

# The phylogenetic problem of *Huia* (Amphibia: Ranidae)

Bryan L. Stuart \*

*The Field Museum, Department of Zoology, Division of Amphibians and Reptiles, 1400 S. Lake Shore Drive, Chicago, IL 60605-2496, USA*  
*University of Illinois at Chicago, Department of Biological Sciences (MIC 066), 845 W. Taylor, Chicago, IL 60607-7060, USA*

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## Abstract

A taxonomic consensus for the diverse and pan-global frog family Ranidae is lacking. A recently proposed classification of living amphibians [Frost, D.R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M., Wheeler, W.C., 2006. The amphibian tree of life. *B. Am. Mus. Nat. Hist.* 297, 1–370] included expansion of the Southeast Asian ranid frog genus *Huia* from seven to 47 species, but without having studied the type species of *Huia*. This study tested the monophyly of this concept of *Huia* by sampling the type species and putative members of *Huia*. Molecular phylogenetic analyses consistently recovered the type species *H. cavitympanum* as the sister taxon to other Bornean-endemic species in the genus *Meristogenys*, rendering all previously published concepts of *Huia* as polyphyletic. Members of *Huia* sensu [Frost, D.R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M., Wheeler, W.C., 2006. The amphibian tree of life. *B. Am. Mus. Nat. Hist.* 297, 1–370.] appear in four places within the family Ranidae. A clade containing the type species of *Odorrana* is phylogenetically unrelated to the type species of *Huia*, and *Odorrana* is removed from synonymy with *Huia*. These findings underscore the need to include relevant type species in phylogenetic studies before proposing sweeping taxonomic changes. The molecular phylogenetic analyses revealed a high degree of homoplasy in larval and adult morphology of Asian ranid frogs. Detailed studies are needed to identify morphological synapomorphies that unite members in these major clades of ranid frogs.

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## 1. Introduction

The frog family Ranidae (sensu Frost et al., 2006, equivalent to Raninae sensu Bossuyt et al., 2006) contains over 300 species and occurs in most temperate and tropical parts of the world (Frost, 2007). The family exhibits tremendous ecological, morphological, and developmental diversity across its wide geographic range. Despite the significance of ranid frogs to almost all fields of comparative biology, a consensus for ranid taxonomy is lacking (Dubois, 1987, 1992, 2005; Inger, 1996; Roelants et al., 2004; Chen

et al., 2005; Bossuyt et al., 2006; Frost et al., 2006; Che et al., 2007a). A significantly revised classification of the family proposed by Dubois (1987, 1992) based on comparisons of overall similarity has limited concordance with evolutionary relationships revealed by phylogenetic analyses of molecular and morphological characters (Chen et al., 2005; Scott, 2005; van der Meijden et al., 2005; Bossuyt et al., 2006; Frost et al., 2006; Cai et al., 2007; Che et al., 2007a,b).

Recently, Frost et al. (2006; “the amphibian tree of life”) proposed a new taxonomy of living amphibians based on the largest phylogenetic analysis of amphibians presented to date (532 terminals representing 522 species). Although Frost et al. (2006) stated that the primary purpose of their undertaking was to test the monophyly of all family-group taxa, the authors also recommended numerous taxonomic

\* Present address: Museum of Vertebrate Zoology, 3101 Valley Life Sciences Building, University of California, Berkeley, CA 94720-3160, USA. Fax: +1 510 643 8238.

E-mail address: [bstuart@berkeley.edu](mailto:bstuart@berkeley.edu)

rearrangements above and below the family rank based on their findings. Importantly, Frost et al. (2006) proposed a number of taxonomic changes without having studied the type species of the affected genera (Dubois, 2007; Wiens, 2007), a potential source of error that they accepted as the cost of the scale of their study. This shortcoming could allow the phylogenetic position of a species that had been assigned earlier to the wrong genus to determine the taxonomic fate of that genus (or even family) in the revised classification. A notable example of this potential pitfall in Frost et al. (2006) is the Southeast Asian ranid genus *Huia* Yang, 1991.

Inger (1966) united a suite of Asian ranid species having torrent-adapted tadpoles with a raised, sharply defined abdominal sucker into the genus *Amolops* Cope, 1865. Yang (1991) recovered three species groups within *Amolops* sensu Inger (1966) in a phylogenetic analysis of morphological characters, and these were split into three genera, *Amolops* and the newly named genera *Meristogenys* Yang, 1991 and *Huia*. The genus *Meristogenys* was based on the single synapomorphy of having larvae with a divided upper beak that is ribbed on its outer surface (Yang, 1991). The genus *Huia* was based on the single synapomorphy of having larvae with “scattered glands on the back” (Yang, 1991). Although some species of *Huia* do have larvae with scattered dorsal glands, this character is actually absent in the type species of *Huia*, *H. cavitympanum* (e.g., FMNH 241615, 250132–35, personal observation; Inger, 1985). Yang’s (1991) failure to identify a morphological synapomorphy when he named and defined the genus *Huia* has led to considerable uncertainty in the use of this name and suggests that *Huia* may not represent a natural group.

Species of *Huia* sensu Yang, 1991 are distributed from southern China to Java, Indonesia, and occur along swift, forested streams. Stuart and Chan-ard (2005) described two new species from Thailand and Laos, bringing the total number of recognized species of *Huia* from five to seven. Subsequent molecular phylogenetic analyses (Chen et al., 2005; Ngo et al., 2006; Cai et al., 2007; Che et al., 2007a) have shown that *H. nasica* is phylogenetically nested within *Odorrana* (Fei et al., 1991), a diverse genus of Southeast Asian ranid frogs that also occurs along swift, forested streams. Frost et al. (2006) recovered a well-supported monophyletic group containing the four species *H. nasica*, *A. chapaensis*, *Eburana chloronota*, and *O. grahami*. The authors provided evidence that *chapaensis* had been erroneously referred to *Amolops* by Dubois (1987), leaving *Huia* as the oldest of the three remaining available names for that clade. Frost et al. (2006) then greatly expanded the genus *Huia* to include *Odorrana* and *Eburana*, and transferred 47 species into *Huia*. These 47 species all occur along swift forested streams, but exhibit considerable heterogeneity in adult and larval morphology, including tadpoles with and without abdominal suckers. Frost’s et al. (2006) taxonomic rearrangement was based on the assumption that *H. nasica* is phylogenetically closely related to the

type species *H. cavitympanum*, and that all referred species are also closely related to *H. cavitympanum*. The authors explicitly recognized the potential problems of making this sweeping taxonomic change without having studied the relevant type species or additional putative members of the group, but felt that generating such a hypothesis of relationships was preferable to the alternative of maintaining the prevailing classification based on comparisons of overall similarity. Molecular phylogenetic studies using mitochondrial DNA sequence data by Matsui et al. (2006) and Cai et al. (2007) included the type species *H. cavitympanum*, but its relationship to other *Huia* and members of the Ranidae was not resolved. However, Cai et al. (2007) showed that *H. cavitympanum* was not closely related to *H. nasica*, casting serious doubt on the monophyly of *Huia* sensu Frost et al. (2006).

This study proposes to test Frost’s et al. (2006) concept of *Huia* with a molecular phylogenetic analysis of two nuclear genes that includes the type species *H. cavitympanum*, improved taxon sampling of *Huia* sensu Frost et al. (2006), and broad representation of the family Ranidae.

## 2. Materials and methods

### 2.1. Sampling

Specimens were collected in the field in Cambodia, Laos, Thailand, and Vietnam and preserved in 10% buffered formalin after fixing pieces of liver in 95% ethanol or 20% DMSO-salt saturated storage buffer. Specimens and tissue samples were deposited at The Field Museum (FMNH), Chicago, and specimens were transferred to 70% ethanol upon arrival there. Specimens and tissue samples from additional localities in Southeast Asia were borrowed from the holdings of the FMNH and other institutions (Table 1). Homologous sequences of additional taxa were downloaded from GenBank (Table 1).

This study includes representatives of every genus of Ranidae recognized by Frost et al. (2006) except *Humerana* Dubois, 1992 and *Pterorana* Kiyasetuo and Khare, 1986. This study includes six of the seven species of *Huia* (missing *H. modiglianii*, a species known only by the type specimens from Sumatra, Indonesia) that were recognized prior to Frost et al. (2006), and includes the same four *Huia* species (*chapaensis*, *chloronota*, *grahami*, and *nasica*) that were studied by Frost et al. (2006). The taxon sampling in this study differs most notably from that of Frost et al. (2006) by having greater representation of *Huia*, including the type species *H. cavitympanum*, and species that were referred to *Huia*, but not studied, by Frost et al. (2006). The mantellid *Aglyptodactylus madagascariensis* and the nyctibatrachid *Nyctibatrachus major* were used as outgroups based on the findings of van der Meijden et al. (2005) and Frost et al. (2006). Taxonomy follows Frost et al. (2006) unless stated otherwise.

Table 1  
Nuclear DNA sequences used in this study

Species	Source	GenBank Accession numbers		Locality
		RAG-1	Tyrosinase	
<i>Aglyptodactylus madagascariensis</i>	Van der Meijden et al. (2004); Bossuyt and Milinkovitch (2000)	AY571640	AF249166	Madagascar
<i>Amolops archotaphus</i>	FMNH 261712	EF088234	EU076756	Chiang Mai Prov., Thailand
<i>Amolops compotrix</i>	FMNH 256500 holotype	EF088235	EU076757	Khammouan Prov., Laos
<i>Amolops cremnobatus</i>	FMNH 255601	EF088236	EU076758	Nghe An Prov., Vietnam
<i>Amolops cucae</i>	AMNH 168726 paratype	EF088237	EU076759	Lao Cai Prov., Vietnam
<i>Amolops daorum</i>	ROM 38501 paratype	EF088238	EU076760	Lao Cai Prov., Vietnam
<i>Amolops iriodes</i>	AMNH 163925 holotype	EF088239	EU076761	Ha Giang Prov., Vietnam
<i>Amolops larutensis</i>	Bossuyt et al. (2006)	DQ347279	DQ347186	Thailand
<i>Amolops mantzorum</i>	FMNH 233124	EF088240	EU076762	Sichuan Prov., China
<i>Amolops marmoratus</i>	CAS 230228	EF088241	EU076763	Kachin State, Myanmar
<i>Amolops ricketti</i>	AMNH 168687	EF088242	EU076764	Lao Cai Prov., Vietnam
<i>Amolops spinapectoralis</i>	FMNH 254436	EF088243	EU076765	Gia Lai Prov., Vietnam
<i>Amolops vitreus</i>	FMNH 258180 paratype	EF088244	EU076767	Phongsaly Prov., Laos
<i>Babina adenopleura</i>	Che et al. (2007a,b)	Missing	DQ360041	Sichuan Prov., China
<i>Babina chapaensis</i>	FMNH 256531	EU076752	EU076792	Xieng Khouang Prov., Laos
<i>Babina pleuraden</i>	Che et al. (2007a,b)	Missing	DQ360042	Yunnan Prov., China
<i>Clinotarsus curtipes</i>	VUB 0085	EU076753	EU076794	Western Ghats, India
<i>Glandirana minima</i>	Che et al. (2007a,b)	Missing	DQ360052	Fujian Prov., China
<i>Huia cavitypanum</i>	FMNH 237299	EF088246	EU076769	Sabah, Malaysia
<i>Huia masonii</i>	TNHC 59914	EF088247	EU076770	Java, Indonesia
<i>Huia melasma</i>	THNHM 00218 paratype	EF088248	EU076771	Prachuap Kirikhan Prov., Thailand
<i>Huia sumatrana</i>	FMNH 267448	EF088249	EU076772	Sumatra, Indonesia
<i>Hydrophylax chalconotus</i>	UTA 53685	EF088266	EU076791	Sumatra, Indonesia
<i>Hydrophylax mocquardii</i>	JAM 5019	EF088275	EU076803	Sulawesi, Indonesia
<i>Hydrophylax galamensis</i>	Bossuyt et al. (2006); Roelants et al. (2004)	DQ347260	AY322337	Kenya
<i>Hydrophylax igorota</i>	FMNH 259496	EU076754	EU076798	Luzon, Kalinga Prov., Philippines
<i>Hydrophylax luzonensis</i>	FMNH 259477	EU076755	EU076801	Luzon, Kalinga Prov., Philippines
<i>Hylarana erythraea</i>	FMNH 257282	EF088268	EU076795	Siem Reap Prov., Cambodia
<i>Lithobates berlandieri</i>	Van der Meijden et al. (2005)	DQ019510	Missing	Coahuila, Mexico
<i>Lithobates clamitans</i>	Bossuyt et al. (2006)	DQ347262	DQ347169	USA
<i>Lithobates palmipes</i>	Bossuyt et al. (2006)	DQ347263	DQ347170	South America
<i>Lithobates palustris</i>	Bossuyt et al. (2006)	DQ347264	DQ347171	USA
<i>Lithobates sylvaticus</i>	Van der Meijden et al. (2005); Bossuyt et al. (2006)	DQ019511	DQ347185	NY, USA
<i>Lithobates tarahumarae</i>	Bossuyt et al. (2006)	DQ347267	DQ347174	USA
<i>Meristogenys kinabaluensis</i>	FMNH 243585	EF088250	EU076773	Sabah, Malaysia
<i>Meristogenys orphnocnemis</i>	FMNH 235548	EF088251	EU076774	Sabah, Malaysia
<i>Meristogenys poecilus</i>	FMNH 251719	EF088252	EU076775	Sarawak, Malaysia
<i>Meristogenys whiteheadi</i>	FMNH 238286	EF088253	EU076776	Sabah, Malaysia
<i>Nasirana alticola</i>	FMNH 263424	EU076751	EU076789	Prachuap Kirikhan Prov., Thailand
<i>Nyctibatrachus major</i>	Van der Meijden et al. (2004); Bossuyt and Milinkovitch (2000)	AY571655	AF249176	India
<i>Odorrana absita</i>	FMNH 258107 holotype	EF088245	EU076768	Xe Kong Prov., Laos
<i>Odorrana bacboensis</i>	FMNH 255611 paratype	EF088254	EU076777	Nghe An Prov., Vietnam
<i>Odorrana chapaensis</i>	AMNH 168685	EF088255	EU076778	Lao Cai Prov., Vietnam
<i>Odorrana chloronota</i>	CAS 220186	EF088256	EU076779	Rakhine State, Myanmar
<i>Odorrana grahami</i>	CAS 207505	EF088257	EU076780	Yunnan Prov., China
<i>Odorrana hmongorum</i>	ROM 26370 paratype	EF088258	EU076781	Lao Cai Prov., Vietnam
<i>Odorrana hosii</i>	FMNH 235650	EF088259	EU076782	Sabah, Malaysia

(continued on next page)

Table 1 (continued)

Species	Source	GenBank Accession numbers		Locality
		RAG-1	Tyrosinase	
<i>Odorrana khalam</i>	FMNH 258172 holotype	EF088272	EU076783	Xe Kong Prov., Laos
<i>Odorrana livida</i>	FMNH 263415	EF088260	EU076784	Prachuap Kirikhan Prov., Thailand
<i>Odorrana margaretae</i>	FMNH 233029	EF088261	EU076785	Sichuan Prov., China
<i>Odorrana megatympanum</i>	FMNH 255644	EF088262	EU076786	Nghe An Prov., Vietnam
<i>Odorrana morafkai</i>	ROM 39907 paratype	EF088263	EU076787	Gia Lai Prov., Vietnam
<i>Odorrana nasica</i>	FMNH 256472	EF088264	EU076788	Khammouan Prov., Laos
<i>Odorrana tormota</i>	PMN, no voucher	EU076750	EU076766	Anhui Prov., China
<i>Pelophylax esculenta</i>	Bossuyt et al. (2006); GenBank (unpublished data)	DQ347235	DQ841551	Belgium
<i>Pelophylax lateralis</i>	FMNH 255345	EF088273	EU076800	Champasak Prov., Laos
<i>Pulchrana baramica</i>	FMNH 266574	EF088265	EU076790	Sarawak, Malaysia
<i>Pulchrana glandulosa</i>	FMNH 266573	EF088270	EU076797	Sarawak, Malaysia
<i>Pulchrana signata</i>	Roelants et al. (2004); Bossuyt et al. (2006)	DQ347218	AY322354	Borneo, Malaysia
<i>Rana aurora</i>	Van der Meijden et al. (2005); Frost et al. (2006)	DQ019509	DQ282944	CA, USA
<i>Rana boylei</i>	Bossuyt et al. (2006)	DQ347277	DQ347184	CA, USA
<i>Rana johnsi</i>	FMNH 261882	EF088271	EU076799	Mondolkiri Prov., Cambodia
<i>Rana temporaria</i>	Hoegg et al. (2004); Bossuyt and Milinkovitch (2000)	AY323776	AF249182	Belgium
<i>Rana weiningensis</i>	Che et al. (2007a,b)	Missing	DQ360050	Yunnan Prov., China
<i>Sanguirana sanguinea</i>	Bossuyt et al. (2006)	DQ347273	DQ347180	Palawan, Philippines
<i>Staurois latopalmaris</i>	FMNH 239088	EF088277	EU076805	Sabah, Malaysia
<i>Staurois natator</i>	Bossuyt et al. (2006)	DQ347250	DQ347155	Mindanao, Philippines
<i>Staurois tuberinguis</i>	FMNH 243096	EF088278	EU076806	Sabah, Malaysia
<i>Sylvirana cubitalis</i>	FMNH 265818	EF088267	EU076793	Loei Prov., Thailand
<i>Sylvirana daemeli</i>	Frost et al. (2006)	Missing	DQ282948	Northern Territory, Australia
<i>Sylvirana faber</i>	FMNH 267767	EF088269	EU076796	Pursat Prov., Cambodia
<i>Sylvirana maosonensis</i>	FMNH 255637	EF088274	EU076802	Nghe An Prov., Vietnam
<i>Sylvirana nigrovittata</i>	FMNH 255434	EF088276	EU076804	Bolikhamsay Prov., Laos
<i>Sylvirana</i> sp.	Bossuyt et al. (2006)	DQ347229	DQ347137	New Guinea

Institutional abbreviations used are AMNH, American Museum of Natural History; CAS, California Academy of Sciences; FMNH, The Field Museum; ROM, Royal Ontario Museum; JAM, Jimmy A. McGuire field series, uncataloged voucher at Museum Zoologicum Bogoriense; PMN, Peter M. Narins' Auditory Science Lab, University of California, Los Angeles; THNHM, Thailand Natural History Museum, Thailand National Science Museum; TNHC, Texas Natural History Collections, Texas Memorial Museum, University of Texas; UTA, University of Texas, Arlington; VUB, Vrije Universiteit Brussel.

## 2.2. Extraction, amplification, and DNA sequencing

Total genomic DNA was extracted from tissues using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc.). A 1233 bp fragment of nuclear DNA that encodes part of the recombination activating protein 1 (RAG-1) gene was amplified by PCR (the polymerase chain reaction; 94 °C 45 s, 55 °C 30 s, 72 °C 1 min) for 35 cycles using the primers L-RAG1Ran (5'-CTGGTCCGTCAGATCTTTCAGC-3') and H-RAG1Ran (5'-GCAAACCGTTGAGAGTGATAAC-3'). A 532 or 601 bp fragment of nuclear DNA that encodes part of exon 1 of the tyrosinase gene was amplified by PCR (94 °C 45 s, 49 °C 30 s, 72 °C 1 min) for 35 cycles using the primer pairs Tyr1C/Tyr1G or Tyr1B/Tyr1G (Bossuyt and Milinkovitch, 2000), respectively. PCR products were electrophoresed in a 1% low melt agarose TALE gel stained with ethidium bromide and visualized under ultraviolet light. The bands containing DNA were excised and agarose was digested from bands using GELase (Epicentre Technologies). PCR products were sequenced in both directions by direct double strand cycle sequencing using Big Dye version 3 chemistry (Perkin-Elmer). The amplifying primers and the RAG-1 internal primers L-RAG1Ranint (5'-GGAAATTGGTGGAAATCCTCAG-3') and H-RAG1Ranint (5'-ATATAGATAGAGCCTGAGGC-3') were used in cycle sequencing reactions. The internal primer L-RAG1Stint (5'-TCCAGTGAACATCATGTTGGAG-3') was substituted for L-RAG1Ran-int with *Stauroids*. Cycle sequencing products were precipitated with ethanol, 3 M sodium acetate, and 125 mM EDTA, and sequenced with a 3730 DNA Analyzer (ABI). Sequences were edited and aligned using Sequencher v. 4.1 (Genecodes). Sequences were deposited in GenBank under accession numbers EF088234–EF088278 and EU076750–EU076806 (Table 1).

## 2.3. Phylogenetic analysis

Phylogenies were reconstructed using maximum parsimony and maximum likelihood optimality criteria, and mixed-model Bayesian inference.

Maximum parsimony analysis was performed using PAUP\* 4.0b10 (Swofford, 2002). A heuristic search was performed with equal weighting of nucleotide substitutions, stepwise addition with 10,000 random addition replicates, and TBR branch swapping. Nodal support was evaluated with 1000 nonparametric bootstrap pseudoreplications (Felsenstein, 1985) using the heuristic search option with TBR branch swapping limited to 10,000,000 rearrangements per replicate.

Maximum likelihood analysis was performed using GARLI 0.95 (Zwickl, 2006). Five independent analyses were performed under the GTR+I+G model using the default settings. Nodal support was evaluated with 500 nonparametric bootstrap pseudoreplications. To test the null hypothesis that Frost's et al. (2006) concept of *Huia* forms a monophyletic group, five independent likelihood

analyses were performed using the same settings in GARLI, but with *Huia* sensu Frost et al. (2006) constrained to be monophyletic. The likelihood score of the best constrained tree was compared against that of the best unconstrained tree using the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) with RELL optimization implemented in PAUP\* 4.0b10.

Mixed-model Bayesian analysis was performed using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). The data were partitioned by gene and by codon position. The model of sequence evolution that best described each of the six data partitions was inferred using the Akaike Information Criterion as implemented in Modeltest 3.7 (Posada and Crandall, 1998). The selected models were HKY+I+G for the RAG-1 first codon position partition, GTR+I+G for the RAG-1 second codon position partition, TrN+G for the RAG-1 third codon position partition, TVM+G for the tyrosinase first codon position partition, TIM+I+G for the tyrosinase second codon position partition, and TVM+G for the tyrosinase third codon position partition. The TrN, TVM, and TIM models are not implemented in MrBayes 3.1, and so the next more complex model available in the program (GTR) was used for those partitions. Four independent Bayesian analyses were performed. In each analysis, four chains were run for 20,000,000 generations using the default priors, trees were sampled every 4000 generations, and the first 25% of trees were discarded as 'burn-in.' A 50% majority rule consensus of the sampled trees was constructed to calculate the posterior probabilities of the tree nodes.

## 3. Results

### 3.1. Sequence data

The alignment contained two outgroup taxa, 72 ingroup taxa, and 1834 characters. The complete 1233 bp fragment of RAG-1 was obtained for all newly generated sequences. The 523 bp fragment of tyrosinase was obtained for *Huia absita*, *Stauroids tuberilinguis*, and *Sylvirana nigrovittata*, and the 601 bp fragment of tyrosinase was obtained for all other newly generated sequences. Some sequences downloaded from GenBank contained missing data in the alignment. All sequences translated into amino acids without stop codons and the alignment contained no insertion–deletions. Of the 1834 characters, 685 were variable and 462 were parsimony-informative.

### 3.2. Phylogenetic analysis

The heuristic search in the maximum parsimony analysis recovered 312 equally most parsimonious trees ( $L = 1665$ ;  $CI = 0.535$ ;  $RI = 0.691$ ; Fig. 1). The score of the best likelihood tree ( $-\ln L 12,153.32876$ ; Fig. 2) was within two likelihood units of the best tree recovered in each of the other four runs, suggesting that the five runs had converged. The standard deviation of split frequencies among

the four Bayesian runs (Fig. 3) was 0.002649, and trace plots of clade probabilities viewed using AWTY (Wilgenbusch et al., 2004) were relatively stationary. These two measures suggest that the four runs had converged and that topologies were sampled in proportion to their true posterior probability distribution.

Eight major clades were recovered in all analyses (Figs. 1–3). A clade containing the type species of *Stauroides* (A in Fig. 1) is the sister clade to all other ranids. A clade containing *Meristogenys* and the type species of *Huia*, *Clinotarsus*, and *Nasirana* (B in Fig. 1) is the sister clade to all other ranids except *Stauroides*. A clade containing the type species of

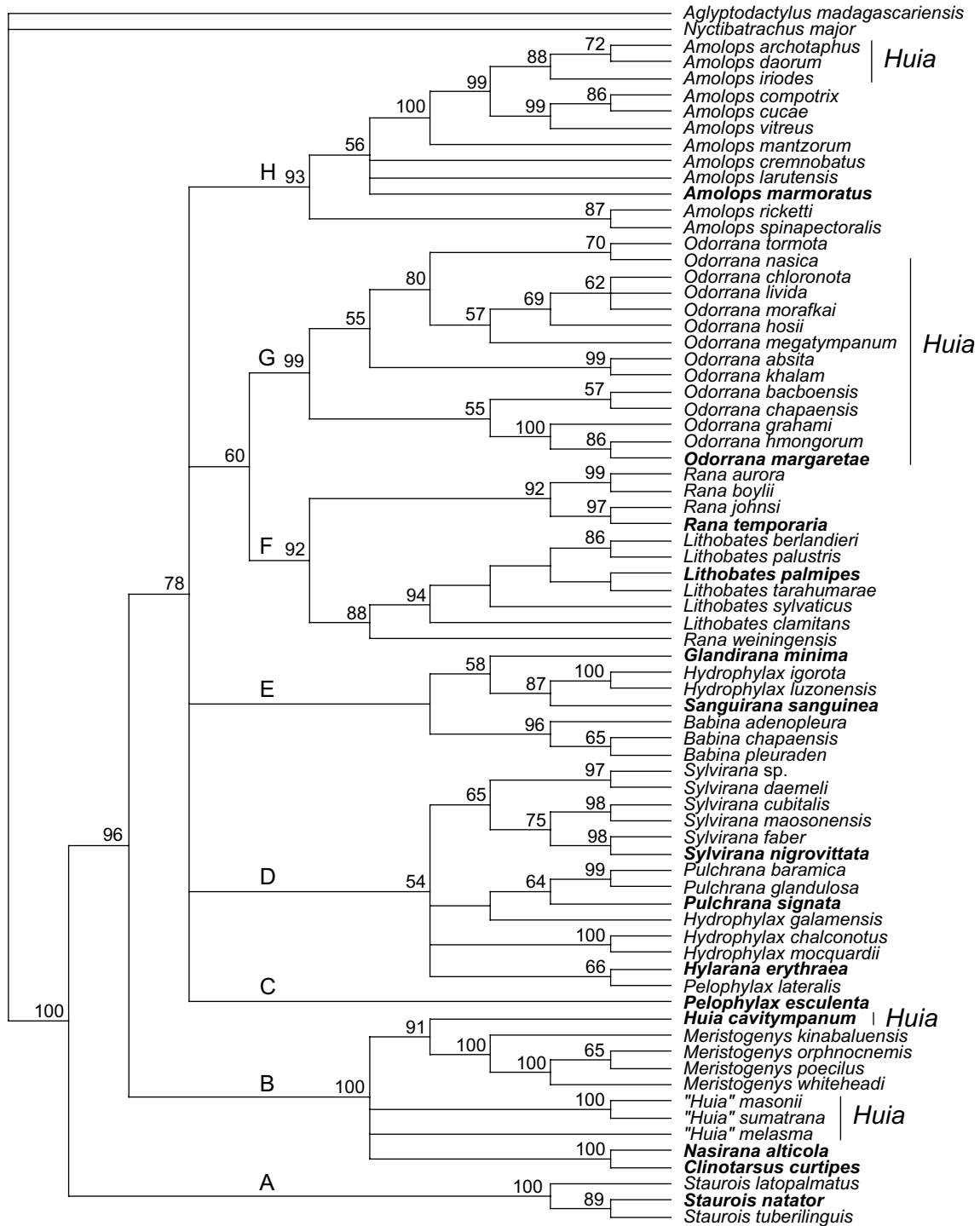


Fig. 1. Strict consensus of the 312 shortest trees resulting from maximum parsimony analysis of 1834 bp of the nuclear RAG-1 and tyrosinase genes from frogs of the family Ranidae sensu Frost et al. (2006). Values next to nodes are nonparametric bootstrap values  $\geq 50$ . Terminals in boldface font are the type species of their genus. Species referred to *Huia* by Frost et al. (2006) are noted on the right.

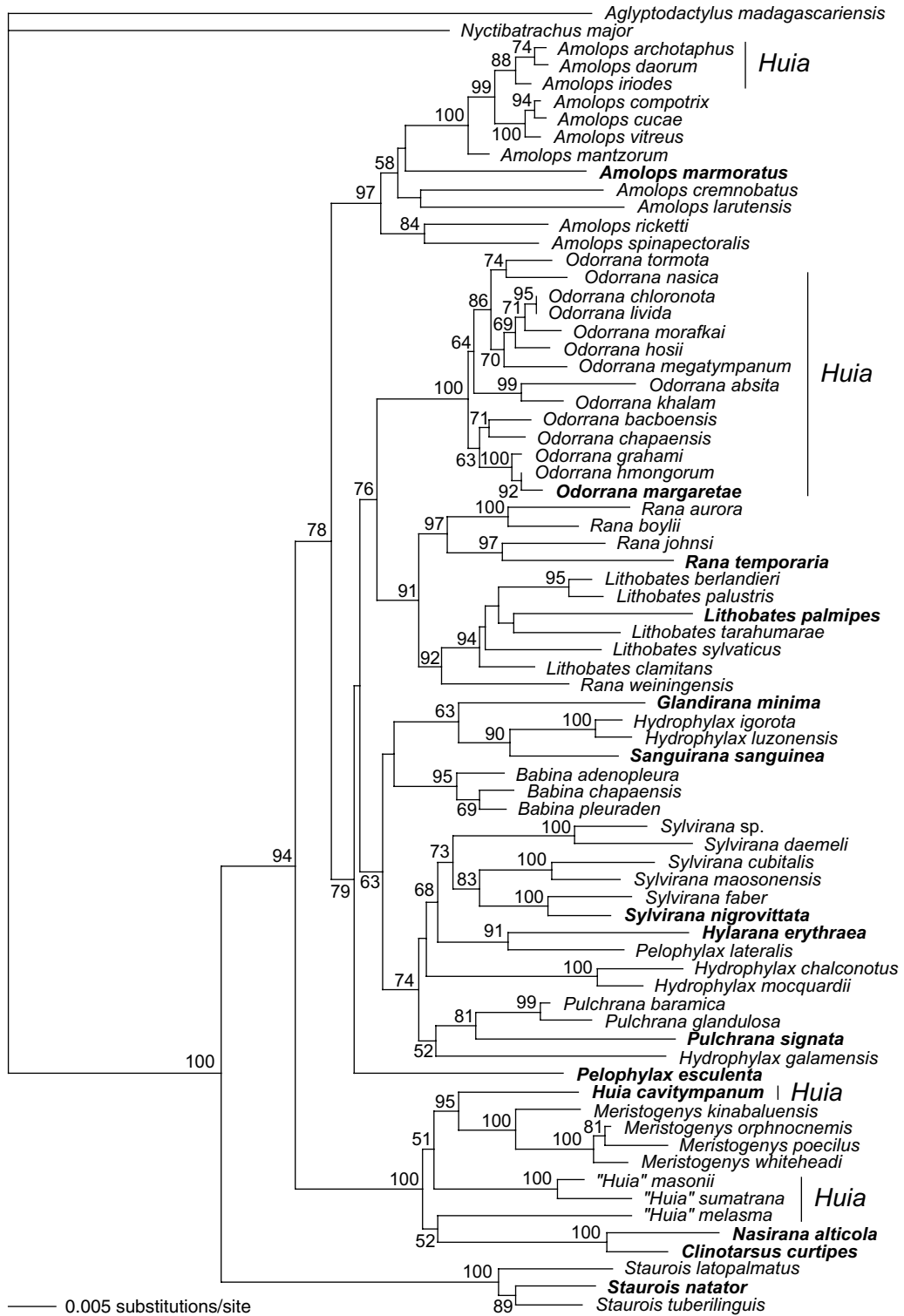


Fig. 2. The best maximum likelihood tree ( $-\ln L$  12,153.32876) based on 1834 bp of the nuclear RAG-1 and tyrosinase genes from frogs of the family Ranidae sensu Frost et al. (2006). Values next to nodes are nonparametric bootstrap values  $\geq 50$ . Terminals in boldface font are the type species of their genus. Species referred to *Huia* by Frost et al. (2006) are noted on the right.

*Pelophylax* (C in Fig. 1) was recovered. A clade containing *Hydrophylax*, *Pelophylax*, and the type species of *Hylarana*, *Pulchrana*, and *Sylvirana* (D in Fig. 1) was recovered. A clade containing *Hydrophylax*, *Babina*, and the type species of

*Glandirana* and *Sanguirana* (E in Fig. 1) was recovered. A clade containing the type species of *Rana* and *Lithobates* (F in Fig. 1) is the sister clade to a clade containing the type species of *Odorrana* (G in Fig. 1). The remaining clade con-

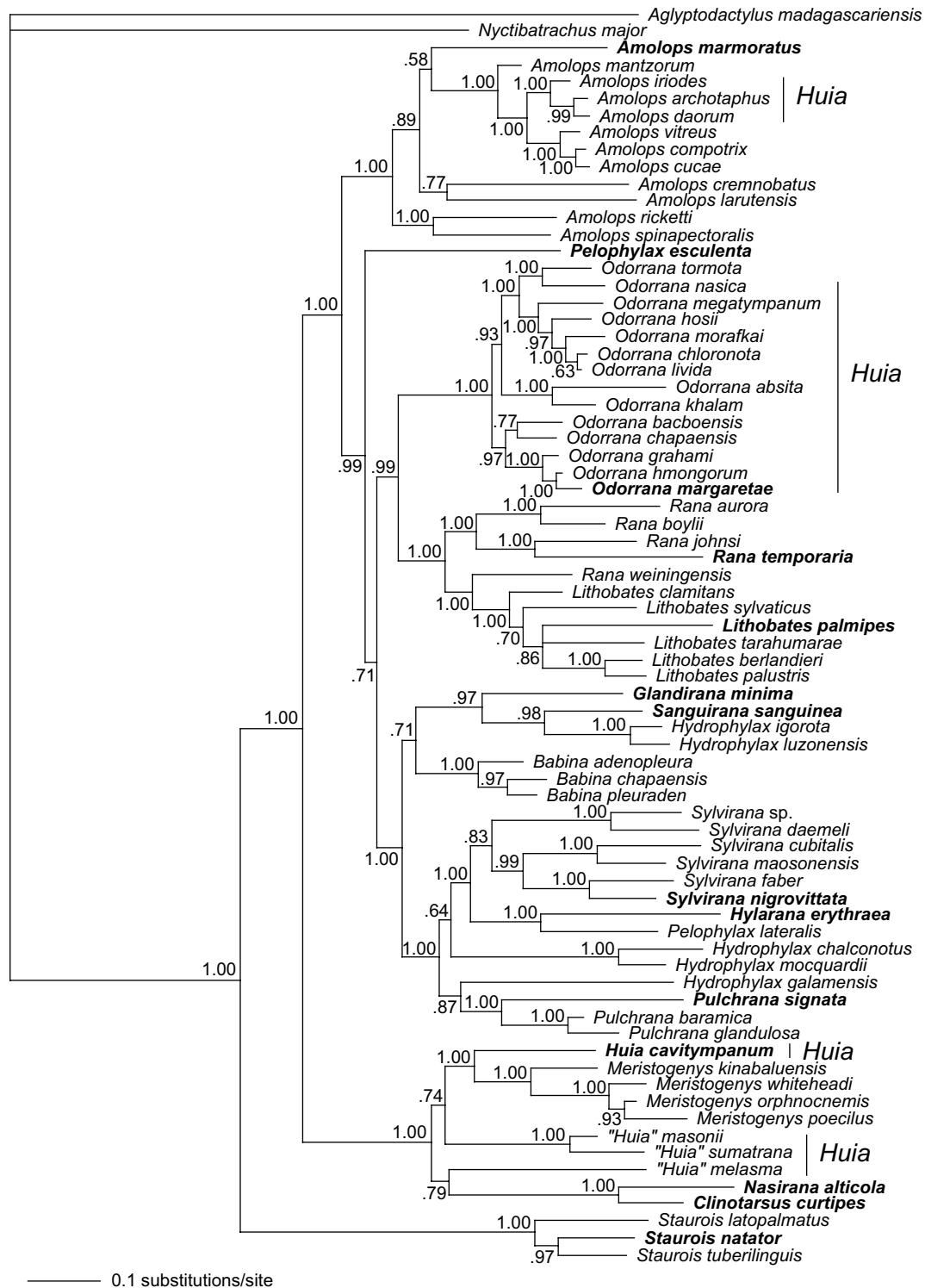


Fig. 3. Fifty percent majority-rule consensus phylogram resulting from mixed-model Bayesian analysis of 1834 bp of the nuclear RAG-1 and tyrosinase genes from frogs of the family Ranidae sensu Frost et al. (2006). Values next to nodes are posterior probabilities. Terminals in boldface font are the type species of their genus. Species referred to *Huia* by Frost et al. (2006) are noted on the right.

tained the type species of *Amolops* (H in Fig. 1). The monophyly of the clade containing *Hydrophylax*, *Babina*, and the type species of *Glandirana* and *Sanguirana* was not statistically supported in any of the analyses. The monophyly of the clade containing *Hydrophylax*, *Pelophylax*, and the type

species of *Hylarana*, *Pulchrana*, and *Sylvirana* was only weakly supported in the maximum parsimony and maximum likelihood analyses.

The monophyly of *Huia* sensu Frost et al. (2006) was rejected. The SH test showed the best unconstrained tree



( $-\ln L$  12,153.32876; Fig. 2) had a significantly better likelihood score than did the best tree constrained to have a monophyletic *Huia* sensu Frost et al. (2006;  $-\ln L$  12,534.5069,  $p < 0.0001$ ). *Huia* sensu Frost et al. (2006) consistently appeared in four places in all tree topologies (Figs. 1–3). The type species *H. cavitympanum* from Borneo is the sister taxon to the Bornean genus *Meristogenys* and does not form a monophyletic group with any other species of *Huia*. *Huia masonii* is the sister species to *H. sumatrana*, but their relationship and that of *H. melasma* to other members of the clade was unresolved. The type species of *Odorrana* and its close relatives are phylogenetically unrelated to *H. cavitympanum*. *Huia absita* and *H. nasica* belong to the clade containing the type species of *Odorrana*, and are phylogenetically unrelated to *H. cavitympanum*. Three species referred to *Huia* by Frost et al. (2006; *archotaphus*, *daorum*, and *iriodes*) are nested within the clade containing the type species of *Amolops*.

## 4. Discussion

### 4.1. The polyphyly of *Huia*

Every published concept of *Huia* (Yang, 1991; Dubois, 1992; Stuart and Chan-ard, 2005; Frost et al., 2006) is polyphyletic. Most notably, the species assigned to *Huia* by Frost et al. (2006) appear in three of the eight major clades of ranids recovered in this study (Figs. 1–3). The type species *H. cavitympanum* is not the sister taxon of any species that have been previously referred to *Huia*, but rather is most closely related to other frogs endemic to the island of Borneo in the genus *Meristogenys*. In agreement with Cai et al. (2007), *H. cavitympanum* is phylogenetically unrelated to *Odorrana* (formerly *Huia*) *nasica*, the single species of “*Huia*” that formed the basis of Frost’s et al. (2006) referral of 47 species to *Huia*. These findings underscore the need to include relevant type species in phylogenetic studies before proposing sweeping taxonomic changes at the generic level.

### 4.2. Morphological homoplasy

Ranid frogs exhibit substantial morphological homoplasy in both larvae and adults (Bossuyt and Milinkovitch, 2000), underscoring the serious limitations of using taxonomic classifications based largely on comparisons of overall similarity (e.g., Dubois, 1987, 1992). This study reveals a number of taxonomic problems that have resulted from a taxonomic emphasis on morphological characters shared among members of phylogenetically unrelated lineages. In a cladistic analysis, Yang (1991) failed to identify a single morphological synapomorphy that unites species of *Amolops* or species in his new genus *Huia* (see Section 1). Fei et al. (1991) also failed to identify a morphological synapomorphy that unites species in their new genus *Odorrana* (see Frost et al., 2006). There are currently no known morphological synapomorphies that diagnose *Huia*, *Amolops* and *Odorrana*, and as a result, some species of *Odorrana*

have been confused with *Huia* and *Amolops*, and some species of *Amolops* have been confused with *Odorrana*. A re-examination of morphological variation needs to be undertaken in the light of recent molecular phylogenetic studies to identify what morphological synapomorphies, should they exist, diagnose these clades of ranid frogs.

#### 4.2.1. *Odorrana* resembling *Huia*

*Huia nasica* was placed in that genus by Yang (1991) when he named *Huia*, and this species was the sole representative of *Huia* studied by Frost et al. (2006). This study supports the findings of other recent molecular analyses that *H. nasica* is phylogenetically nested within *Odorrana* (Chen et al., 2005; Ngo et al., 2006; Cai et al., 2007; Che et al., 2007a). Stuart and Chan-ard (2005) described *H. absita* as a new species from Laos, and provisionally assigned it to the genus *Huia* owing to its morphological similarity to adults of other species of *Huia*. In particular, adults of *H. absita* very closely resemble adults of *H. masonii* from Java, differing only by the condition of tubercles on the hands and feet, and the relative lengths of the third and fifth toes (Stuart and Chan-ard, 2005). Owing to this remarkable morphological similarity, Stuart and Chan-ard (2005) hypothesized that the two were sister species, despite being geographically separated in Laos and Java by approximately 2500 km. That hypothesis is rejected in this study. Rather, the holotype of *H. absita* is phylogenetically nested within *Odorrana*, and *H. masonii* is phylogenetically related to *Huia*, *Clinotarsus*, *Meristogenys*, and *Nasirana*.

#### 4.2.2. *Odorrana* resembling *Amolops*

Dubois (1987) treated *chapaensis* as an *Amolops* owing to its similarity in adult morphology with other *Amolops*, and this generic allocation went unchallenged until recent molecular analyses showed that *A. chapaensis* is phylogenetically nested within *Odorrana* (Cai et al., 2007; Che et al., 2007a; Ngo et al., 2006). *Amolops tormotus* is unique among amphibians in its ability to communicate with ultrasounds (Feng et al., 2006) and was recently described as a new monotypic genus, *Wurana* Li, Lu, Lü, 2006. This study supports the finding of Cai et al. (2007) that *A. tormotus* is also phylogenetically nested within *Odorrana*. Feng et al. (2006) showed that a second species of frog, *O. livida*, also has the ability to detect ultrasound, although its ability to communicate with ultrasound is not known. This suggests the intriguing possibility that ultrasonic communication might be a synapomorphy uniting members of the genus *Odorrana*. Lividins, a novel family of peptides found in *O. livida* skin secretions (Zhou et al., 2006) might also prove to be a non-DNA synapomorphy for *Odorrana*.

#### 4.2.3. *Amolops* resembling *Odorrana*

Adults of the six species in the *A. archotaphus* group (*archotaphus*, *compotrix*, *cucuae*, *daorum*, *iriodes*, and *vitreus*) superficially resemble *O. livida* and its closest relatives (e.g., *O. chloronota*, and *O. morafkai*) in external morphol-

ogy. Both groups have a greenish dorsum, smooth skin, males with gular pouches and nuptial pads, females with unpigmented ova, and sexual body size dimorphism (Inger and Chan-ard, 1997; Bain et al., 2003, 2006; Bain and Nguyen, 2004). *Amolops archotaphus* and *A. daorum* were both assumed to be closely related to the *O. livida* group in their original descriptions (Inger and Chan-ard, 1997; Bain et al., 2003), with *A. daorum* originally described as a member of the subgenus *Odorrana* (Bain et al., 2003). Frost et al. (2006) did not study any of the species in the *A. archotaphus* group, but referred three of them (*A. archotaphus*, *A. daorum*, and *A. iriodes*) to the genus *Huia*, presumably because of the superficial resemblance of adults to *O. chloronota*, a member of the *O. livida* group that was included in their study. The remaining three species (*A. compotrix*, *A. cucae*, and *A. vitreus*) were only very recently described to science (Bain et al., 2006) and so were not dealt with by Frost et al. (2006).

#### 4.2.4. Larval homoplasy

Species of *Amolops*, *Huia*, and *Meristogenys* have larvae with a raised, sharply defined abdominal sucker (Inger, 1966; Yang, 1991) and, partly on this basis, were united within the genus *Amolops* by Inger (1966) and Dubois (1992). *Rana sauteri* also has larvae with an abdominal sucker, and although less developed than in *Amolops*, was thought to indicate a close phylogenetic relationship between *R. sauteri* and *Amolops* (Kuramoto et al., 1984). *Rana sauteri* was not included in this study, but is phylogenetically nested within a clade containing three species that were studied, *R. aurora*, *R. johnsi*, and *R. temporaria* (Frost et al., 2006; Che et al., 2007a). Taxa having larvae bearing abdominal suckers are thus represented in three of the eight major clades of ranids recovered in this study (B, F, and H in Fig. 1) and do not form a monophyletic group, in agreement with the findings of other recent molecular phylogenetic studies (Roelants et al., 2004; Bossuyt et al., 2006; Cai et al., 2007; Che et al., 2007a; Ngo et al., 2006; Frost et al., 2006; Matsui et al., 2006). This non-monophyly implies that the larval abdominal sucker was repeatedly gained or lost during the evolution of the Ranidae (Frost et al., 2006; Ngo et al., 2006). Detailed morphological studies are needed to determine if the abdominal sucker found in members of unrelated clades of ranids is even a homologous structure (Kuramoto et al., 1984). The abdominal sucker is presumably an adaptation for living in rocky torrents, although it is absent in the torrent-dwelling larvae of *Staurois* and *Odorrana* (Liu, 1950; Inger, 1966; Malkmus et al., 2002; Fei et al., 2005).

#### 4.3. Taxonomic recommendations

*Odorrana* is a strongly supported monophyletic group that is phylogenetically unrelated to *H. cavitympanum*, and it should be removed from the synonymy of *Huia*, where it was placed by Frost et al. (2006). Based on the findings of this study, the genus *Odorrana* includes the fol-

lowing species that are closely related to the type species *O. margaretae* (Liu, 1950): *O. absita* (Stuart and Chan-ard, 2005), *O. bacboensis* (Bain et al., 2003), *O. chapaensis* (Bouret, 1937), *O. chloronota* (Günther, 1875), *O. grahami* (Boulenger, 1917), *O. hmongorum* (Bain et al., 2003), *O. hosii* (Boulenger, 1891), *O. khalam* (Stuart et al., 2005), *O. livida* (Blyth, 1856), *O. megatympanum* (Bain et al., 2003), *O. morafkai* (Bain et al., 2003), *O. nasica* (Boulenger, 1903a), and *O. tormota* (Wu, 1977). The genus *Odorrana* also contains the following species, based on phylogenetic analyses by Jiang and Zhou (2001, 2005), Chen et al. (2005), Matsui et al. (2005), Ngo et al. (2006), Cai et al. (2007), and Che et al. (2007a,b): *O. amamiensis* (Matsui, 1994), *O. andersonii* (Boulenger, 1882), *O. banaorum* (Bain et al., 2003), *O. hejiangensis* (Deng and Yu, 1992), *O. ishikawae* (Stejneger, 1901), *O. narina* (Stejneger, 1901), *O. schmackeri* (Boettger, 1892), *O. supranarina* (Matsui, 1994), *O. swinhoana* (Boulenger, 1903b), *O. tiannanensis* (Yang and Li, 1980), *O. utsunomiyaorum* (Matsui, 1994), and *O. versabilis* (Liu and Hu, 1962). Additional species closely resemble these morphologically and probably also belong to *Odorrana* (e.g., *Rana indeprensa* Bain and Stuart, 2005 and *Rana bolavensis* Stuart and Bain, 2005), but they are not transferred here to that genus prior to phylogenetic analysis.

The following six species are transferred from *Huia* sensu Frost et al. (2006) or *Rana* sensu lato according to their original descriptions into *Amolops*: *A. archotaphus* (Inger and Chan-ard, 1997), *A. compotrix* (Bain et al., 2006), *A. cucae* (Bain et al., 2006), *A. daorum* (Bain et al., 2003), *A. iriodes* (Bain and Nguyen, 2004), and *A. vitreus* (Bain et al., 2006).

The type species of *Huia*, *H. cavitympanum*, is the sister taxon to other Bornean species currently referred to the genus *Meristogenys*. As a result, the name *Huia* cannot be applied to any of the remaining 46 species that are treated as *Huia* by Frost et al. (2006) without recognizing a paraphyletic group. Species in the genus *Meristogenys* form a well-supported monophyletic group that is united by the strong synapomorphy of having larvae with a divided upper beak that is ribbed on its outer surface (Yang, 1991). Although the type species of *Meristogenys*, *M. jerboa*, was not included in this study, Matsui et al. (2006) found *M. jerboa* to be phylogenetically nested between *M. kinabaluensis* and *M. orphnocnemis*, two species that were included in this study. Although monotypic genera are generally undesirable because they provide no information on phylogenetic relationships (Parham and Feldman, 2002), the desire to retain the genus *Meristogenys* requires that *Huia* be recognized as a monotypic genus containing the sole species *H. cavitympanum*. However, the monotypic genera *Clinotarsus* and *Nasirana* are strongly supported sister species that share several unique larval characters (Hiragond et al., 2001; Grosjean et al., 2003), and continuing to recognize both genera is unnecessary. *Nasirana* Dubois, 1992 should therefore be treated as a junior synonym of *Clinotarsus* Mivart, 1869. The three species “*H.*”

*melasma*, “*H.*” *masonii*, and “*H.*” *sumatrana* are clearly members of the clade containing the type species of *Huia*, *Meristogenys*, *Clinotarsus*, and *Nasirana*. However, their phylogenetic relationships to those taxa were unresolved in this study, and so generic revision of those three species (and the single missing species of “*Huia*,” *H. modiglianii*) is unwarranted at this time.

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