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## PHYLOGENETIC SYSTEMATICS OF THE *AMOLOPS MONTICOLA* GROUP (AMPHIBIA: RANIDAE), WITH DESCRIPTION OF A NEW SPECIES FROM NORTHWESTERN LAOS

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**ABSTRACT:** The *Amolops monticola* group contains approximately 12 morphologically similar species distributed across much of mainland Southeast Asia. Approximately half of the species in the group were described only during the past 6 yr, and several have been erroneously placed in the genus *Odorrana*. A molecular phylogenetic analysis is performed using three mitochondrial and two nuclear genes sequenced from eight of the 12 putative members of the group. The group is shown to be monophyletic within the genus *Amolops*. The Indochinese species *A. daorum* is removed from the synonymy of *A. mengyangensis*, is shown to be a member of the genus *Amolops* and not *Odorrana*, and is found to be paraphyletic with respect to *A. iriodes*. A newly discovered population in northwestern Laos is described as a new species on the basis of its morphological and molecular distinctiveness.

**Key words:** *Amolops*; Laos; New species; *Odorrana*; Phylogeny

THE RANID frog genus *Amolops* Cope, 1865, contains approximately 42 species distributed throughout mainland Southeast Asia (Frost, 2008). These species inhabit swift, rocky streams, including torrents and waterfalls, enabled by abdominal suckers in larvae and enlarged digital pads in adults. The monophyly of *Amolops* has been established previously (Cai et al., 2007; Chen et al., 2005; Stuart, 2008), but relationships within the genus remain poorly understood owing to relatively limited taxon sampling in available phylogenetic analyses (Cai et al., 2007; Chen et al., 2005; Jin et al., 2005; Matsui et al., 2006; Ngo et al., 2006; Stuart, 2008).

A suite of *Amolops* species, referred to here as the *A. monticola* group, differs in external adult morphology from other species in the genus by having the combination of smooth skin, the side of head dark with a light-colored upper lip stripe extending to the shoulder, and a dorsolateral fold. At least 12 described species fit this definition and are putative members of the *A. monticola* group: *A. aniqiaoensis* (Dong et al., 2005); *A. archotaphus* (Inger and Chanard, 1997); *A. bellulus* (Liu et al., 2000); *A. chakrataensis* Ray, 1992;

*A. chunganensis* (Pope, 1929); *A. compotrix* (Bain et al., 2006); *A. cucae* (Bain et al., 2006); *A. daorum* (Bain et al., 2003); *A. mengyangensis* Wu and Tian, 1995; *A. monticola* (Anderson, 1871); *A. iriodes* (Bain and Nguyen, 2004); and *A. vitreus* (Bain et al., 2006). Fei et al. (2005) also placed *A. gerbillus* (Annandale, 1912) in this group, but the species is excluded here due to its divergent morphology from the remaining species (*A. gerbillus* has “large compressed longitudinal tubercles” on the dorsum, and lacks a dark side of head with a light-colored upper lip stripe extending to the shoulder; Annandale, 1912). The monophyly of the *A. monticola* group has not been tested, although there is evidence that at least some of these species are close relatives. *Amolops chunganensis* has been shown to be the sister species of *A. monticola* (see Pang and Liu, 1992) or *A. bellulus* (see Cai et al., 2007; Ngo et al., 2006). *Amolops archotaphus*, *A. compotrix*, *A. cucae*, *A. daorum*, *A. iriodes*, and *A. vitreus* formed a monophyletic group with respect to other species of *Amolops* (see Stuart, 2008).

Members of the *A. monticola* group have posed vexing taxonomic problems at both shallow and deep taxonomic levels. Six of the putative 12 species in the group have been

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described since 2003 (and 10 of 12 since 1992) as a result of new fieldwork and re-evaluations of existing specimens in natural history collections, suggesting that species diversity in the group is only now being realized. All members of the *A. monticola* group share a very similar external adult morphology, and molecular tools are likely to be particularly helpful in identifying distinct evolutionary lineages (and species) in this group (Stuart et al., 2006). Generic allocation has also been problematic. Members of the *A. monticola* group are more similar in external adult morphology to some species in the genus *Odorrana* Fei, Ye and Huang, 1991, than they are to other *Amolops*. Owing to this similarity, *A. archotaphus*, *A. daorum*, and *A. iriodes* were thought to be close relatives of *O. livida* (Blyth, 1856) or *O. chloronota* (Günther, 1875) in their original descriptions (Bain and Nguyen, 2004; Bain et al., 2003; Inger and Chanard, 1997). Compounding this issue, Chen et al. (2005) obtained a mitochondrial DNA (mtDNA) sequence of *A. daorum* that closely matched a sequence of *O. hmongorum* (Bain et al., 2003); consequently, *daorum* was placed within the genus *Odorrana* and away from other species of *Amolops*. Ngo et al. (2006) and Cai et al. (2007) duplicated this finding by incorporating the same mitochondrial sequence, available through GenBank, in their analyses. In contrast, nuclear DNA sequences of a different sample of *daorum* placed this species deep within *Amolops* and close to other members of the *A. monticola* group (Stuart, 2008).

Recent fieldwork in northwestern Laos by three of us resulted in the discovery of a putative member of the *A. monticola* group that does not conform to any other described species. This discovery, and the taxonomic problems outlined above, motivated us to undertake a molecular phylogenetic analysis of all members of the *A. monticola* group for which tissues are available. This phylogeny is used to test the monophyly of the group within *Amolops*; evaluate evolutionary relationships among species in the group; determine whether the new Laos population represents a distinct evolutionary lineage; and address outstanding taxonomic problems in these frogs.

## MATERIALS AND METHODS

### Sampling

Institutional abbreviations follow Leviton et al. (1985), except ZISP = Zoological Institute of St. Petersburg (St. Petersburg, Russia), CUMZ = Chulalongkorn University Museum of Zoology (Bangkok, Thailand), and SCUM = Sichuan University Museum (Chengdu, China).

Specimens were collected by the authors in Laos, Vietnam, Cambodia, and Thailand and fixed in 10% buffered formalin after preserving tissue in 95% ethanol or 20% dimethyl sulfoxide salt-saturated storage buffer. Specimens were later transferred to 70% ethanol. Specimens and tissue samples were deposited at the FMNH and AMNH and were borrowed from the holdings of these and other institutions (Fig. 1; Table 1; Appendix I). In addition, data for *A. aniqiaoensis* were taken from Dong et al. (2005); for *A. chakrataensis* from Ray (1999); for *A. mengyangensis* from Ohler (2007), Wu and Tian (1995), and G. Wu (personal communication); for *A. monticola* from Anderson (1871) and Boulenger (1920); and for *A. bellulus* from Liu et al. (2000).

Eight of the 12 species putatively belonging to the *A. monticola* group were included in the molecular phylogenetic analyses. Missing were *A. aniqiaoensis* with type locality of Aniqiao, Mêdog County, Tibet (Dong et al., 2005); *A. chakrataensis* with type locality of Chakrata, Uttar Pradesh, India (Ray, 1992); *A. mengyangensis* with type locality of Mengyang, Yunnan Province, China (Wu and Tian, 1995); and the nominate *A. monticola* with type locality of Darjeeling, West Bengal, India (Anderson, 1871). The first three of the four unsampled species remain known only from the type specimens (but see Ohler, 2007). Samples from Sichuan Province, China, have conventionally been referred to *A. chunganensis* (e.g., Cai et al., 2007; Fei, 1999; Matsui et al., 2006; Ngo et al., 2006; Zhao and Adler, 1993), but the large geographic distance between Sichuan and the type locality in Fujian Province casts doubt on this identification. The Sichuan and Hubei Province samples are referred here to *A. cf. chunganensis* to indicate this taxonomic uncertainty. All species known from the vicinity of the new population in northwestern Laos (Laos, Thai-

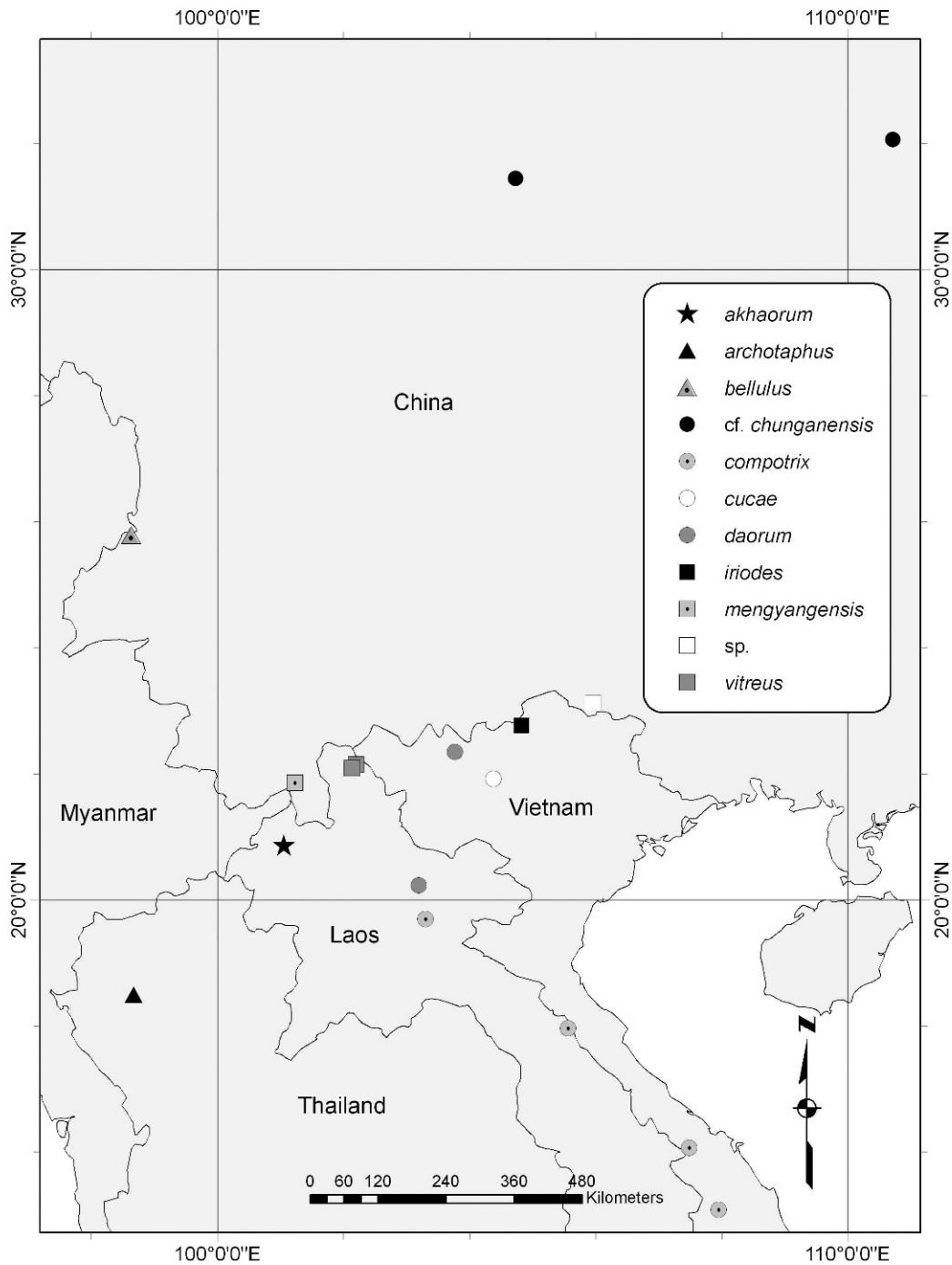


FIG. 1.—Map illustrating the type locality of *Amolops akhaorum* sp. nov. (star), the type locality of *A. mengyangensis* (square enclosing black dot), and sampling localities of tissues of the *A. monticola* group used in this study (remaining symbols).

land, Vietnam, and southern China), except *A. mengyangensis*, were sampled.

Six species of *Amolops* that represent the other major clades known within the genus (Cai et al., 2007; Matsui et al., 2006; Ngo et al., 2006) were included in the phylogenetic analyses to test the monophyly of the *A. monticola* group.

Multiple hypotheses of the sister clade to *Amolops* have been proposed (Bossuyt et al., 2006; Che et al., 2007; Chen et al., 2005; Frost et al., 2006; Stuart, 2008). Therefore, the mantellid *Mantella madagascariensis* and a diversity of ranids (sensu Frost et al., 2006) that represent most of the family's major clades (Bossuyt et al., 2006; Che et al., 2007; Chen et al., 2005; Frost et al., 2006; Stuart, 2008) were used as outgroups.

#### Molecular Data

Total genomic DNA was extracted from tissues using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc., Minneapolis, Minnesota, USA). A 1233-bp fragment of nuclear (nu) DNA that encodes part of the recombination activating protein 1 (RAG-1) gene and a 601-bp fragment of nu DNA that encodes part of exon 1 of the tyrosinase gene were amplified by the polymerase chain reaction (PCR) following Stuart (2008). A 597–605-bp fragment of mtDNA that encodes part of the 16S rRNA gene and a 1059–1063-bp fragment of mtDNA that encodes part of the tRNA methionine gene, the complete NADH dehydrogenase subunit 2 gene, and part of the tRNA tryptophan gene (ND2), and a 466–467-bp fragment of mtDNA that encodes part of the cytochrome oxidase c subunit III gene, the complete tRNA glycine, the complete NADH dehydrogenase subunit 3 gene, and part of the tRNA arginine (ND3) were amplified following Stuart et al. (2006). Three taxa (*A. archotaphus*, *A. cremnobatus*, and the new population from northwestern Laos) did not amplify for ND3 using these primers. Rather, these taxa were amplified under the same PCR conditions using the primers L-COXIII5' (Inger et al., 2009) and Arg-HND3III (Stuart et al., 2006). PCR products were electrophoresed, cycle-sequenced, precipitated, and sequenced following Stuart et al. (2006). Internal se-

quencing primers for RAG-1 followed Stuart (2008). The primers L-COXIII (Stuart et al., 2006) and H-GlyND3 (Inger et al., 2009) were used as internal sequencing primers in the ND3 fragment amplified from *A. archotaphus*, *A. cremnobatus*, and the northwestern Laos population. Sequences were edited and aligned using Sequencher® version 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA), and deposited in GenBank under accession numbers FJ417117–FJ417329 (Table 1).

Phylogenies were reconstructed under the maximum parsimony criterion and mixed-model Bayesian inference. A heuristic search for the most parsimonious tree was performed using PAUP\* 4.0b10 (Swofford, 2002) with equal weighting of nucleotide substitutions, stepwise addition with 10,000 random addition replicates, tree bisection-reconnection branch swapping, and gaps treated as missing data. Nodal support was evaluated with 1000 nonparametric bootstrap pseudoreplications (Felsenstein, 1985). Mixed model Bayesian analysis was performed using MrBayes 3.12 (Ronquist and Huelsenbeck, 2003). Data were partitioned by tRNA, rRNA, and codon position by gene. The cytochrome oxidase c subunit III gene was grouped with the NADH dehydrogenase subunit 3 gene due to its small size (17–18 bp per codon position). The model of sequence evolution that best described each of the 14 data partitions was inferred using the Akaike Information Criterion as implemented in MrModeltest 2.2 (Nylander, 2004). Four independent Bayesian analyses were performed. In each analysis, four chains were run for 15,000,000 generations using the default priors and temp = 0.1, trees were sampled every 3000 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. Trace plots of clade probabilities were viewed using AWTY (Wilgenbusch et al., 2004).

#### Morphological Data

Measurements were taken from studied voucher specimens (Table 1; Appendix I) with dial calipers to the nearest 0.1 mm. Abbreviations used are as follows: SVL = snout–vent length; HDL = head length from tip of snout to

TABLE 1.—Mitochondrial and nuclear DNA sequences used in this study. ID refers to terminals in Fig. 2.

| Species                          | ID | Source                  | Locality                                                                         | GenBank accession numbers |          |          |          |            |
|----------------------------------|----|-------------------------|----------------------------------------------------------------------------------|---------------------------|----------|----------|----------|------------|
|                                  |    |                         |                                                                                  | ND3                       | ND2      | 16S      | RAG-1    | Tyrosinase |
| <i>Analops abhaorum</i> sp. nov. | 1  | FMNH 271355<br>paratype | Laos, Luang Namtha Prov., Vieng Phou Kha Dist., 20° 52' 08.0" N 101° 03' 19.0" E | FJ417256                  | FJ417207 | FJ417158 | FJ417291 | FJ417324   |
| <i>Analops abhaorum</i> sp. nov. | 2  | FMNH 271406<br>holotype | Laos, Luang Namtha Prov., Vieng Phou Kha Dist., 20° 52' 08.0" N 101° 03' 19.0" E | FJ417257                  | FJ417208 | FJ417159 | FJ417292 | FJ417325   |
| <i>Analops abhaorum</i> sp. nov. | 3  | FMNH 271357<br>paratype | Laos, Luang Namtha Prov., Vieng Phou Kha Dist., 20° 52' 08.0" N 101° 03' 19.0" E | FJ417258                  | FJ417209 | FJ417160 | FJ417293 | FJ417326   |
| <i>Analops archotaphus</i>       | 4  | CUMZ A 2000.69          | Thailand, Chiang Mai Prov., Doi Inthanon, 18° 29' 54.1" N 98° 40' 33.7" E        | FJ417221                  | FJ417172 | FJ417123 | FJ417264 | FJ417297   |
| <i>Analops archotaphus</i>       | 5  | CUMZ A 2000.62          | Thailand, Chiang Mai Prov., Doi Inthanon, 18° 29' 54.1" N 98° 40' 33.7" E        | FJ417222                  | FJ417173 | FJ417124 | FJ417265 | FJ417298   |
| <i>Analops archotaphus</i>       | 6  | FMNH 261712             | Thailand, Chiang Mai Prov., Doi Inthanon, 18° 29' 54.1" N 98° 40' 33.7" E        | FJ417223                  | FJ417174 | FJ417125 | EF088234 | EU076756   |
| <i>Analops bellulus</i>          | 7  | CAS 233986              | China, Yunnan Prov., Teng Chong Co., 25° 47' 10.7" N 98° 36' 56.2" E             | FJ417224                  | FJ417175 | FJ417126 | FJ417266 | FJ417299   |
| <i>Analops bellulus</i>          | 8  | CAS 233991              | China, Yunnan Prov., Teng Chong Co., 25° 47' 7.2" N 98° 36' 45.9" E              | FJ417225                  | FJ417176 | FJ417127 | FJ417267 | FJ417300   |
| <i>Analops cf. chunganensis</i>  | 9  | KIZ YP 0202-1           | China, Hubei Prov., Fangxian Co.                                                 | FJ417226                  | FJ417177 | FJ417128 | FJ417268 | FJ417301   |
| <i>Analops cf. chunganensis</i>  | 10 | SCUM 045818HX           | China, Sichuan Prov., Anxian Co.                                                 | FJ417228                  | FJ417179 | FJ417130 | FJ417270 | FJ417303   |
| <i>Analops compotrix</i>         | 11 | FMNH 271369             | Laos, Xieng Khouang Prov., Pek Dist., 19° 42' 07.8" N 103° 18' 05.3" E           | FJ417229                  | FJ417180 | FJ417131 | FJ417271 | FJ417304   |
| <i>Analops compotrix</i>         | 12 | AMNH 169315             | Vietnam, Thua Tien Hue Prov., A Luoi Dist., 16° 04' 28" N 107° 29' 14" E         | FJ417230                  | FJ417181 | FJ417132 | FJ417272 | FJ417305   |
| <i>Analops compotrix</i>         | 13 | ZISP A7367<br>paratype  | Vietnam, Kon Tum Prov., Dak Glei Dist., 15° 05' 30" N 107° 57' 10" E             | FJ417240                  | FJ417191 | FJ417142 | FJ417281 | FJ417314   |
| <i>Analops compotrix</i>         | 14 | FMNH 256496<br>paratype | Laos, Khammouan Prov., Nakai Dist., 17° 58' N 105° 34' E                         | FJ417231                  | FJ417182 | FJ417133 | FJ417273 | FJ417306   |
| <i>Analops compotrix</i>         | 15 | FMNH 256501<br>paratype | Laos, Khammouan Prov., Nakai Dist., 17° 58' N 105° 34' E                         | FJ417235                  | FJ417186 | FJ417137 | FJ417277 | FJ417310   |
| <i>Analops compotrix</i>         | 16 | FMNH 256502<br>paratype | Laos, Khammouan Prov., Nakai Dist., 17° 57' N 105° 34' E                         | FJ417236                  | FJ417187 | FJ417138 | FJ417278 | FJ417311   |
| <i>Analops compotrix</i>         | 17 | FMNH 256500<br>holotype | Laos, Khammouan Prov., Nakai Dist., 17° 58' N 105° 34' E                         | FJ417239                  | FJ417190 | FJ417141 | EF088235 | EU076757   |
| <i>Analops compotrix</i>         | 18 | FMNH 256504<br>paratype | Laos, Khammouan Prov., Nakai Dist., 17° 57' N 105° 34' E                         | FJ417238                  | FJ417189 | FJ417140 | FJ417280 | FJ417313   |
| <i>Analops compotrix</i>         | 19 | FMNH 256497<br>paratype | Laos, Khammouan Prov., Nakai Dist., 17° 58' N 105° 34' E                         | FJ417232                  | FJ417183 | FJ417134 | FJ417274 | FJ417307   |
| <i>Analops compotrix</i>         | 20 | FMNH 256498<br>paratype | Laos, Khammouan Prov., Nakai Dist., 17° 58' N 105° 34' E                         | FJ417233                  | FJ417184 | FJ417135 | FJ417275 | FJ417308   |

TABLE 1.—Continued.

| Species                         | ID | Source                  | Locality                                                                  | GenBank accession numbers |          |          |          |            |
|---------------------------------|----|-------------------------|---------------------------------------------------------------------------|---------------------------|----------|----------|----------|------------|
|                                 |    |                         |                                                                           | ND3                       | ND2      | 16S      | RAG-1    | Tyrosinase |
| <i>Analops compotrix</i>        | 21 | FMNH 256499<br>paratype | Laos, Khammouan Prov., Nakai Dist.,<br>17° 58' N 105° 34' E               | FJ417234                  | FJ417185 | FJ417136 | FJ417276 | FJ417309   |
| <i>Analops compotrix</i>        | 22 | FMNH 256503<br>paratype | Laos, Khammouan Prov., Nakai Dist.,<br>17° 57' N 105° 34' E               | FJ417237                  | FJ417188 | FJ417139 | FJ417279 | FJ417312   |
| <i>Analops crennobatus</i>      | 23 | FMNH 258377             | Vientiane Prov., Kasi Dist., 19° 04' 39"<br>N 102° 08' 44" E              | FJ417241                  | FJ417192 | FJ417143 | FJ417282 | FJ417315   |
| <i>Analops cucae</i>            | 24 | AMNH 168729<br>paratype | Vietnam, Lao Cai Province, Van Ban Dist.,<br>21° 55' 23" N 104° 22' 43" E | FJ417243                  | FJ417194 | FJ417145 | FJ417284 | FJ417317   |
| <i>Analops cucae</i>            | 25 | AMNH 168727<br>paratype | Vietnam, Lao Cai Province, Van Ban Dist.,<br>21° 55' 23" N 104° 22' 43" E | FJ417242                  | FJ417193 | FJ417144 | FJ417283 | FJ417316   |
| <i>Analops cucae</i>            | 26 | AMNH 168726<br>paratype | Vietnam, Lao Cai Province, Van Ban Dist.,<br>21° 55' 23" N 104° 22' 43" E | FJ417244                  | FJ417195 | FJ417146 | EF088237 | EU076759   |
| <i>Analops daorum</i>           | 27 | FMNH 255353<br>paratype | Laos, Huaphanh Prov., Vieng Tong Dist.,<br>20° 14' N 103° 12' E           | FJ417245                  | FJ417196 | FJ417147 | FJ417285 | FJ417318   |
| <i>Analops daorum</i>           | 28 | FMNH 255354             | Laos, Huaphanh Prov., Vieng Tong Dist.,<br>20° 14' N 103° 12' E           | FJ417246                  | FJ417197 | FJ417148 | FJ417286 | FJ417319   |
| <i>Analops daorum</i>           | 29 | FMNH 255355             | Laos, Huaphanh Prov., Vieng Tong Dist.,<br>20° 14' N 103° 12' E           | FJ417247                  | FJ417198 | FJ417149 | FJ417287 | FJ417320   |
| <i>Analops daorum</i>           | 30 | ROM 38501<br>paratype   | Vietnam, Lao Cai Prov., Sa Pa, 22° 20' 58" N<br>103° 46' 14" E            | FJ417248                  | FJ417199 | FJ417150 | EF088238 | EU076760   |
| <i>Analops daorum</i>           | 31 | ROM 38503<br>paratype   | Vietnam, Lao Cai Prov., Sa Pa, 22° 20' 58" N<br>103° 46' 14" E            | FJ417249                  | FJ417200 | FJ417151 | FJ417288 | FJ417321   |
| <i>Analops triodes</i>          | 32 | AMNH 163926<br>paratype | Vietnam, Ha Giang Prov., Vi Xuyen Dist.,<br>22° 46' 10" N 104° 49' 53" E  | FJ417250                  | FJ417201 | FJ417152 | FJ417289 | FJ417322   |
| <i>Analops triodes</i>          | 33 | AMNH 163928<br>paratype | Vietnam, Ha Giang Prov., Vi Xuyen Dist.,<br>22° 46' 10" N 104° 49' 53" E  | FJ417251                  | FJ417202 | FJ417153 | FJ417290 | FJ417323   |
| <i>Analops triodes</i>          | 34 | AMNH 163925<br>holotype | Vietnam, Ha Giang Prov., Vi Xuyen Dist.,<br>22° 46' 10" N 104° 49' 53" E  | FJ417252                  | FJ417203 | FJ417154 | EF088239 | EU076761   |
| <i>Analops mantzorum</i>        | 35 | FMNH 233124             | China, Sichuan Prov., Hongya Xian Co.                                     | FJ417253                  | FJ417204 | FJ417155 | EF088240 | EO076762   |
| <i>Analops marmoratus</i>       | 36 | CAS 230228              | Myanmar, Kachin State, Putao Dist.,<br>27° 16' 19.4" N 97° 35' 18.0" E    | FJ417254                  | FJ417205 | FJ417156 | EF088241 | EU076763   |
| <i>Analops ricketti</i>         | 37 | AMNH 168687             | Vietnam, Lao Cai Prov., Van Ban Dist.,<br>21° 55' 23" N 104° 22' 43" E    | FJ417255                  | FJ417206 | FJ417157 | EF088242 | EU076764   |
| <i>Analops spinapectoralis</i>  | 38 | FMNH 254436             | Vietnam, Gia Lai Prov., An Khe Dist.                                      | FJ417259                  | FJ417210 | FJ417161 | EF088243 | EU076765   |
| <i>Analops</i> sp.              | 39 | KU 292045               | China, Guangxi Prov., Jing Xin Co.,<br>23.1222992° N 105.9636002° E       | FJ417257                  | FJ417178 | FJ417129 | FJ417269 | FJ417302   |
| <i>Analops tubero depressus</i> | 40 | CAS 234058              | China, Yunnan Prov., Teng Chong Co.,<br>25° 47' 40.2" N 98° 37' 45.5" E   | FJ417260                  | FJ417211 | FJ417162 | FJ417294 | FJ417327   |



TABLE 1.—Continued.

| Species                          | ID | Source                  | Locality                                                                    | GenBank accession numbers |           |           |          |            |
|----------------------------------|----|-------------------------|-----------------------------------------------------------------------------|---------------------------|-----------|-----------|----------|------------|
|                                  |    |                         |                                                                             | ND3                       | ND2       | 16S       | RAG-1    | Tyrosinase |
| <i>Anolops citreus</i>           | 41 | FMNH 258183<br>paratype | Laos, Phongsaly Prov., Phongsaly Dist.,<br>22° 09' 04" N 102° 12' 19" E     | FJ417261                  | FJ417212  | FJ417163  | FJ417295 | FJ417328   |
| <i>Anolops citreus</i>           | 42 | FMNH 258187<br>paratype | Laos, Phongsaly Prov., Phongsaly Dist.,<br>22° 05' 44" N 102° 08' 10" E     | FJ417262                  | FJ417213  | FJ417164  | FJ417296 | FJ417329   |
| <i>Anolops citreus</i>           | 43 | FMNH 258180<br>paratype | Laos, Phongsaly Prov., Phongsaly Dist.,<br>22° 05' 38" N 102° 12' 50" E     | FJ417263                  | FJ417214  | FJ417165  | EF088244 | EU076767   |
| <i>Huia cavatimpanum</i>         | 44 | FMNH 237299<br>paratype | Malaysia, Sabah, Tenom Dist.                                                | FJ417216                  | FJ417167  | FJ417118  | EF088246 | EU076769   |
| <i>Mantella madagascariensis</i> | 45 | GenBank                 | Madagascar                                                                  | NC_007888                 | NC_007888 | NC_007888 | DQ019500 | AF249164   |
| <i>Meristogenys whiteheadi</i>   | 46 | FMNH 238286             | Malaysia, Sabah, Tenom Dist.                                                | FJ417217                  | FJ417168  | FJ417119  | EF088253 | EU076776   |
| <i>Odorrana hmongorum</i>        | 47 | ROM 26370<br>paratype   | Vietnam, Lao Cai Prov., Sa Pa, 22° 20' 09" N<br>103° 50' 14" E              | FJ417218                  | FJ417169  | FJ417120  | EF088258 | EU076781   |
| <i>Rana johnsi</i>               | 48 | FMNH 261882             | Cambodia, Mondolkiri Prov., Pichrada Dist.,<br>12° 29' 49" N 107° 29' 33" E | FJ417219                  | FJ417170  | FJ417121  | EF088271 | EU076799   |
| <i>Staurois latopalmatus</i>     | 49 | FMNH 239088             | Malaysia, Sabah, Tenom Dist.                                                | FJ417215                  | FJ417166  | FJ417117  | EF088277 | EU076805   |
| <i>Sylvirana cubitalis</i>       | 50 | FMNH 265818             | Thailand, Loei Prov., Phu Rua Dist.,<br>17° 20' 02.8" N 101° 30' 32.4" E    | FJ417220                  | FJ417171  | FJ417122  | EF088267 | EU076793   |

rear of the jaws; HDW = maximum head width; IND = internarial distance; SNT = snout length from tip of snout to the anterior corner of the eye; EYE = diameter of the exposed portion of the eyeball; IOD = interorbital distance at narrowest point; TMP = horizontal diameter of tympanum; TEY = tympanum–eye distance from anterior edge of tympanum to posterior corner of the eye; TIB = tibia length; FEM = femur length, from vent to outer edge of knee; HND = hand length, from base of palm to tip of Finger III; F3DSC = width of Finger III digital disc; and FTL = foot length, from proximal edge of inner metatarsal tubercle to tip of fourth toe. All measurements were taken by the second author for consistency.

RESULTS

Molecular Data

All genes were obtained from all individuals, except only 121 bp of the ND3 fragment (containing the cytochrome oxidase c subunit III gene and the tRNA glycine, but missing the NADH dehydrogenase subunit 3 gene and the tRNA arginine) was obtained from the new northwestern Laos population and *A. archotaphus* owing to a putative mitochondrial gene rearrangement.

The results of the parsimony and Bayesian analyses were highly concordant (Fig. 2). The *A. monticola* group was resolved as a well-supported monophyletic group with respect to remaining species of *Amolops*. Paratypes of *A. daorum* were deeply nested within *Amolops* as members of the *A. monticola* group and were phylogenetically unrelated to a paratype of *O. hmongorum*. All species represented by more than one individual were found to be monophyletic, except *A. daorum* was paraphyletic with respect to *A. iriodes*.

The northwestern Laos population was found to be a distinct lineage within the *A. monticola* group and the sister lineage to *A. archotaphus*, with which it shares a putative mitochondrial gene rearrangement (Fig. 2). Uncorrected pairwise sequence distances between the northwestern Laos population and its sister taxon *A. archotaphus* were 0.08–0.24% for RAG-1, 0.67% for tyrosinase, 4.67% for 16S, and 13.18–13.28% for ND2.



*Amolops bellulus* was found to be the sister taxon to all other species in the *A. monticola* group. The remaining species were recovered in two major clades. The first clade contained *A. archotaphus*, the new population from north-western Laos, *A. daorum*, and *A. iriodes*. The second clade contained *A. vitreus*, *A. cucae*, *A. compotrix*, and an unnamed lineage from Guangxi, China. The most northerly sampled species, *A. cf. chunganensis*, was resolved as the sister taxon to the second clade in the Bayesian analysis, but this relationship was unresolved in the parsimony analysis (Fig. 2).

### Morphological Data

The northwestern Laos population differed from all other named species in the *A. monticola* group by having large, raised, round, black tubercles on an otherwise smooth dorsum, and by having black pigment, without a nuptial pad, on the base of Finger I. This population is therefore genetically and morphologically distinct from all other known species. These independent data sets provide a corroborated hypothesis that this population represents a species, which is described here as new, as follows.

#### *Amolops akhaorum* sp. nov.

**Holotype.**—FMNH 271406 (field number BLS 10528), adult male, from Laos, Luang Namtha Province, Vieng Phou Kha District, Nam Ha National Protected Area, Nam Maye Stream, near 20° 52' 08.0" N 101° 03' 19.0" E, 1000-m elevation, collected 22 March 2007 by Bryan L. Stuart, Somphouthone Phimmachak, and Kelly Spence (Figs. 3 and 4).

**Paratypes.**—Eight adult males (FMNH 271355, 271357, 271364, 271398–400, 271407, 271408) from the type locality, collected 21–28 March 2007 by the same collectors.

**Diagnosis.**—An *Amolops* having the combination of smooth skin, with large, raised, round, black tubercles on an otherwise smooth dorsum; glandular dorsolateral fold, without spinules; side of head black (dark brown in preservative) from snout tip to posterior of axilla, continuing as a thin band below dorsolateral fold to near sacrum; gold (creamy white in preservative) upper lip stripe extending to posterior of axilla; gular pouches; and males with SVL 34.9–37.2.

**Comparisons.**—The new species differs from all other members of the *A. monticola* group (defined above) by having large, round, raised, black dorsal tubercles on an otherwise smooth dorsum, and black pigment, without a nuptial pad, on the base of Finger I. *Amolops akhaorum* is most closely related to *A. archotaphus*, *A. daorum*, and *A. iriodes* (Fig. 2) but further differs from these species by lacking at least one large white gland on flank (present in *daorum* and *iriodes*); lacking distinct white spinules on the dorsolateral fold and in a cluster on the temporal region, tympanic region, and posterior corner of the upper eyelid (present in *daorum*); and lacking an outer metatarsal tubercle (present in *archotaphus*).

*Amolops akhaorum* superficially resembles some species in the genus *Odorrana* but differs by having Finger I shorter than Finger II (opposite condition in *Odorrana*, except for *O. chapaensis*). *Amolops akhaorum* differs from *O. chapaensis* by having much smaller males (*akhaorum* SVL 34.9–37.2; *chapaensis* SVL > 70) and dorsolateral folds (absent in *chapaensis*).

**Description of holotype.**—Habitus moderately slender; head narrow, longer than wide; snout obtusely pointed in dorsal view, projecting beyond lower jaw, round in profile, not depressed; nostril lateral, slightly closer to eye than tip of snout; canthus rostralis distinct, slightly constricted behind nostrils; lores concave and oblique; eye diameter 72% of snout length; interorbital distance greater than width of upper eyelid; pineal body visible; distinct, round tympanum, covered by layer of skin, 46% eye diameter, not depressed relative to skin of temporal region, tympanic rim not elevated relative to tympanum; vomerine teeth weakly developed, on two oblique, crescentic ridges, closer to each other than choanae; tongue cordiform, deeply notched posteriorly, free for approximately two thirds its length; vocal sac opening on floor of mouth at corner; sac-like gular pouch, front margin positioned near to level of center of orbit.

Tips of all four fingers expanded with circummarginal grooves, horizontal grooves absent; width of Finger III disc about one and a half times width of phalanx, approximately

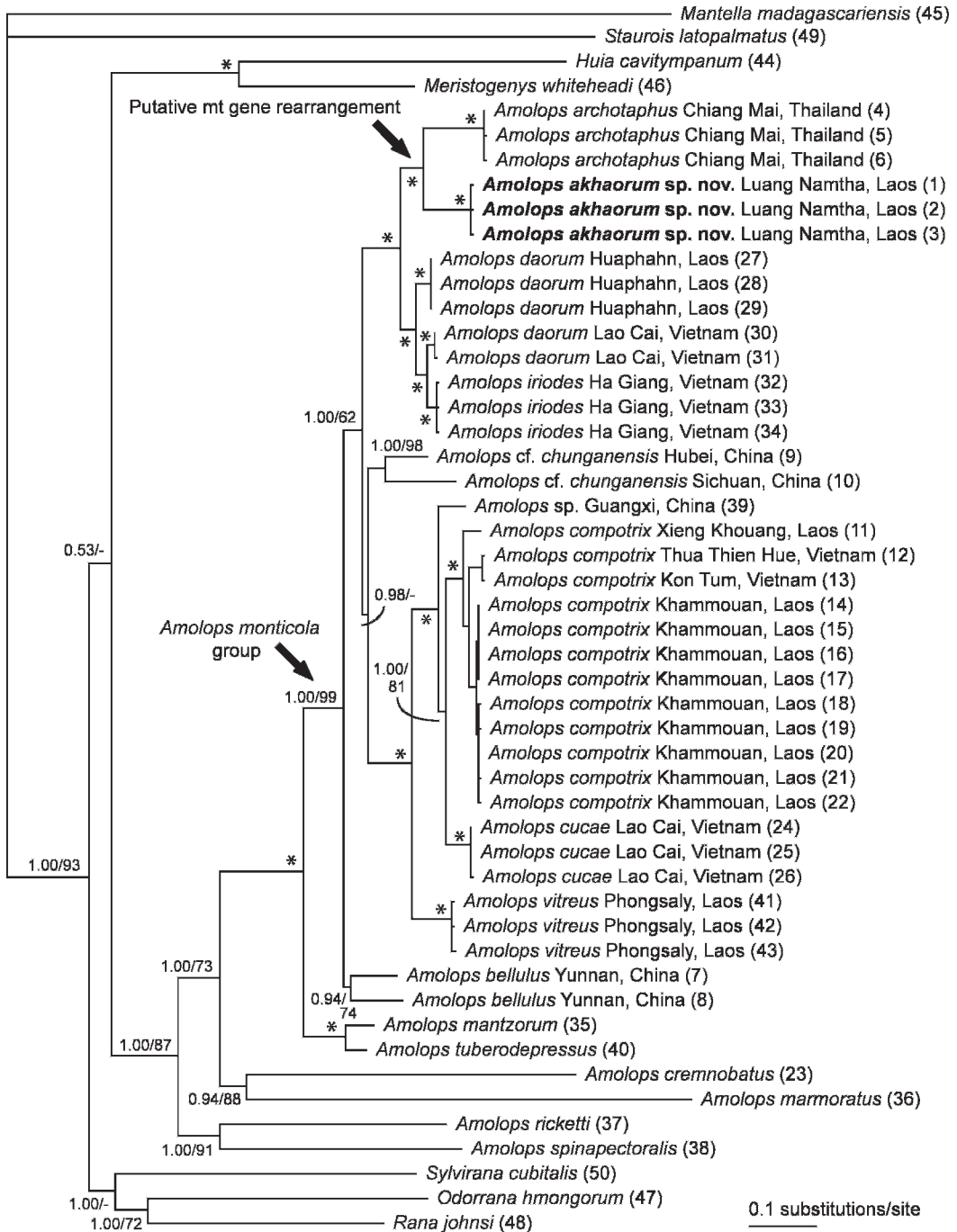


FIG. 2.—Fifty percent majority-rule consensus phylogram resulting from mixed model Bayesian analysis of mitochondrial and nuclear DNA of frogs of the genus *Amolops* and outgroups. Numbers at nodes are Bayesian posterior probabilities/parsimony bootstrap values > 50. An asterisk (\*) indicates a Bayesian posterior probability of 1.00 and parsimony bootstrap value of 100.



FIG. 3.—Holotype of *Amolops akhaorum* (FMNH 271406) in life.

80% diameter of tympanum; relative finger lengths  $I < II < IV < III$ ; ventral callous pad on Fingers II, III, and IV from distal edge of proximal subarticular tubercle to base of disc; no movable flap of skin on preaxial side of II and III; one subarticular tubercle on I and II, two subarticular tubercles on III and IV; one supernumerary tubercle proximal to proximal subarticular tubercle on II, III, and IV; no palmar tubercles; large, glandular nuptial excrecence on preaxial side of I, skin not differentiated into a pad; forearm robust.

Tips of toes expanded, width of Toe IV disc less than Finger III disc; Toe III shorter than V; Toes I, II, V, and postaxial side of III fully webbed to base of discs; Toe IV and preaxial side of III fully webbed to distal subarticular tubercle with narrow extension to base of disc; movable flap of skin on preaxial side of Toe I and postaxial side of V to level of proximal subarticular tubercle; elongate, oval inner metatarsal tubercle; no outer metatarsal tubercle.

Skin smooth on all surfaces except lightly granular on posterior surface of thigh; large, raised, round, glandular tubercles on otherwise smooth dorsum, lower in preservative; no supratympanic fold; one low rictal gland, continuous with upper lip; no humeral gland; dorsolateral fold distinct, glandular, from rear of tympanum to near vent.

Measurements (mm) of holotype: SVL 37.2; HDL 16.5; HDW 11.8; IND 4.5; SNT 5.8; EYE 4.1; IOD 3.7; TMP 1.9; TEY 1.0; TIB 21.8; FEM 20.4; HND 11.3; F3DSC 1.5; FTL 19.2.

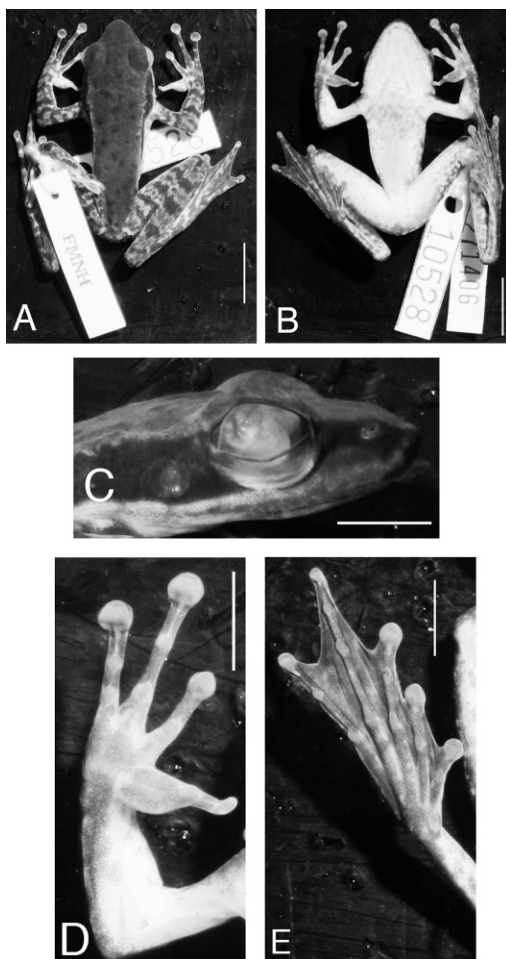


FIG. 4.—Holotype of *Amolops akhaorum* (FMNH 271406) in preservative: (A) dorsal and (B) ventral view of body (scale = 10 mm); (C) head in lateral view (scale = 5 mm); (D) palmar view of right hand (scale = 5 mm); (E) palmar view of right foot (scale = 5 mm).

*Coloration of holotype in life.*—Dorsum dark green, with raised black tubercles; pineal body same color as dorsum; narrow gold stripe on canthus, from tip of snout along margin of upper eyelid, continuing above edge of dorsolateral fold to sacrum; side of head black from snout tip, diffusing posterior to axilla, continuing as a thin band below dorsolateral fold, diffusing anterior to sacrum; cream blotch with brown dusting in contact with upper lip at base of lores; gold upper lip stripe, dusted with brown from snout tip to level of eye, becoming light gold without dusting from level of eye to posterior edge of

arm insertion; iris bronze, with red wash at posterior and anterior edges of pupil; upper portion of flank dark green with black spots, merging with black tympanic region anterior to level of arm insertion, becoming a black and green network posteriorly; ventral margin of flank marbled black with cream; dorsal surface of limbs bronze, as in dorsolateral fold, with black banding and some yellow marbling; gold spots on upper surface of fingers and finger and toe discs; black humeral spot; anterior portion of thigh marbled black with yellow-green; venter creamy with dark gray marbling; ventral surfaces of limbs greenish gray.

*Coloration of holotype in preservative.*—Dorsum fades to dark gray-blue, with slightly raised, black tubercles; pineal spot white; narrow stripe from canthus to sacrum fades to tan; side of head and lower portion of dorsolateral fold fades to dark brown; anterior portion of lip line mottled dark gray on cream, posterior portion of lip line creamy white with less mottling; upper half of flank dark gray-blue, as on dorsum, lower half mottled with cream; limbs banded dark brown on stippled dark gray-blue; dark gray-blue humeral spot on anterior portion of arm; nuptial excrescence on Finger I with distinct black pigment and yellow subcutaneous glands; axial area white; anterior surface of thigh near groin dark brown-black, with whitish gray marbling; inguinal area creamy white with dark gray mottling; posterior surface of thigh black with creamy marbling; yellow subcutaneous glands concentrated on axillary region; throat speckled dusty brown; chest and upper portion of belly spotted brown; ventral surface of hands, feet dark brown, hindlimbs fade to creamy white with dark brown speckling; foot webbing gray with dark gray flecking, outer margin white.

*Variation.*—Measurements of paratypes summarized in Table 2. All paratypes conform to description of holotype, with the following exceptions. FMNH 271408 with weak supratympanic fold on right side. Rictal glands as one strong gland on each side (FMNH 271400), two on each side (FMNH 271399), one on right side (FMNH 271398), one on left side (FMNH 271407), one on right side, two on left side (FMNH 271364), three on right

TABLE 2.—Measurements (mm) of adult male type specimens of *Amolops akhaorum* sp. nov. Abbreviations defined in the text.

| Measurement | Range; mean $\pm$ SD ( $n = 9$ ) |
|-------------|----------------------------------|
| SVL         | 34.9–37.2; 36.1 $\pm$ 0.7        |
| HDL         | 13.3–16.5; 14.4 $\pm$ 1.2        |
| HDW         | 10.9–11.9; 11.4 $\pm$ 0.4        |
| IND         | 3.8–4.5; 4.1 $\pm$ 0.2           |
| SNT         | 5.4–6.4; 5.8 $\pm$ 0.3           |
| EYE         | 4.0–4.9; 4.4 $\pm$ 0.3           |
| IOD         | 3.5–4.0; 3.8 $\pm$ 0.2           |
| TMP         | 1.9–2.4; 2.1 $\pm$ 0.2           |
| TEY         | 0.9–1.5; 1.2 $\pm$ 0.2           |
| TIB         | 21.0–22.2; 21.6 $\pm$ 0.4        |
| FEM         | 19.4–21.8; 20.6 $\pm$ 0.8        |
| HND         | 11.3–11.9; 11.7 $\pm$ 0.2        |
| F3DSC       | 1.4–1.9; 1.6 $\pm$ 0.2           |
| FTL         | 18.9–19.9; 19.4 $\pm$ 0.3        |
|             | Range; Median ( $n = 9$ )        |
| HDL:HDW     | 1.17–1.43; 1.23                  |
| SNT:HDL     | 0.35–0.46; 0.41                  |
| TMP:EYE     | 0.46–0.55; 0.48                  |
| EYE:SNT     | 0.67–0.87; 0.77                  |
| TIB:SVL     | 0.58–0.64; 0.59                  |
| HND:SVL     | 0.30–0.33; 0.32                  |
| FTL:SVL     | 0.52–0.56; 0.54                  |
| HDL:SVL     | 0.37–0.47; 0.38                  |
| F3DSC:TMP   | 0.62–0.92; 0.73                  |

side, two on left side (FMNH 271408), or absent (271355, 271357). Weak outer metatarsal tubercle present on both feet (FMNH 271355, 271399, 271407), left foot only (FMNH 271400), or right foot only (FMNH 271398, 271408). In preservative, pineal spot same color as adjacent area on head in FMNH 271355. Upper lip stripe extends to mid-level of flank in FMNH 271364, 271398, 271407. Lower portion of flank light gray with yellow marbling in FMNH 271355. Yellow subcutaneous glands present on Finger I and intermandibular and axillary regions of FMNH 271355, 271357, 271400, 271407.

*Etymology.*—The specific epithet honors the ethnic Akha people of Ban Phou Yea Mai Village who greatly assisted the fieldwork that resulted in discovery of the new species.

*Remarks.*—All individuals were collected at night (1921 to 2230 h) in hill evergreen forest perched on rocks, plant leaves, a mossy vine, or a fallen tree trunk 0.25–1.5 m above 0.5–4-m-wide swift, rocky sections of the Nam Maye Stream (Fig. 5) and its smaller tributaries. None were observed calling. Females, eggs, and larvae of the new species are unknown.





FIG. 5.—Nam Maye Stream, the type locality of *Amolops akhaorum*.

#### DISCUSSION

##### *Phylogenetic Relationships of the A. monticola Group*

Species of *Amolops* united by having the combination of smooth skin, the side of head dark with a light-colored upper lip stripe extending to the shoulder, and a dorsolateral fold were found to be a natural group, although only two thirds of the approximately 12 species in the group were sampled in this study. The phylogenetic positions of the four missing species *A. aniqiaoensis*, *A. chakrataensis*, *A. mengyangensis*, and the nominate *A. monticola* remain untested, although their inclusion in this group is likely owing to their great similarity in external morphology with verified members of the group.

The *A. monticola* group exhibits a complicated pattern of diversification, with phylogenetically unrelated species occurring in geographic proximity, e.g., *A. daorum* to *A.*

*compotrix* and *A. cucae* (Fig. 1). At least six species occur near the Laos–Vietnam frontier with China. No members of the *A. monticola* group are currently known to occur in sympatry. Most species in the *A. monticola* group have relatively small known geographic distributions. *Amolops compotrix* has the largest known distribution within Indochina, extending from Kon Tum Province in central Vietnam to Xieng Khouang Province (a new provincial record reported here) in northeastern Laos (Fig. 1).

##### *Taxonomic Status of Amolops daorum Bain, Lathrop, Murphy, Orlov and Ho, 2003*

The five individuals of *A. daorum* (including two paratypes) used in this study were phylogenetically placed into *Amolops* rather than *Odorrana*, in agreement with Stuart (2008) but in disagreement with Chen et al. (2005), Ngo et al. (2006), and Cai et al. (2007). However, the mitochondrial sequence of

"*daorum*" reported by Chen et al. (2005), and subsequently used by Ngo et al. (2006) and Cai et al. (2007) via GenBank download, is in error. Chen et al.'s (2005) specimen of "*daorum*" (ROM field number 19053 = ROM 26374) is actually a female paratype of *O. hmongorum*; consequently, that sequence closely matches a second sequence of *O. hmongorum* used in the same studies (uncorrected pairwise distance of 0.36%).

Ohler (2007) synonymized *A. daorum* with *A. mengyangensis*, a species known only from the type series of two males and one female collected in 1957, on the basis of their superficial similarity and overlap in body size measurements. Using the same criteria, most species in the *A. monticola* group could also be synonymized. *Amolops daorum* is readily differentiated from *A. mengyangensis* by having smaller males (*daorum* SVL 32.0–38.1; *mengyangensis* SVL 39.0–40.0); at least one large white gland on flank (absent in *mengyangensis*); distinct white spinules on the dorsolateral fold and in a cluster on the temporal region, tympanic region, and posterior corner of the upper eyelid (absent in *mengyangensis*); and a humeral spot (absent in *mengyangensis*; Bain et al., 2003, 2006; Wu and Tian, 1995; G. Wu, personal communication). The male type specimens of both species have nuptial pads and presumably were in breeding condition when collected. The taxonomic status of *A. mengyangensis* is uncertain owing to limited information in its original description and lack of fresh material (notably tissues), but the evidence strongly suggests that it is not conspecific with *A. daorum*. We therefore remove *A. daorum* from the synonymy of *A. mengyangensis*.

#### *Taxonomic Status of Amolops iriodes Bain and Nguyen, 2004*

This study found that paratypes of *A. daorum* from northern Vietnam were more closely related to the holotype and paratypes of *A. iriodes* from northern Vietnam than to other samples of *A. daorum* from northern Laos. There are three possible explanations for this pattern. First, *A. daorum* may be a complex of at least two species. We found no morphological evidence separating the Vietnamese and Lao populations of *A. daorum* to support this

explanation. Second, *A. iriodes* may be a junior synonym of *A. daorum*. *Amolops daorum* is readily distinguished from *A. iriodes* by having distinct white spinules on the dorsolateral fold and in a cluster on the temporal region, tympanic region, and posterior corner of the upper eyelid, which are absent in *A. iriodes* (Bain et al., 2003, 2006). The type series of *A. daorum* and *A. iriodes* were collected in northern Vietnam in early to mid-May, and both type series contained males with nuptial pads and gravid females (Bain et al., 2003; Bain and Nguyen, 2004), implying that the presence of spinules is not an artifact of seasonality. Third, *A. daorum* and *A. iriodes* may both be valid species, but *A. daorum* has not yet achieved monophyly with respect to *A. iriodes*. Nonmonophyletic species are common, often because of incomplete lineage sorting (Funk and Omland, 2003). Pending further information, *A. daorum* and *A. iriodes* are recognized here as distinct species on the basis of their morphological distinctiveness. Further investigations into the relationship of these two species are warranted, and data on advertisement calls and larvae (currently unknown for both species) may be particularly helpful in resolving this taxonomic uncertainty.

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- ## APPENDIX I
- ### *Specimens Examined*
- Amolops archotaphus*: Thailand, Chiang Mai Province, Doi Inthanon: FMNH 214074, male holotype; FMNH 187447–48, 214073, 214075–76, 216072–73, male paratypes; FMNH 214072, 216074, female paratypes.
- Amolops bellulus*: China, Yunnan Province, Teng Chong County: CAS 233986, 233991, males.
- Amolops chunganensis*: China, Fujian (=Fukien) Province, Kuatun: AMNH 30479, male holotype; AMNH 30407, 30418–19, 30423, 30434, 30443, 30448, 30453, 30456, 30461, 30466, 30481–82, male paratypes; FMNH 24658, female paratype.
- Amolops cucae*: Vietnam, Lao Cai Province, Van Ban District: AMNH 168730, male holotype; AMNH 168729, 168731, 168803/IEBR 1582 male paratypes; AMNH 168726–28, 168802/IEBR 1583, female paratypes.
- Amolops compotrix*: Laos, Khammouan Province, Nakai District: FMNH 256500, male holotype; FMNH 256496–99, 256501–02, 256504 male paratypes; FMNH 256503 female paratype; Laos, Xieng Khouang Province, Pek District: FMNH 271369, male. Vietnam, Kon Tum Province, Dak Glei District: ZISP A7365, A7367–69, male paratypes; A7364, A7366, female paratypes.
- Amolops daorum*: Vietnam, Lao Cai Province, Sa Pa: ROM 26381, female holotype, ROM 38500, 38503, 38507, 38512, 38516–17, 38526, 38530, 38538, female paratypes; ROM 26382–97, 38501–02, 38504–38506, 38508–38511, 38513–38515, 38518–25, 38527–29, 38532–37, 38539–40, 38542–43, 38546, 38548–61, male paratypes. Laos, Huaphahn Province, Vieng Tong District: FMNH 255353–55, males.
- Amolops iriodes*: Vietnam, Ha Giang Province, Vi Xuyen District: AMNH 163925/IEBR 70, male holotype; AMNH 163924/IEBR 69, AMNH 163928, male paratypes; AMNH 163926, female paratype.
- Amolops monticola*: India, West Bengal, Darjeeling: CAS 15362, male.
- Amolops vitreus*: Laos, Phongsaly Province, Phongsaly District: FMNH 258182, male holotype; FMNH 258180, 258183–87, male paratypes.

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