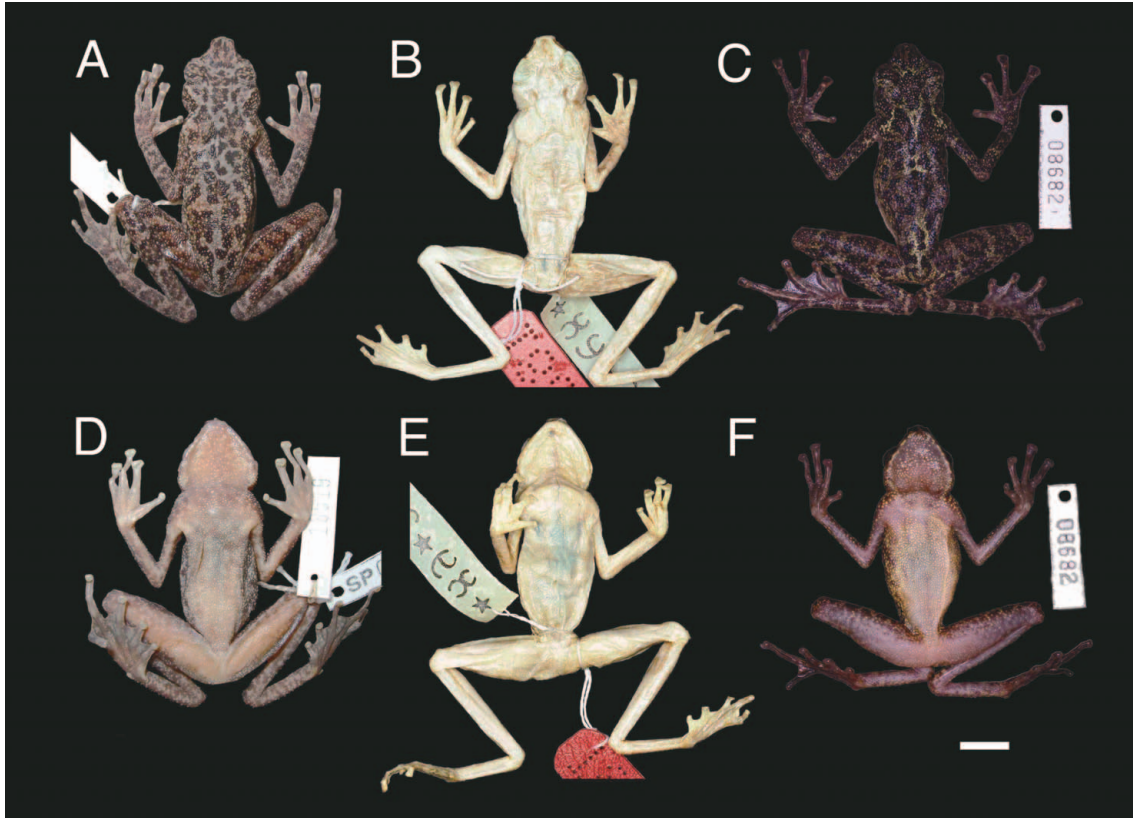


Fig. 2. Dorsal (A–C) and ventral (D–F) views of (A, D) *Ansonia anotis* (holotype, SP 01762), (B, E) *Nectophryne maculata* (syntype, MNHNP 1899-267), and (C, F) *Pedostibes maculatus* (KUHE 38506 [formerly BORNEENSIS 08682]). Scale bar=10 mm.

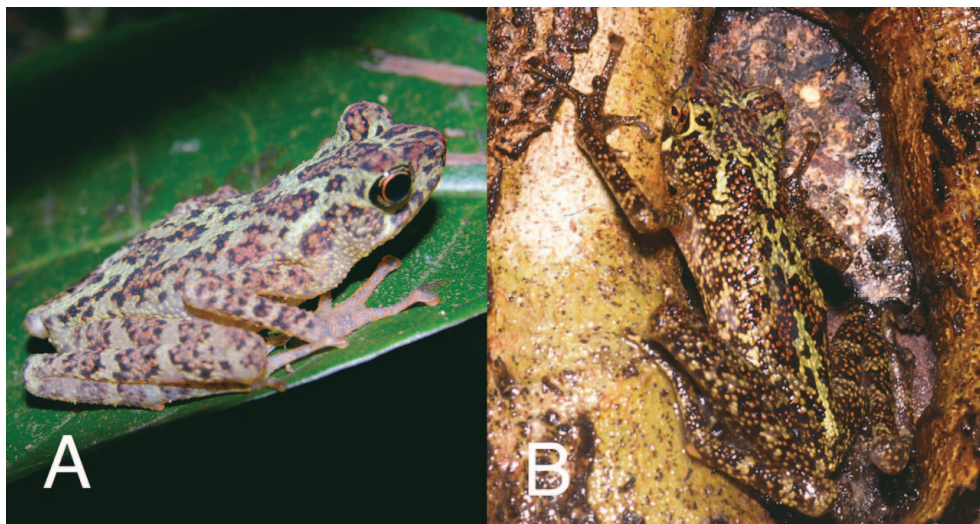


Fig. 3. Views of living (A) *Ansonia anotis* (SP 26033 from Riou Hutan Simpan, Kiau, Mt. Kinabalu) and (B) *Pedostibes maculatus* (KUHE 38505 [formerly BORNEENSIS 08681], from Trail 5 of Ulu Kimanis, Crocker Range).

The holotype of *A. anotis* is a female with SVL 52.6 mm, which is slightly larger than that given in the original description (52.1 mm: Inger *et al.*, 2001). The other two specimens considered as males are 39.0 and 35.5 mm in SVL (Table 2). Of three syntypes of *N. maculata*, two females are 51.5 and 45.4 mm in SVL, and an individual of unknown sex is 37.5 mm in SVL. Of the *P. maculatus* collected from the Crocker, the largest one is the female 48.6 mm in SVL; the male is 30.4 mm in SVL. The remaining two specimens of unknown sex are 31.9 and 39.7 mm in SVL. Thus, *A. anotis*, *N. maculata*, and *P. maculatus* do not differ from each other in SVL in either sex, although the maturity of males is not clear. These species also had similar relative size in head (median of RHW=29.0, 28.5, and 27.9%, respectively), and hindlimb characters (medians: RTHIGH=51.9, 52.4, and 55.1%; RTL=59.2, 56.6, and 61.4%; RFL=43.7, 43.7, and 45.5%, respectively; Table 2).

As described by Inger *et al.* (2001), the holotype of *A. anotis* in preservative is slight gray to brown on dorsal and lateral surfaces, scattered with many small, roundish dark spots, and pale brown on ventral surfaces. Limbs are barred with dark bands (Fig. 2). In the two males, the ground color of the dorsum was light green in life (Fig. 3). Mocquard's (1890) original description for *N. maculata*, "Dorsum grey brown scattered with small irregular black spots, isolated or confluent; transverse bands on limbs; ventral surface a little lighter grey brown", was barely evident because the three specimens are all faded (Fig. 2). Color of *P. maculatus* both in preservative and in life (Fig. 2, 3) was almost identical to that of *A. anotis*.

In addition, *A. anotis* was nearly identical with *N. maculata* and *P. maculatus* in morphological characteristics other than

the morphometric and color traits. The following characteristics were found in common with them: lacking bony crests on head, parotoid gland, tympanum, columella, and eustachian tube; snout projecting beyond lower jaw; hand very large, first finger much shorter than second; tips of outer fingers expanded into distinct, spatulate discs; web extending beyond distal subarticular tubercles of third and fifth toes. Males lack vocal sac opening, nuptial pad, and mandibular spines.

DNA sequences

We obtained sequences of 888–908 bp for 12S, 69–73 bp for tRNA^{val}, and 1,372–1,403 bp for 16S, and the aligned 12S (926 bp), tRNA^{val} (73 bp), and 16S (1,428 bp) data set yielded 2,427 nucleotide positions. Of these sites, 1,077 were variable and 722 were informative for parsimony analyses. *Ansonia anotis* and *P. maculatus* differed only slightly genetically. The sequence divergences between them (K2p=0.015 in 12S, 0.014 in tRNA^{val}, and 0.032 in 16S) were much smaller than divergences between *A. anotis* and four other *Ansonia* species (minimum K2p=0.111, 0.075, and 0.156, respectively) or between *P. maculatus* and other two *Pedostibes* species (minimum K2p=0.098, 0.143, and 0.145, respectively).

We obtained six most parsimonious trees with 311 evolutionary steps, with a consistency index of 0.521 and a retention index of 0.335. All phylogenetic analyses resulted in the similar topologies (only the Bayesian tree from the combined data set is shown in Fig. 4). The following relationships were indicated, with high support, by all three methods employed: (1) monophyly of bufonid species other than *Atelopus* with respect to *Dendrobates* (BS=97% in MP,

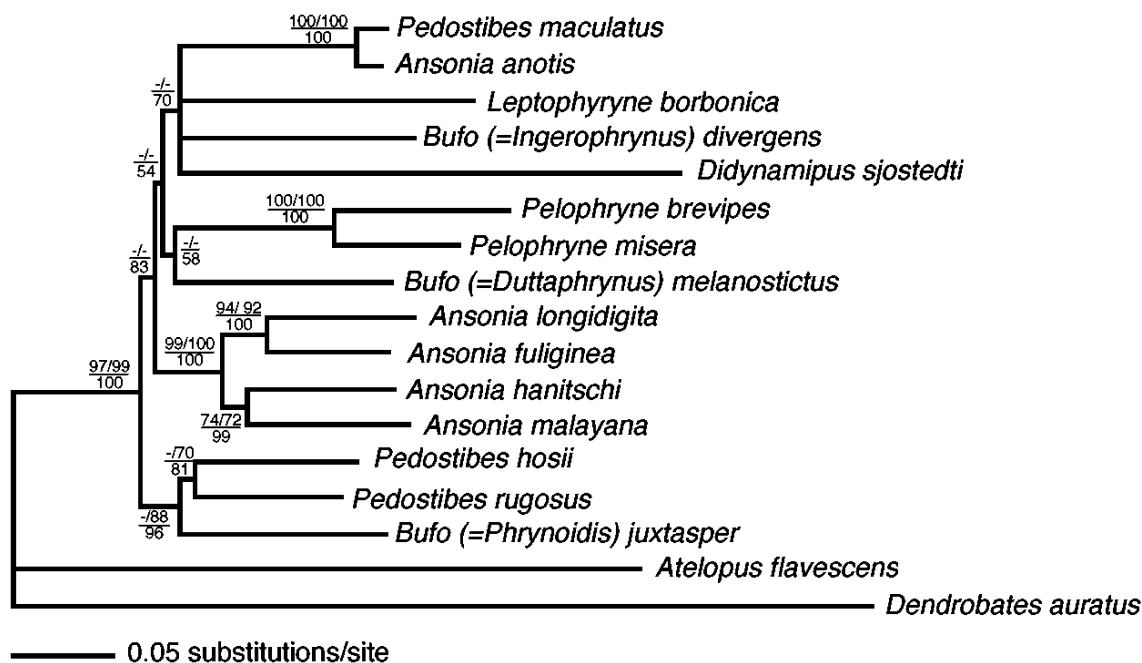


Fig. 4. Bayesian tree based on 2,427 bp of the combined 12S rRNA, tRNA^{val}, and 16S rRNA genes for species of *Ansonia*, *Pedostibes*, *Pelophryne*, *Leptophryne*, *Bufo*, *Didynamipus*, and *Atelopus*, with *Dendrobates* as an outgroup. Numbers above branches represent bootstrap support for MP (1,000 replicates)/NJ (1,000) inference, and numbers below branches indicate posterior probabilities for Bayesian inference.

BS=99% in NJ, BPP=100%); (2) monophyly of four species of *Ansonia* other than *A. anotis* (99%, 100%, 100%, respectively); (3) sister-group relationship of two species of *Pelophryne* (100% in all); (4) sister-group relationship of *A. anotis* and *P. maculatus* (BS=100% in all). In addition, monophyly of two species of *Pedostibes* other than *P. maculatus* was supported in NJ (BS=70%), and monophyly of these two species with *Bufo juxtasper* was supported in the NJ and Bayesian trees (BS=88% and BPP=96%).

DISCUSSION

Comparisons of *Ansonia anotis* and *Pedostibes maculatus*

All the characters of *P. maculatus* originally listed by Mocquard (1890, as *Nectophryne*: see above) and expanded by Inger (1966) apply to *A. anotis* (see above diagnosis), except for one point. According to Inger (1966), a male stored in Zoologisches Museum zu Berlin (now Museum für Naturkunde der Humboldt-Universität zu Berlin, ZMB 29466) has a blackish nuptial pad covering the dorsal surface of the first finger and the medial edge of the entire metacarpal. Because we could not definitely determine the maturity of available males that had no secondary sex characters, we admit the possibility that fully mature *A. anotis* may have a nuptial pad.

Ecologically, the two species also resemble one another; Inger *et al.* (2001) noted that the holotype and a male paratype were found on a log beside stream, and on a rock at the bank of a stream, respectively. However, a juvenile paratype was obtained from 2 m above ground in a small hole in the trunk of a large tree. Similarly, all four specimens of *P. maculatus* from the Crocker range were found 1–2 m above ground on the trunk of a huge tree, 10 m from the edge of a small stream (width=3 m). Two individuals were found just above a small hole in the trunk (Fig. 3).

As shown above, the results of the present study clearly indicate that *A. anotis* and *P. maculatus* are morphologically, genetically, and ecologically hardly distinguishable, and that the former should be synonymized with the latter, pending the question of the male nuptial pad. Slight genetic differentiation found between the Kinabalu and Crocker samples indicates the presence of some degree of isolation between the localities. A similar, but larger, degree of genetic differentiation between these two regions has been detected in a stream-living ranid, *Meristogenys kinabaluensis* (Inger, 1966) (Shimada, unpublished data).

Taxonomic history of *Pedostibes maculatus*

Mocquard (1890) without any reason placed *maculata* in *Nectophryne* Buchholz and Peters in Peters, 1875, but Barbour (1938) later moved the species to *Pelophryne* with some consideration. Barbour thought that several bufonid species from Asia, then included in *Nectophryne*, are closely related and differ from African members. He divided the Asian species into two genera: *Pedostibes* Günther, 1876 (type species *P. tuberculosus* Günther, 1876 from Malabar, India) and his new genus *Pelophryne* Barbour, 1938. The diagnostic characteristics Barbour (1938) listed for *Pelophryne* include usually seven, and perhaps occasionally six, presacral vertebrae, fusion of the coccyx (=urostyle) to the sacrum, and presence of coccygeal expansions. Barbour (1938) placed *N. maculata* in *Pelophryne*, although

he noted that because of the hidden tympanum, this species might turn out not to be congeneric with any species of *Pelophryne*. Barbour (1938) probably believed *N. maculata* to have the coccyx expanded dorsally and fused to the sacrum, and seven presacral vertebrae, like other members of *Pelophryne*.

Inger (1966) examined the syntypes of *N. maculata* and clarified Barbour's (1938) mistakes. He ascertained that the species has a coccyx that is not equally expanded dorsally nor fused to the sacrum, and eight presacral vertebrae. These traits were confirmed by our examination of radiographs. Inger (1966) also noted that two of the three syntypes (MHNP 89.266–267) have numerous small ova (more than 50 per ovary, also confirmed in the present study), unlike members of *Pelophryne*, with a small number of large eggs, but like *Pedostibes*. Inger (1966) thus moved *Pelophryne maculata* to *Pedostibes*, even though the species lacks a parotoid gland, unlike its congeners. All subsequent authors (*e.g.*, Inger and Tan, 1996; Malkmus *et al.*, 2002; Matsui, 2006) have followed this classification.

Notwithstanding close morphological similarities with *P. maculatus* in metamorphs, Inger *et al.* (2001) described *A. anotis* as a new species of *Ansonia*, whose tadpoles are famous for their unique conformation of the sucker-type oral disc (Inger, 1960, 1966). Inger (1992) reported from Sabah a unique bufonid tadpole, which he assigned to the genus *Ansonia* and called the *Ansonia* “sucker”. It has a large oral disc only slightly narrower than the widest part of the body, like other *Ansonia* larvae (Inger, 1966), but at the same time lacks an upper jaw sheath, unlike congeneric species. What is more unique is that it characteristically has a sharply defined abdominal sucker immediately behind the oral disc, similar to that found in the torrent-dwelling ranids *Meristogenys* Yang, 1991 and *Huia* Yang, 1991 (not sensu Frost *et al.*, 2006), but which has never been seen in Bornean bufonids.

Because Inger *et al.* (2001) found no species of *Ansonia* other than the juvenile paratype of *A. anotis*, and no form of larval *Ansonia* other than the *Ansonia* “sucker” (1992) at Purlon, one of the known habitats of *A. anotis* in the Crocker Range, they associated these (metamorph and larva) as conspecific. In most *Ansonia* species, the tympanum is exposed, but it is hidden under the skin in *A. mcgregori* (Taylor, 1922) and *A. muelleri* (Boulenger, 1887) from the Philippines. Inger *et al.* (2001) considered the complete absence of tympanum in *A. anotis* to be a more extreme condition, within a generic morphocline, than in the two Philippine species. Also, the larval upper jaw sheath in *Ansonia* varies from large to very small, as if leading to the absence in *A. anotis*. Furthermore, postdental portion of the suctorial lip also varies from small to large, and the abdominal sucker found in *A. anotis* can be viewed as an extension of such a morphocline. Thus, Inger *et al.* (2001) concluded that both the metamorph and the larva represent extremes of morphological specialization among *Ansonia* species, although they admitted the possibility that *A. anotis* warrants distinct generic status.

Designation of lectotype of *Nectophryne maculata* and new generic allocation

To prevent future taxonomic confusion, we select

NMHN 1899-267 as the lectotype of *Nectophryne maculata* Mocquard, 1890. This specimen is the largest of the syntypes and is considered to be that figured in the original description. The remaining two specimens, NMHN 1899-266 and 268, thus automatically become paralectotypes of this name. The lectotype is shown in Fig. 2B, E, and measurements are shown in Table 2.

The results of our genetic analyses strongly indicate that the species forms a distinct lineage among Southeast Asian bufonids, and, because the species is also morphologically unique, it cannot be allocated to any currently known genus. Therefore, we here propose to establish a new monotypic genus for *N. maculata*, with *A. anotis* as a synonym.

Sabaphrynus new genus

Diagnosis

A bufonid genus lacking tympanum annulus, columella, and eustachian tube; head without bony crests; no parotoid gland; tips of outer fingers expanded into distinct, spatulate discs; distal phalanges T-shaped; subarticular tubercles normal, though weak; toe webbing moderately developed; male without vocal sac opening or mandibular spines; ova numerous, small and unpigmented; coccyx neither expanded dorsally nor fused to the sacrum, movable; eight presacral vertebrae; quadratojugal complete: pectoral girdle arciferal. If correctly identified, the tadpole is also unique in the possession of expanded mouthparts and abdominal sucker, and absence of upper jaw sheath.

Comparisons

Among the above diagnostic characters, absence of a tympanum is the most easily confirmed visually. An invisible tympanum is not rare among various bufonid lineages; in many cases, the tympanum is actually present but hidden under the skin (e.g., in some *Bufo* [sensu lato], some *Ansonia*, *Nannophryne* Günther, 1870, and probably *Bufoides* Pillai and Yazdani, 1973). Actual absence of a tympanum and columella is more limited to some lineages mostly from Africa (*Altiphrynoidea* Dubois, 1987; *Capensibufo* Grandison, 1980; *Didynamipus* Andersson, 1903; *Laurentophryne* Tihen, 1960; *Mertensophryne* Tihen, 1960; *Nectophryne*; part of *Nectophrynoidea* Noble, 1926; *Nimbaphrynoidea* Dubois, 1987; *Werneria* Poche, 1903; and *Wolterstorffina* Mertens, 1939) (Grandison, 1978, 1981) and Middle to South America (some *Atelopus*; *Crepidophryne* Cope, 1889; *Dendrophryniscus* Jiménez de la Espada, 1871; *Melanophryniscus* Gallardo, 1961; *Oreophrynella* Boulenger, 1895; *Osornophryne* Ruiz-Carranza and Hernández-Camacho, 1976; and some *Rhamphophryne* Trueb, 1971) (Savage and Kluge, 1961; Cannatella, 1986).

The new genus is differentiated from most of these by its larger body size and by widely expanded, T-shaped phalangeal tips (vs. slightly modified, T-shaped or knobbed phalangeal tips in *Altiphrynoidea*, *Nectophrynoidea*, and *Werneria*) (Grandison, 1978); by eight presacral vertebrae (vs. up to seven in *Didynamipus*, *Laurentophryne*, *Mertensophryne*, some *Nectophryne*, *Nimbaphrynoidea*, *Oreophrynella*, *Osornophryne*, and most of *Rhamphophryne*) (Trueb, 1971; Grandison, 1978; Cannatella, 1986); by the absence of sacro-coccygeal fusion (vs. fused sacrum and coccyx in *Wolterstorffina*) (Grandison, 1978); and by the

arciferal pectoral girdle (vs. arciferal-firmisternal in some *Atelopus*, *Dendrophryniscus*, *Didynamipus*, and *Melanophryniscus*) (Trueb, 1971; Cannatella, 1986). It also differs from *Capensibufo* by the absence of parotoid gland, presence of toe webs, and a large clutch of small eggs (vs. presence of parotoid gland, absence of toe webbing, and a small clutch of large eggs) (Grandison, 1981), and from *Crepidophryne* by normal phalangeal formulae (vs. phalanges reduced) (Savage and Kluge, 1961).

Etymology

The taxon is named for the state of Sabah, Malaysia from where the unique species has been recorded. The suffix *-phrynus* is from the Greek, *phrynos*, meaning a toad.

Type species

Nectophryne maculata Mocquard, 1890.

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REFERENCES

- Barbour T (1938) Notes on "*Nectophryne*". Proc Biol Soc Wash 51: 191–195
- Cannatella, DC (1986) A new genus of bufonid (Anura) from South America, and phylogenetic relationships of the neotropical genera. Herpetologica 42: 197–205
- Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. Cladistics 10: 315–319
- Frost DR *et al.* (2006) The amphibian tree of life. Bull Amer Mus Nat Hist 297: 1–370
- Grandison AGC (1978) The occurrence of *Nectophrynoidea* (Anura Bufonidae) in Ethiopia. A new concept of the genus with a description of a new species. Monit Zool Ital New Ser Suppl 11: 119–172
- Grandison AGC (1981) Morphology and phylogenetic position of the west African *Didynamipus sjoestedti* Andersson, 1903 (Anura: Bufonidae). Monit Zool Ital New Ser Suppl 15: 187–215
- Graybeal A, Cannatella DC (1995) A new taxon of Bufonidae from Peru, with descriptions of two new species and a review of the phylogenetic status of supraspecific bufonid taxa. Herpetologica 51: 105–131
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95–98
- Hedges SB (1992) The number of replications needed for accurate

- estimation of the bootstrap P value in phylogenetic studies. *Mol Biol Evol* 9: 366–369
- Hedges SB, Maxson LR (1993) A molecular perspective on lissamphibian phylogeny. *Herpetol Monogr* 7: 27–42
- Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA (1996) Nucleic acids IV: sequencing and cloning. In “Molecular Systematics” Ed by DM Hillis, C Moritz, BK Mable, Sinauer Associates, Sunderland, pp 321–378
- Huelsenbeck JP, Hillis DM (1993) Success of phylogenetic methods in the four-taxon case. *Syst Biol* 42: 247–264
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755
- Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310–2314
- Inger RF (1958) Comments on the definition of genera. *Evolution* 12: 370–384
- Inger RF (1960) A review of the oriental toads of the genus *Ansonia* Stoliczka. *Fieldiana Zool* 39: 473–503
- Inger RF (1966) The systematics and zoogeography of the Amphibia of Borneo. *Fieldiana Zool* 52: 1–402
- Inger RF (1992) Variation of apomorphic characters in stream-dwelling tadpoles of the bufonid genus *Ansonia* (Amphibia: Anura). *Zool J Linn Soc* 105: 225–237
- Inger RF (1999) Distribution of amphibians in Southern Asia and adjacent islands. In “Patterns of Distribution of Amphibians: A Global Perspective” Ed by WE Duellman, Johns Hopkins University Press, Baltimore, pp 445–482
- Inger RF, Tan FL (1996) Checklist of the frogs of Borneo. *Raffles Bull Zool* 44: 551–574
- Inger RF, Tan FL, Yambun P (2001) A new species of toad of the genus *Ansonia* (Anura: Bufonidae) from Borneo. *Raffles Bull Zool* 49: 35–37
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111–120
- Kueh BH, Sudin A, Matsui M, Maryati M (2004) Notes on the anurans of Crocker Range Park. In “Crocker Range Scientific Expedition 2002” Ed by M Maryati, Z Hamzah, T Tachi, N Jamili, Universiti Malaysia Sabah, Kota Kinabalu, pp 103–112
- Larget B, Simon DL (1999) Markov chain monte carlo algorithms for the Bayesian analysis of phylogenetic trees. *Mol Biol Evol* 16: 750–759
- Malkmus R, Manthey U, Vogel G, Hoffman P, Kosuch J (2002) Amphibians and Reptiles of Mount Kinabalu (North Borneo). ARG Gantner Verlag Kommanditgesellschaft, Ruggell
- Matsui M (1984) Morphometric variation analyses and revision of the Japanese toad (genus *Bufo*, Bufonidae). *Contr Biol Lab Kyoto Univ* 26: 209–428
- Matsui M (2006) Anuran inventory in Sabah—Past and future. *Cur Herpetol* 25: 1–14
- Mocquard F (1890) Recherches sur la faune herpetologique des Iles de Borneo et de Palawan. *Nouv Arch Mus Nat Hist Nat Ser 3* 2: 115–168
- Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818
- Rambaut A, Drummond AJ (2003) Tracer v1.0.1. Available from <http://evolve.zoo.ox.ac.uk/>
- Savage JM, Kluge AG (1961) Rediscovery of the strange Costa Rica toad, *Crepidius epiocticus* Cope. *Rev Biol Trop* 9: 39–51
- Shaffer HB, McKnight ML (1996) The polytypic species revisited: genetic differentiation and molecular phylogenetics of the tiger salamander *Ambystoma tigrinum* (Amphibia: Caudata) complex. *Evolution* 50: 417–433
- Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland
- Trueb L (1971) Phylogenetic relationships of certain neotropical toads with the description of a new genus (Anura: Bufonidae). *Contr Sci Los Angeles Co Mus* 216: 1–40

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