A New Species of Amolops from Thailand (Amphibia, Anura, Ranidae)

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We describe a new species of torrent-dwelling ranid frog of the genus Amolops from western to peninsular Thailand. Amolops panhai, new species, differs from its congeners by the combination of: small body, males 31–34 mm, females 48–58 mm in snout-vent length; head narrower than long; tympanum distinct; vomerine teeth in short, oblique patches; first finger subequal to second; disc of first finger smaller than that of second, with circumbasal groove; no wide fringe of skin on third finger; toes fully webbed; outer metatarsal tubercle present; supratympanic fold present; dorsolateral fold indistinct; axillary gland present; horny spines on back, side of head and body, and chest absent; large tubercles on side of anus absent; glandular fold on ventral surface of tarsus absent; nuptial pad and paired gular pouches present in male; white band along the upper jaw extending to shoulder absent; larval dental formula 7(4-7)/3(1). This new species is the second anuran discovered which has a disjunct distribution around the Isthmus of Kra.

Key words: Amolops, new species, Southeast Asia, tadpole, taxonomy, zoogeography

INTRODUCTION

Oriental ranid frogs related to Amolops Cope, 1865 (sensu lato) are characterized by their peculiar larvae, which inhabit mountain torrents using an abdominal, suctorial disk (Inger, 1966). After Inger (1966) confirmed this genus as valid, Yang (1991) split it into the genera Amolops (sensu stricto), Huia, and Meristogenys. In contrast, Dubois (1992) relegated Yang’s (1991) three genera to subgenera of the genus Amolops (sensu lato) and added the subgenus Amo. Currently, Yang’s (1991) classification is more popular (e.g., Frost, 2004), but Matsui et al. (2006) recently evaluated these two different taxonomic systems. Through analyses of mitochondrial cyt-b gene sequences, Matsui et al. (2006) confirmed the monophyly of Meristogenys and the invalidity of Amo, although they did not show monophyly for either Amolops (sensu stricto) or Huia.

Two species assigned to Amolops (sensu stricto) have previously been recorded from Thailand (Taylor, 1962): A. marmoratus (Blyth, 1855) from the northernmost area [Chiang Mai; as Staurois afghanus (Günther, 1858)] and A. larutensis (Boulenger, 1899) from disjunct areas of the southern (Pattani and Yala) and northern (Chumphon) peninsular regions (as S. larutensis). During our faunal surveys of Thailand between 1995 and 1997, we collected larval and adult specimens of an apparently undescribed species of this genus from the western (Kanchanaburi) and the northern and central peninsular regions (Prachuap Khiri Khan and Ranong), which we describe below as a new species.

MATERIALS AND METHODS

A field survey was conducted in western and peninsular Thailand between December 1995 and January 1997. In the field, we recorded calls using a cassette tape recorder (Sony TC-D5) with an external microphone (Sony ECM-23). After collecting specimens, we took tissues for later biochemical analysis, and fixed them as vouchers. Metamorphosed specimens were fixed in 10% formalin and later preserved in 70% ethanol. Larvae were fixed and preserved in 5% formalin. Assignment of larvae to the new species was based upon the occurrence of adults of that species where the larvae were collected.

For preserved metamorphosed specimens, we took the following 18 body measurements (Table 1), mainly following Matsui (1984), to the nearest 0.1 mm with dial calipers under a binocular dissecting microscope: snout-vent length (SVL); head length (HL) from tip of snout to hind border of the angle of jaw (not measured parallel with the median line); snout length (SL); eye length (EL); tympanum-eye distance (T-EL); tympanum diameter (TD); head width (HW); internarial distance (IND); interorbital distance (IOD); upper eyelid width (UEW); forelimb length (FLL); third finger disk diameter (3FDW); fourth toe disk diameter (4TDW); hindlimb length (HLL); tibia length (TL); foot length (FL) from proximal end of inner metatarsal tubercle to tip of fourth toe; first toe length (1TL) from distal end of inner metatarsal tubercle to tip of first toe; inner metatarsal tubercle length (IMTL).

For larvae, the following 17 measurements to the nearest 0.01 mm were taken using a binocular dissecting microscope equipped with a micrometer: total length (TOTL); head-body length (HBL); maximum head-body width (HBW); body depth; eye-snout distance; eyeball diameter; internarial distance; interorbital distance; oral disk width; abdominal disk length; abdominal disk width; spiracle length; tail length; maximum tail depth; maximum tail muscle depth; maximum dorsal fin depth; maximum ventral fin depth. Measurements
were made mainly following Inger (1985), and staging followed Gosner’s (1960) table. For oral apparatus terminology, we followed Altig and McDiarmid (1999).

For comparisons, we examined larvae and metamorphs of *A. larutensis* and *A. marmoratus* (see Appendix 1). We analyzed recorded calls using SoundEdit Vers. 2 and SoundEdit Pro (MacroMind-Paracomp, Inc.) software on a Macintosh computer.

**SYSTEMATICS**

*Amolops panhai* sp. nov. (Fig. 1)


**Diagnosis**

A small form of *Amolops*, males 31–34 mm, females 48–58 mm in SVL; head narrower than long; tympanum distinct; vomerine teeth grouped in short, oblique patches; first finger subequal to second; disc of first finger smaller than disc of second finger, with circummarginal groove; no wide fringe of skin on third finger; toes fully webbed; outer metatarsal tubercle present; supratympanic fold present; axillary gland present; supratympanic fold absent or incomplete; horny spines absent on back, side of head and body, and chest; no large tubercles on side of anus; ventral surface of tarsus without glandular fold; male with nuptial pad and paired gular pouches; no white band along the upper jaw extending to shoulder; larvae with the denticle formula of 7(4-7)/3(1).

**Etymology**

The specific name is dedicated to Dr. Somsak Panha of Chulalongkorn University, Bangkok, who greatly helped us during our survey in Thailand.

**Material examined**

*Holotype*: CUZM (Chulalongkorn University, Zoological Museum) (A) 5255, an adult male from Pa Lao U, Prachuap Khiri Khan Province, Thailand (99°31' E, 12°33' N), collected on 13 December 1995 by M. Matsui.

*Paratypes*: Five males, CUZM (A) 5256, 5257, KUHE 20138, 20153, 20198; ten females, CUZM (A) 5258–5262, KUHE 20143, 20163–20165, 20199; fifteen juveniles, CUZM (A) 5263–5269, KUHE 20132, 20133, 20137, 20158, 20161, 20162, 20168, 20169; all paratopotypes with the same collection data as the holotype. One male (KUHE 20022), two juveniles (KUHE 19936, 20021) from Pilok, near Thong Pha Phum, Kanchanaburi Province, Thailand (98°30' E, 14°33' N), collected on 5 December 1995 by M. Matsui, T. Hikida, and J. Nabhitabhata.

**Description of holotype (measurements in mm)**

Body moderately stocky (Fig. 1), SVL 31.7; head trian-
gular, longer (12.8) than wide (11.6); snout moderately short (5.3), shorter than eye (5.9), rounded at tip in dorsal view, rounded in profile, projecting beyond lower jaw; canthus distinct, constricted; lore slightly oblique, concave; nostril lateral, below canthus, nearer to tip of snout than to eye; inter-
narial distance (3.7) wider than interorbital (3.2); latter slightly narrower than upper eyelid (3.4); eye elevated,

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Table 1. Measurements of 18 characters in *Amolops panhai*. SVL (±1SD, in mm) and medians of ratios (R) of other characters to SVL, followed by ranges in parenthesis. See text for character abbreviations.
Individual variation in size and body proportions is given in Table 1. In adult type series, females are significantly larger (\(\bar{x}=53.9\) mm) than males (\(\bar{x}=32.6\) mm; Tukey-Kramer comparison test, \(p<0.01\)). Relative lengths of head, eye, tympanum, and interorbital, and relative width of head, are significantly greater in males, while relative length of eye-tympanum is greater in females. Thus, tympanum is smaller than half eye diameter in females. In some males, snout is shorter than eye. In some individuals, nostril is midway between tip of snout and eye. In females, samples from Petchaburi have significantly narrower head and wider fourth toe disk, both relative to SVL, than those from Ranong (Dunn’s multiple comparison test, \(p<0.05\)).

Some individuals have bead-like interrupted dorsolateral fold, while the others completely lack it. Dorsum is sometimes scattered with flat granules. Individuals are slightly variable in coloration, with the greatest variation in the extent of the dark spots on the throat and in the development of markings on the back. More than half the females have many dark spots, while most males have no spots on the throat. Samples from Ranong have much clearer dorsal markings than those from Phetchaburi. Juveniles at the time of metamorphosis have SVL of 20.2–21.3 mm.

**Eggs**

One female contained various sizes of ova in the ovaries. The diameter of five larger ova ranged from 1.3–1.4 (mean±2SE=1.35±0.05) mm. The animal pole and the vegetal pole are cream in color.

**Larvae**

Five tadpoles of stages (Gosner, 1960) 25 (N=2, TOTL=20.5–34.8 mm, HBL=8.4–12.9 mm), 28 (N=2, 39.6–43.9 mm, 14.1–15.9 mm; Fig. 2), and 31 (43.6 mm, 16.1 mm) from the type locality were examined. Head-body oval, broadly rounded at snout, flat below, HBW maximum at level of spiracle 66–71% (median=67.3%) of HBL; depth 56–69% (median=66.1%) of HBW; eyes dorsolateral, not visible from below, pointing outward, eyeball 11–13% (median=11.8%) of HBL; interorbital 182–235% (median=200.0%) of eye diameter; eye-snout distance 30–35% (median=31.5%) of HBL; nostril open, rim not raised, closer to eye than to tip of snout; internarial 69–90% (median=78.6%) of interorbital.

Oral disk ventral, width 60–71% (median=62.6%) of HBW; upper lip separated from snout by a groove; lips without papillae except for small papillae in a single row at corners of oral disk, with a second, shorter row of larger papillae antero-medially; denticles 7(4-7)/3(1); beaks heavy, completely black except for outer margins; outer surface smooth; margin finely serrate, about 60 serrae on upper beak; upper beak M-shaped, lower V-shaped; neither beak divided; a large suctorial abdominal disk following oral disk; transverse band of horny tissue absent behind oral disk and indistinct inside lateral and posterior margins of disk; length 53–58% (median=56.2%) of HBL; width 75–86% (median=81.1%) of HBW.

Spiracle sinistral; tube moderately long, length subequal to length of eyeball, pointing upward and backward, free of body wall for half its length; anal tube median, free of tail; tail heavily muscled, dorsal margin strongly convex, deepest before middle, tapering to slightly pointed tip; tail length 144–176% (median=171.1%) of HBL, maximum depth 25–30% (median=24.7%) of length; caudal muscle deeper than fins in basal half; dorsal fin origin behind body, fin deeper...
than ventral fin except in final fourth; ventral fin origin at end of proximal third of tail; head-body with two pairs of glandular clusters; an anterior cluster about an eye length behind eye, with about 25 glands; a posterior cluster of about five glands at posterior corner of body; no glands in fins; head-body scattered dorsally with minute protuberances posterior to eye; the area occupied by spinules and their density increasing with stage of development; lateral line pores indistinct, a short row beginning on snout, running below nostril and eye.

Head-body dark brown dorsally and laterally, sometimes scattered with small, black spots dorsally; caudal muscle dark with light mottling; fins with a fine, dark network.

Larvae from Ranong have the body shape similar to those from Prachuap Khiri Khan, but tend to have a shorter abdominal disk [46–53% (median=48.7%) of HBL], and all lack small, dark spots on dorsum. In addition, dark and light marking on the tail is more conspicuous in Ranong samples.

**Comparisons**

Two species of *Amolops* hitherto recorded from Thai-
land, *Amolops larutensis* and *A. marmoratus*, resemble *A. panhai* in having an outer metatarsal tubercle. However, they differ in some dimensions of body. In both sexes, *A. panhai* is not different from *A. larutensis* in SVL, but is significantly smaller than *A. marmoratus* (Tukey-Kramer test, *p*<0.05). In dimensions relative to SVL, *A. panhai* has wider head than *A. larutensis* in male, and has narrower head than *A. marmoratus* and longer tibia than *A. larutensis* in female (Dunn’s multiple comparison test, *p*<0.05). *Amolops larutensis* is similar to *A. panhai* in having axillary gland, but characteristically has a transverse groove on ventral surface of digital disks, which is absent in *A. panhai*. The larval dental formula of *A. panhai* [7(4-7)/3(1)] also differs from that of *A. larutensis* [8(4-8)/5(1)] and *A. marmoratus* [8(5-8)/3(1)] (our observation).

*Amolops panhai* differs from the other *Amolops* species in the following characteristic (data from Andersson, 1938; Fei, 1999; Fei et al., 2005; Inger and Kottelat, 1998; Inger et al., 1999; Liu et al., 2000; Liu and Yang, 2000; Ray, 1992; Yang, 1991); (1) presence of auxiliary gland (absent in all others including *A. nepalicus* Yang, 1991); (2) presence of vomerine teeth [absent in *A. dayiunensis* (Liu and Hu, 1975), *A. hainanensis* (Boulenger, 1900), *A. hongkongensis* (Pope and Romer, 1951), *A. torrentis* (Smith, 1923), and *A. wuyiensis* (Liu and Hu, 1975)]; (3) presence of distinct, normal tympanum [deeply concave in *A. gerbillus* (Annandale, 1912), indistinct in *A. formosus* (Ray, 1977) and *A. lifanensis* (Liu, 1945)]; (4) presence of outer metatarsal tubercle [absent in *A. chakrataensis* Ray, 1992, *A. longimanus* (Andersson, 1938), and *A. spinapectoralis* Inger, Orlav, and Darevsky, 1999]; (5) presence of nuptial pad in males (absent in *A. hainanensis* and *A. torrentis*); (6) presence of external vocal sacs in males [absent in *A. kantingensis* (Liu, 1950), *A. loloensis* (Liu, 1950), *A. mantzorum* (David, 1872), *A. ricketti* (Boulenger, 1889), *A. tuberodepressus* Liu and Yang, 2000, *A. viridimaculatus* (Jiang, 1983), *A. dayiunensis*, *A. hainanensis*, *A. lifanensis*, and *A. wuyiensis*]; (7) head narrow, length greater than width (head wider than long in *A. jaunari* Ray, 1992); (8) absence of distinct dorsoalateral fold [present in *A. bellius* Liu, Yang, Ferraris, and Matsu, 2000, *A. chunganensis* (Pope, 1929), *A. mengyangensis* Wu and Tian, 1995, *A. monticola* (Anderson, 1871), *A. chakrataensis*, *A. gerbillus*, and *A. tormotus*]; (9) absence of horny spinules on head, shoulder, or chest [present in *A. himalayanus* (Boulenger, 1888), and *A. spinapectoralis*]; (10) absence of wide fringe of skin on the third finger [present in *A. chaopaeensis* (Bouret, 1937), *A. formosus* (Günther, 1876), *A. kaubacki* (Smith, 1940), and *A. ricketti*]; (11) absence of a pair of large tubercles on sides of anus [present in *A. jinjiangensis* Su, Yang, and Li, 1986 and *A. liangshanensis* (Wu and Zhao, 1984)]; and (12) absence of distinct glandular ridge under the tarsus (present in *A. cremnobatus* Inger and Kottelat, 1998, *A. hainanensis*, *A. hongkongensis*, and *A. tormotus*).

**Calls**

Calls were recorded at Ranong on 21 January 1997 at air and water temperatures of 23.5°C and 22.1°C, respectively. The call consists of a single or a pair of un pulsed short notes each lasting 68–76 msec. When the call is paired, the note gap is about 820 msec. The call has a marked frequency modulation rising from 5900 hz to 7000 hz, with the dominant being 6500 hz.

**Range**

Besides the type locality, Pa Lao U, Prachup Khiri Khan, northern peninsular Thailand, *Amolops panhai* n. sp. is also known from Pilok, near Thong Pha Phum, Kanchanaburi, western Thailand, and Punyaban waterfall, Ranong, in central peninsular Thailand (Fig. 3). *Amolops larutensis* recorded from Chumphon (Smith, 1930; Taylor, 1962) is probably actually *A. panhai* (see below).

**Natural History**

In Kanchanaburi, a number of juveniles just metamorphosed were collected among stones on riverbeds and on rocks in a wide stream (width >5 m) in early December, but few adults were seen. Tadpoles seen included two groups, those without limb buds, and those just metamorphosing. In Prachup Khiri Khan, a small number of tadpoles was collected from a stream (width=5 m) in mid December. One of ten females collected had small ovaries with developing ova (see above). In Ranong, adults of *A. panhai* were found along the bank of a stream (width >5 m), perching on rocks at night in late January. Some males were calling, but no amplexant pairs were found. Older tadpoles were collected along with metamorphosing ones. Tadpoles were found in groups, clinging to bare rock in a shallow portion (depth <20 cm) of the stream, just below a big waterfall; these soon escaped to deeper water when disturbed.

**DISCUSSION**

Recent intensive herpetofaunal surveys in Thailand have greatly clarified amphibian diversity in this country. Some species formerly considered wide-ranging have been separated as distinct forms, and some cryptic species have been detected [e.g., the Thai population of *Leptobrachium hasselti* was described as *L. smithi* (Matsui et al., 1999); *Rana archotaphus* was separated from *R. livida* (Inger andChan-ard, 1997); and *Ansonia kraensis* was split from *A. malayana* (Matsui et al., 2005)]. The discovery of *A. panhai* is a case similar to the last. Because the adult morphology of *A. panhai* resembles that of *A. larutensis*, old records of the latter species from Chumphon, Isthmus of Kra (Smith, 1930; Taylor, 1962) are likely based on misidentifications of *A. panhai*.

*Amolops panhai* has a distributional range between that of *A. marmoratus* (northern Thailand) and *A. larutensis* (southern peninsular Thailand to Malaysia). Based on 1346-bp sequences of the mitochondrial 12S and 16S rRNA genes, Matsui et al. (2006) showed a sister relationship of *A. panhai* (as undescribed A. sp. from Thailand) with *A. marmoratus*. In contrast, *A. larutensis* formed a clade, though not very strongly, with *A. cremnobatus* from Vietnam (Matsui et al., 2006). These results indicate that an invasion of the *A. panhai* lineage occurred from north to south along western Thailand.

The present discovery of a new *Amolops* in Thailand from Kanchanaburi (western region) to Ranong (Isthmus of Kra) is significant biogeographically. As already suggested by the geographical separation of species of stream-toads, e.g., *Ansonia kraensis* from the Isthmus of Kra and *A.*
malayana from the northern part of Peninsular Malaysia (Matsui et al., 2005), the occurrence of different species between the northern and southern regions of the Malay Peninsula (i.e., A. panhai and A. larutensis) contradicts the idea that many amphibian species co-occur in the two regions (Inger, 1999).

Finally, A. panhai differs in dorsal color pattern between northern (Kanchanaburi and Prachup Khiri Khan) and southern (Ranong) populations. Relevant to this, Matsui et al. (2006) found comparatively high sequence divergence between populations of A. panhai from Prachup Khiri Khan (mistakenly reported as Phetchaburi) and Kanchanaburi (5.5%). Further detailed study is required to elucidate the nature of these interpopulation differences.

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Appendix 1. Specimens of A. larutenis and A. marmoratus examined for comparisons.

A. marmoratus: KUHE 19030, 19072–19090, 19111, 19112, and KUHE eight unnumbered tadpoles, from Doi Inthanon, Chiang Mai, Thailand; KUHE 19153–19159, 19162, 19217, 19235 from Doi Suthep-pui, Chiang Mai, Thailand; KUHE 19813, 19814, 19828 from Huay Sa and Kud and Pang Tong, Mae Hong Son, Thailand.