

# A New Tree Frog of the Genus *Gracixalus* from Thailand (Amphibia: Rhacophoridae)

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We report a new tree frog of the genus *Gracixalus* from western Thailand and describe it as a new species *Gracixalus seesom* based on results of morphological and molecular analyses. The new species is a small-sized *Gracixalus* (male snout-vent length ca. 22 mm) and is morphologically similar to *G. gracilipes*, but is easily distinguished from it by its dorsal tan color in life, absence of white spot on lower lip, and black markings on its foot webbing. The new species also clearly differs from all the other members of the genus by the combination of small body size, triangular snout, and light yellowish brown dorsum without distinct tuberculations. Problems of phylogeny and taxonomy of the genus *Gracixalus* are briefly discussed.

**Key words:** *Gracixalus seesom*, mitochondrial phylogeny, Southeast Asia, systematics, taxonomy

## INTRODUCTION

From Thailand, Khonsue and Thirakhupt (2001) listed a total of 30 species of tree frogs of the family Rhacophoridae, including the following number of species: four *Chirixalus* Boulenger, 1893, one *Nyctixalus* Boulenger, 1882, four *Philautus* Gistel, 1848, five *Polypedates* Tschudi, 1838, 12 *Rhacophorus* Kuhl and Van Hasselt, 1822, and four *Theلودerma* Tschudi, 1838. However, in all of these genera except for *Nyctixalus* and *Theلودerma*, generic status of some species included have been changed recently. *Polypedates feae* (Boulenger, 1893) is now regarded as a member of *Rhacophorus* (see Matsui and Panha, 2006), and the four small species long placed in the genus *Chirixalus* were transferred to *Chirromantis* Peters, 1854 or *Feihyla* Frost, Grant, Faivovich, Bain, Haas, Haddad, de Sá, Channing, Wilkinson, Donnellan, Raxworthy, Campbell, Blotto, Moler, Drewes, Nussbaum, Lynch, Green, and Wheeler, 2006 (Frost, 2014; but see Matsui et al., 2014).

Of the remaining small species, *Philautus carinensis* (Boulenger, 1893) was once moved to *Aquixalus* Delorme, Dubois, Grosjean, and Ohler, 2005, then to *Kurixalus* Ye, Fei, and Dubois, 1999 (Li, et al., 2008), and now recognized as a member of *Gracixalus* Delorme, Dubois, Grosjean, and Ohler, 2005 (Li et al., 2009). *Philautus gracilipes* Bourret, 1937 was also once moved to the genus *Aquixalus* as the type species of its subgenus *Gracixalus* (Delorme et al., 2005), which is now considered as a distinct genus (Li et al., 2008). *Philautus parvulus* (Boulenger, 1893) was once placed in *Pseudophilautus* Laurent, 1943 (Li et al., 2009), but was grouped with many Indian species and moved to *Raorchestes* Biju, Shouche, Dubois, Dutta, and Bossuyt,

2010 (Biju et al., 2010). *Rhacophorus appendiculatus* (Günther, 1859) was recently moved to *Kurixalus* by Yu et al. (2013), while *Rh. bisacculus* Taylor, 1962, once placed in *Aquixalus* (*Aquixalus*) by Delorme et al. (2005), is now also considered as a member of *Kurixalus* (Lie et al., 2008).

Most of these taxonomic changes have been made through recently developed molecular phylogenetic studies (Delorme et al., 2005; Frost et al., 2006; Li et al., 2008, 2009; Biju et al., 2010; Yu et al., 2013). As a result of these significant changes, morphologically diagnostic characters as proposed in traditional taxonomy (e.g., Liem, 1970) are nearly lacking in each of small-sized rhacophorid genera from Southern and Southeastern Asian regions. In our molecular analyses of Thai amphibians, we have already found several candidates of cryptic species (e.g., Pansook et al., 2012) and described some of them (Matsui et al., 2010; Nishikawa et al., 2013). However, there still remain many cases to be studied, and one of them is a tree frog currently identified as *G. gracilipes*. Close examination of its external morphology and subsequent molecular analyses revealed that it is different from all the known congeners, as we describe in detail below.

## MATERIALS AND METHODS

DNA sequence data were newly obtained from tissues preserved in 99% ethanol for specimens of *Gracixalus* sp. from Thailand (KUHE [Graduate School of Human and Environmental Studies, Kyoto University] 35084, 35088, 35089, CU [Chulalongkorn University] K1856), *G. carinensis* (Boulenger, 1893) (KUHE46401, 46402), and *G. jinxiuensis* (Hu, 1978) (Chengdu Institute of Biology [CIB] HN201108200, KUHE 32453). We followed Kuraishi et al. (2013) for methods for DNA extraction, and amplification and sequencing of the mtDNA fragments. We deposited the resultant sequences (ca. 500 base pairs [bp] of partial sequences of mitochondrial 16S rRNA gene) in GenBank (Accession numbers listed in Table 1).

For comparisons, GenBank data of all hitherto known members of the genus, i.e., *G. gracilipes*, *G. lumarius* Rowley, Le, Dau,

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**Table 1.** Sample of *Gracixalus* and outgroup species used for DNA analysis (16S rRNA, 441 bp) in this study together with the information on voucher, collection locality and GenBank accession numbers. Voucher abbreviations: AMNH = American Museum of Natural History; AMS = Australian Museum; BORN = BORNEENSIS, University Malaysia, Sabah; CIB = Chengdu Institute of Biology; CUMZ = Chulalongkorn University Museum of Natural History; IEBR = Institute of Ecology and Biological Resources, Hanoi; KUHE = Graduate School of Human and Environmental Studies, Kyoto University; KIZ = Kunming Institute of Zoology; MNHN = Muséum national d'Histoire naturelle, Paris; MZB = Museum Zoologicum Bogoriense; NHMG = Natural History Museum of Guangxi; VNMN = Vietnam National Museum of Nature; VNUH = Vietnam National University, Hanoi, ZFMK = Zoologisches Forschungs-museum Alexander Koenig.

| Species name                    | Voucher          | Locality               | Reference              | Acc. No. |
|---------------------------------|------------------|------------------------|------------------------|----------|
| <i>Gracixalus quangii</i>       | AMS R173417      | Vietnam, Nghe An       | Rowley et al. (2011)   | JN862539 |
| <i>Gracixalus quangii</i>       | AMS R173426      | Vietnam, Nghe An       | Rowley et al. (2011)   | JN862541 |
| <i>Gracixalus quangii</i>       | IEBR A.2012.5    | Vietnam, Thanh Hoa     | Rowley et al. (2014)   | JX896683 |
| <i>Gracixalus supercornutus</i> | AMS R173887      | Vietnam, Kon Tum       | Rowley et al. (2011)   | JN862545 |
| <i>Gracixalus supercornutus</i> | AMS R173395      | Vietnam, Kon Tum       | Rowley et al. (2011)   | JN862542 |
| <i>Gracixalus gracilipes</i>    | AMNH A163897     | Vietnam, Ha Giang      | Li e al. (2008)        | DQ283051 |
| <i>Gracixalus gracilipes</i>    | MNHN1999.592     | Vietnam, Lao Cai       | Yu et al. (2009)       | AY880504 |
| <i>Gracixalus gracilipes</i>    | KIZ 060821196    | China, Yunnan          | Li e al. (2009)        | GQ285568 |
| <i>Gracixalus quyeti</i>        | VNUH160706       | Vietnam: Quang Binh    | Li e al. (2009)        | EU871428 |
| <i>Gracixalus quyeti</i>        | ZFMK 82999       | Vietnam: Quang Binh    | Nguyen et al. (2013)   | EU871429 |
| <b><i>Gracixalus</i> sp.</b>    | KUHE 35084       | Thailand, Kanchanaburi | This study             | LC011932 |
| <b><i>Gracixalus</i> sp.</b>    | KUHE 35089       | Thailand, Kanchanaburi | This study             | LC011934 |
| <b><i>Gracixalus</i> sp.</b>    | CUMZ K1856       | Thailand, Kanchanaburi | This study             | LC011935 |
| <b><i>Gracixalus</i> sp.</b>    | KUHE 35088       | Thailand, Kanchanaburi | This study             | LC011933 |
| <i>Gracixalus lumarius</i>      | AMS R 176202     | Vietnam, Kon Tum       | Rowley et al. (2014)   | KF918412 |
| <i>Gracixalus nonggangensis</i> | NHMG 20091010    | China, Guangxi         | Mo et al. (2013)       | JX841318 |
| <i>Gracixalus nonggangensis</i> | NHMG1005046      | China, Guangxi         | Mo et al. (2013)       | JX841320 |
| <i>Gracixalus jinxiuensis</i>   | CIB HN 201108200 | China, Hunan           | This study             | LC011936 |
| <i>Gracixalus jinxiuensis</i>   | KUHE 32453       | Laos, Houapan          | This study             | LC011937 |
| <i>Gracixalus waza</i>          | IEBR A.2012.2    | Vietnam, Cao Bang      | Nguyen et al. (2013)   | JX896681 |
| <i>Gracixalus carinensis</i>    | KUHE 46401       | Vietnam, Lao Cai       | This study             | LC011938 |
| <i>Gracixalus carinensis</i>    | KUHE 46402       | Vietnam, Lao Cai       | This study             | LC011939 |
| <i>Kurixalus eiffingeri</i>     | KUHE 12910       | Japan, Iriomote Is.    | Nguyen et al. (2014a)  | AB933305 |
| <i>Kurixalus odontotarsus</i>   | KIZ 201307071    | China, Yunnan          | Nguyen et al. (2014a)  | AB933303 |
| <i>Phyllautus aurifasciatus</i> | MZB 16395        | Indonesia, Java        | Nguyen et al. (2014b)  | KJ802924 |
| <i>Rhacophorus borneensis</i>   | BORN 22410       | Malaysia, Sabah        | Matsui et al. (2013)   | AB781693 |
| <i>Polypedates leucomystax</i>  | BORN 12420       | Malaysia, Sabah        | Kuraishi et al. (2013) | AB728138 |

Hoang, and Cao, 2014, *G. nonggangensis* Mo, Zhang, Luo, Zhou, and Chen, 2013, *G. quangii* Rowley, Dau, Nguyen, Cao, and Nguyen, 2011, *G. quyeti* (Nguyen, Hendrix, Böhme, Vu, and Ziegler, 2008), *G. supercornutus* (Orlov, Ho, and Nguyen, 2004), and *G. waza* Nguyen, Le, Pham, Nguyen, Bonkowski, and Ziegler, 2013 were utilized. No sequence data are available for *G. medogensis* (Ye and Hu, 1984), which was placed in *Gracixalus* simply by its morphological resemblance with *G. jinxiuensis* (Li et al., 2009). As outgroup species, we used sequences of *Rhacophorus borneensis* Matsui, Shimada, and Sudin, 2013, *Kurixalus eiffingeri* (Boettger, 1895), *K. odontotarsus* (Ye and Fei, 1993), *Phyllautus aurifasciatus* (Schlegel, 1837), and *Polypedates leucomystax* (Gravenhorst, 1829). Details of these specimens are shown in Table 1. We followed Kuraishi et al. (2013) for tree construction and calculation of genetic distances (uncorrected p-distance).

We took the following 21 body measurements to the nearest 0.05 mm with a dial caliper under a binocular microscope, following Matsui (1984, 1994): (1) snout-vent length (SVL); (2) head length (HL); (3) head width (HW); (4) internarial distance (IND); (5) inter-orbital distance (IOD); (6) upper eyelid width (UEW); (7) nostril-eyelid length (N-EL); (8) snout length (SL); (9) eye length (EL); (10) eye diameter (ED); (11) tympanum diameter (TD); (12) tympanum-eye length (T-EL); (13) forelimb length (FLL); (14) lower arm and hand length (LAL); (15) first finger length (1FL); (16) inner palmar tubercle length (IPTL); (17) hindlimb length (HLL); (18) tibia length (TL); (19) foot length (FL); (20) inner metatarsal tubercle length (IMTL); and (21) first toe length (1TOEL). Additionally, measurements were taken for finger and toe disks to the nearest 0.01 mm using a bin-

ocular dissecting microscope equipped with a micrometer: (22–25) first to fourth finger disk diameter (1–4FDW); and (26–30) first to fifth toe disk diameter (1–5TDW). We followed the system of description of toe-webbing states used by Savage (1997).

## SYSTEMATICS

In both the maximum-likelihood (ML: Fig. 1A) and Bayesian (BI: Fig. 1B) trees, species of *Gracixalus* formed a clade, although not very strongly supported (BS = 74%, BPP = 0.98). The two trees greatly differed in the placement of *G. lumarius*. In ML tree (Fig. 1A), the species was nested in a clade with *G. jinxiuensis*, *G. carinensis*, *G. nonggangensis*, and *G. waza* (BS = 71%), in opposition to another clade of *G. supercornutus*, *G. quangii*, *G. gracilipes*, *G. quyeti*, and *Gracixalus* sp. from Thailand (BS = 90%). In contrast, in BI tree (Fig. 1B), *G. lumarius* was not included in the

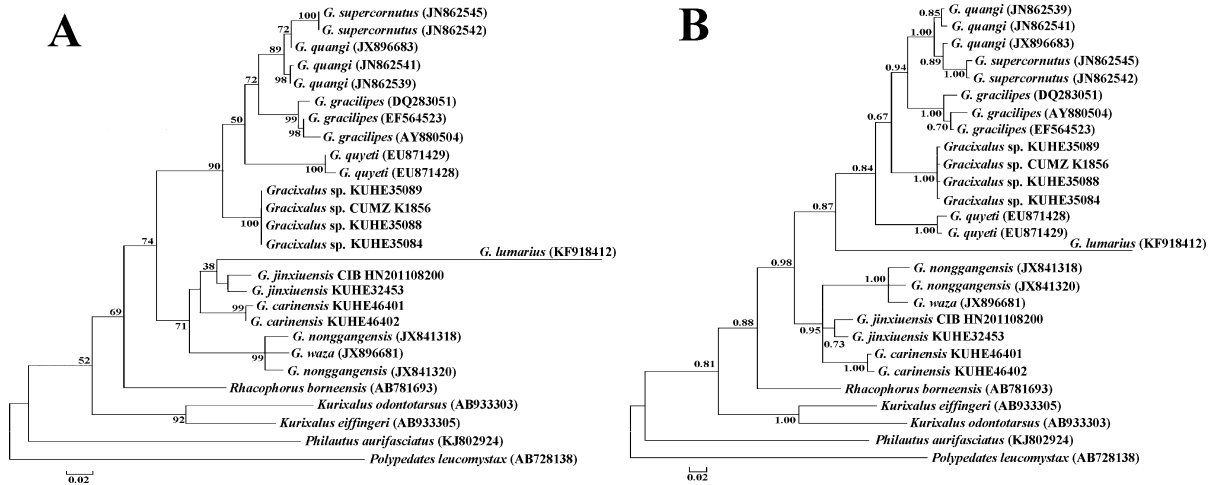
first clade (BPP = 0.95), but positioned at the base of the second group (BPP = 0.87), which did not form a clade (BPP = 0.84). *Gracixalus* sp. from Thailand formed a clade (BS = 100%, BPP = 1.00), and was included in the second group, although the relationships with other species were not resolved. Anyway, these results validate placement of *Gracixalus* sp. from Thailand in the genus *Gracixalus*.

*Gracixalus* sp. from Thailand differed genetically from the nine congeneric species by large genetic distances (5.4–15.0%: Table 2), which were larger than distances between *G. quangii* and *G. supercornutus* (2.0–2.9%) or *G. gracilipes* (4.2–5.4%), and between *G. nonggangensis* and *G. waza* (2.2–2.7%). Furthermore, in congruent with genetic separation, *Gracixalus* sp. from Thailand is clearly separated morphologically from all nominal species of *Gracixalus*. Thus, we conclude *Gracixalus* sp. from Thailand as a distinct new species and describe it as follows:

*Gracixalus seesom* sp. nov.  
(Figs. 2–4)

## Diagnosis

The new species is assigned to the genus *Gracixalus* from its morphological similarity with *G. gracilipes*, the type species of the genus, and inclusion in the clade with the type



**Fig. 1.** Maximum-likelihood (ML) (A) and Bayesian inference (BI) (B) trees from a 441 bp sequence of mitochondrial 16S rRNA gene for *Gracixalus* sp. from Thailand, members of the species currently assigned to *Gracixalus*, and for outgroups. Numbers above or below branches represent bootstrap supports for ML inferences (A) and Bayesian posterior probabilities (B).

**Table 2.** Uncorrected p-distances (in %) for fragment of 16S rRNA among 10 *Gracixalus* and five rhacophorid taxa compared.

|                                   | 1              | 2              | 3              | 4              | 5                | 6         | 7         | 8         | 9       | 10        | 11   | 12   | 13   | 14   |
|-----------------------------------|----------------|----------------|----------------|----------------|------------------|-----------|-----------|-----------|---------|-----------|------|------|------|------|
| 1 <i>Gracixalus quangi</i>        |                |                |                |                |                  |           |           |           |         |           |      |      |      |      |
| 2 <i>Gracixalus supercornutus</i> | 2.0–2.9        |                |                |                |                  |           |           |           |         |           |      |      |      |      |
| 3 <i>Gracixalus gracilipes</i>    | 4.2–5.4        | 5.7–6.9        |                |                |                  |           |           |           |         |           |      |      |      |      |
| 4 <i>Gracixalus quyeti</i>        | 5.4–6.4        | 6.1–6.4        | 6.9–8.6        |                |                  |           |           |           |         |           |      |      |      |      |
| 5 <b><i>Gracixalus</i> sp.</b>    | <b>5.4–5.7</b> | <b>6.4–6.6</b> | <b>5.4–6.4</b> | <b>6.4–7.1</b> |                  |           |           |           |         |           |      |      |      |      |
| 6 <i>Gracixalus lumarius</i>      | 14.3–14.5      | 15.5–15.7      | 14.5–15.5      | 14.0–14.7      | <b>15.0</b>      |           |           |           |         |           |      |      |      |      |
| 7 <i>Gracixalus nonggangensis</i> | 9.6–10.1       | 10.8–11.3      | 10.8–12.5      | 11.1–11.5      | <b>10.1–10.6</b> | 16.0      |           |           |         |           |      |      |      |      |
| 8 <i>Gracixalus jinxiuensis</i>   | 7.6–8.4        | 8.8–9.3        | 9.3–10.6       | 9.1–9.6        | <b>8.4–8.8</b>   | 14.3–14.5 | 5.2–7.4   |           |         |           |      |      |      |      |
| 9 <i>Gracixalus waza</i>          | 10.3–10.6      | 11.5–11.8      | 11.5–12.5      | 11.5–11.8      | <b>10.1</b>      | 16.2      | 2.2–2.7   | 5.4–6.4   |         |           |      |      |      |      |
| 10 <i>Gracixalus carinensis</i>   | 7.6–8.4        | 9.1–9.6        | 8.6–9.8        | 8.1–9.1        | <b>7.6–7.9</b>   | 15.7–16.2 | 6.6–7.6   | 4.7–5.2   | 6.6–6.9 |           |      |      |      |      |
| 11 <i>Kurixalus eiffingeri</i>    | 12.0–12.3      | 12.3–12.5      | 13.3–15.0      | 13.0–13.3      | <b>13.3</b>      | 20.4      | 13.8      | 12.8–13.0 | 14.0    | 12.5–12.8 |      |      |      |      |
| 12 <i>Kurixalus odontotarsus</i>  | 15.0–16.0      | 15.0–15.2      | 17.0–17.9      | 14.5–14.7      | <b>16.0</b>      | 19.2      | 15.5–16.2 | 13.3–13.8 | 15.5    | 13.8–14.0 | 10.3 |      |      |      |
| 13 <i>Rhacophorus borneensis</i>  | 10.8–11.1      | 12.0–12.3      | 10.6–11.8      | 9.8–10.6       | <b>10.3</b>      | 17.9      | 9.8–11.1  | 7.6–7.9   | 10.1    | 8.1–8.4   | 13.5 | 14.0 |      |      |
| 14 <i>Philautus aurifasciatus</i> | 15.7–16.0      | 16.2–16.5      | 16.5–17.2      | 17.0–17.4      | <b>15.2</b>      | 19.9      | 14.7–15.5 | 15.0–15.7 | 15.0    | 15.0–15.5 | 17.0 | 16.5 | 16.2 |      |
| 15 <i>Polypedates leucomystax</i> | 17.0–17.4      | 18.4–18.7      | 18.2–19.2      | 16.7–17.4      | <b>17.2</b>      | 18.4      | 16.5–16.7 | 16.5–17.2 | 16.7    | 17.0–17.2 | 18.2 | 17.4 | 17.2 | 18.9 |

species in the mitochondrial phylogeny. Generic diagnosis used for description of the other congeners (presence of intercalary cartilage between terminal and penultimate phalanges of digits, tips of digits expanded into large disks bearing circummarginal grooves, vomerine teeth absent, horizontal pupil, small size [males < 25 mm SVL], and triangularly pointed snout) applies to the new species, although these are not synapomorphy of the genus.

*Gracixalus seesom* sp. nov. is distinguishable from its congeners by a combination of (1) size small (males 22–23 mm, females 23–25 mm SVL), (2) snout triangularly pointed, (3) dorsal skin nearly smooth, (4) tan dorsally with orange yellow on ventral sides of limbs and anterior and posterior surfaces of thigh, (5) upper eyelid and dorsum lacking spines, (6) upper eyelid and web lacking black marking, (7) flank and ventral surface of thigh lacking brownish black spot, and (8) tibiotarsal projection absent.

**Etymology**

The specific name is from the Thai words, “see”, meaning orangish and “som” (color), alluding to the ventral color of limbs in life of the new species.

**Holotype**

KUHE 35084, an adult female from Pilok, Thong Pha Phum National Park, Kanchanaburi Province, Thailand (14°41’ N, 98°24’ E, 942 m a.s.l.), collected on 3 January 2002 by Masafumi Matsui and Wichase Khonsue.

**Paratypes**

CUMZ K 1855, 1856, and two males and three females, KUHE 35085–35089, same data as the holotype. A male, KUHE 19177 from near Park HQ, Doi Inthanon National Park, Chang Mai Province, Thailand (18°31’ N, 98°32’ E, 1650 m a.s.l.), collected on 6 August 1994 by Masafumi Matsui.

**Referred specimens**

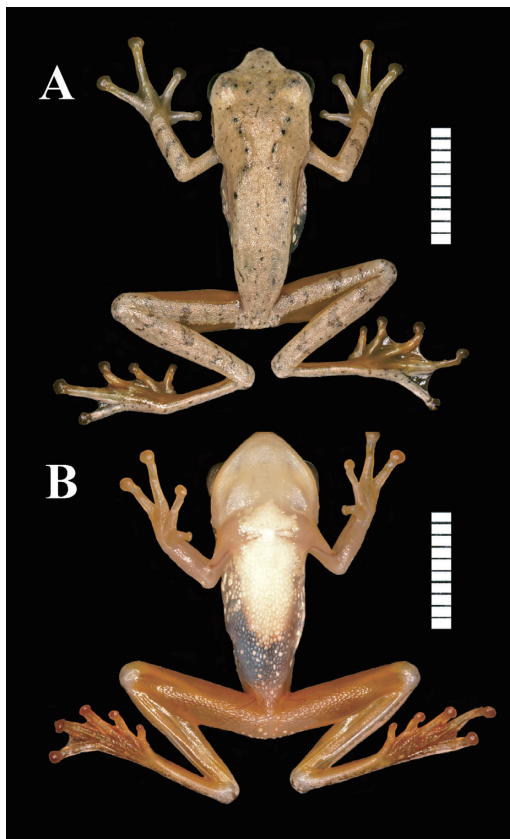
Six males, Natural History Museum, London (BM) 1979.460–465 from Doi Inthanon, alt. 1300 m a.s.l., Chang Mai Province, Thailand.

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

Adult female, SVL 24.6 mm; body dorsoventrally compressed; head slightly longer (HL 9.3, 37.8%SVL) than wide (HW 9.0, 36.6%SVL), wider than body; snout pointed in dor-

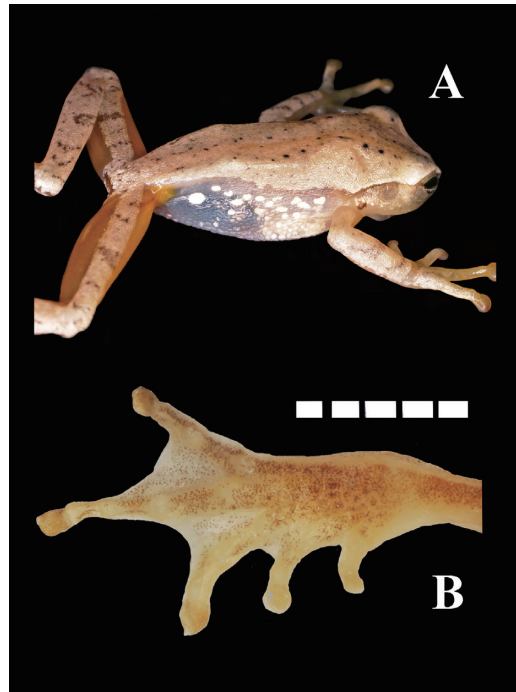


**Fig. 2.** Female holotype (KUHE 35084) of *Gracixalus seesom* sp. nov. in life.



**Fig. 3.** (A) Dorsal and (B) ventral views of female holotype (KUHE 35084) of *Gracixalus seesom* sp. nov. in anesthetized state. Scale bar in (A) and (B) = 10 mm.

sal view, rounded in lateral view, length (SL 3.7, 15.0%SVL) longer than eye length (EL 3.2, 13.0%SVL), projecting beyond mouth; canthus rostralis sharply angular; loreal region vertical and concave; nostril slightly protuberant, nearer to tip of snout (S-NL 1.4, 5.7%SVL) than to eye (N-EL 2.2, 8.9%SVL); internarial distance (IND 2.6, 10.6%SVL) less than interorbital distance (IOD 3.6, 14.6%SVL), which in turn much wider than upper eyelid (UEW 2.2, 8.9%SVL); eye large, pupil horizontal; tympanum distinct, subcircular, diameter (TD 1.2, 4.9%SVL) three-eighths of eye length and separated from eye by one-fourth of tympanum diameter (T-EL 0.3, 1.2%SVL); tympanic rim slightly elevated relative to skin of temporal region; pineal ocellus present at level connecting anterior borders of upper eyelids; vomerine teeth



**Fig. 4.** (A) Lateral view of body in anesthetized state and (B) ventral view of right foot after preservation of female holotype (KUHE 35084) of *Gracixalus seesom* sp. nov. Scale bar in (B) = 10 mm, (A) not to scale.

absent; tongue notched posteriorly.

Forelimb long (FLL 16.8, 68.3%SVL) and slender; hand and forearm long (LAL 13.2, 53.7%SVL); finger length formula: I < II < IV < III; expanded disks each with circummarginal groove and transverse ventral groove; disks on third and fourth fingers (3FDW 1.1, 4.5%SVL; 4FDW 1.3, 5.2%SVL) only slightly wider than those on second (2FDW 1.1, 4.5%SVL) but wider than first (1FDW 0.7, 2.9%SVL), all, but first, subequal to tympanum; remnant of webbing between inner three fingers; subarticular tubercles prominent, rounded, formula 1, 1, 2, 2; inner palmar tubercle flat (IPTL 1.1, 4.4%SVL); outer palmar tubercle weakly divided into two.

Hindlimb long (HLL 42.5, 172.8%SVL); tibiotarsal articulation reaching to point between nostril and eye when fully stretched leg addressed to body; heels overlapping each other when thigh (THIGH 13.7, 55.7%SVL) and tibia (TL 14.4, 58.5%SVL) placed at right angle to body; foot (FL 11.2, 45.5%SVL) much shorter than tibia; toe length formula I < II < III < V < IV; toes bearing expanded disks each with circummarginal groove and transverse ventral groove; widths of outer toe disks (4TDW 1.3, 4.4%SVL; 5TDW 1.0, 4.1%SVL) narrower than those of outer finger disks; webbing formula I 2–2<sup>1</sup>/<sub>2</sub> II 1<sup>1</sup>/<sub>4</sub>–2<sup>1</sup>/<sub>4</sub> III 1<sup>1</sup>/<sub>2</sub>–2<sup>3</sup>/<sub>4</sub> IV 2<sup>1</sup>/<sub>4</sub>–1<sup>1</sup>/<sub>2</sub> V; subarticular tubercles distinct, rounded, formula 1, 1, 2, 3, 2; supernumerary tubercles absent; inner metatarsal tubercle oval, (IMTL 1.0, 4.1%SVL) and flat, about two-fifths length of first toe (1TOEL 2.5, 10.2%SVL); no outer metatarsal tubercle (Fig. 4B).

Dorsal surface nearly smooth, sparsely scattered with minute, blunt tubercles between shoulder and sacral regions; skin of head not co-ossified to forehead; supratym-

panic fold continuing as glandular fold to beyond level of axilla; skin of lower jaw posterolaterally forming short fold at anterior base of upper arm; low, large tubercles on flank, continuing to flat granules on belly; otherwise ventral surface smooth; tarsal fold absent; very weak tubercles at tibiotarsal articulation.

### Color

In life, dorsal surface tan, with small dark brown spots on upper eyelid and across back, forming interrupted  $\chi$  marking (Figs. 2, 3A, 4A); faint lighter line from snout through outer margin of upper eyelid to supratympanic fold, continuing posteriorly to separate tan dorsum and darker flank (Fig. 4A); supratympanic fold margined underneath by brown, extending from eye to axilla, and covering upper three-fourths of tympanum; large white blotches laterally on trunk, becoming smaller and continuing to anterior belly; small white spots less dense on translucent posterior belly; dorsal side of thigh and tibia barred with faint dark brown (Figs. 2, 3A, 4A); ventral surface of throat, chest and anterior belly opaque white; underside of forelimb, posterior to insertion of arm, and underside of hindlimb, continuing to anterior and posterior surfaces of thigh to tarsus, dorsal surface of inner four toes, and groin, bright orange (Figs. 3B, 4A); foot webbing also orange ventrally; iris golden with a network of fine black reticulations concentrated around periphery. In alcohol, dorsum faded to beige and upper eyelid covered by dark brown; ventrally totally cream white, with orange on ventral surface of limbs faded.

### Variation

Morphometric variation is shown in Table 3. Small sample size prohibited statistical comparisons, but in the type series, females (mean SVL  $\pm$  1 SD = 24.3  $\pm$  1.0 mm) are larger than males (22.4  $\pm$  0.7 mm) with no overlap in SVL ranges (23.2–25.4 mm and 21.6–23.0 mm, respectively). Referenced six males in BM collection are slightly larger (22.4–24.0 mm) than paratypic males. The paratypes are generally similar with the holotype in general morphology and coloration, but some have clearer dark spots on dorsum. Males lack nuptial pads, but have a pair of vocal slits on both sides of mouth floor well anterior to jaw commissure.

### Range

Known only from northwestern Thailand, Pilok, Thong Pha Phum, Kanchanaburi Province and Doi Inthanon, Chiang Mai Province. The known localities vary from 942–1650 m in altitude.

### Natural history

At the type locality, specimens were found at night in a small valley, perching on twigs of short shrubs along a dried stream. No calls were heard in early January. Frogs found at the time of the collection of type specimens were *Leptotalax melanoleucus* Matsui, 2006, *Limnonectes limborgi* (Sclater, 1892), *Limnonectes taylori* Matsui, Panha, Khonsue, and Kuraishi, 2010, *Kurixalus bisacculus* (Taylor, 1962), and *Raorchestes parvulus* (Boulenger, 1893). At Doi Inthanon, *Megophrys* sp., *Ansonia inthanon* Matsui, Nabhitabhata, and Panha, 1998, *Rhacophorus bipunctatus* Ahl, 1927, and *R. parvulus* were observed with the new species.

**Table 3.** Measurements of *Gracixalus seesom* sp. nov. SVL (mean  $\pm$  1 SD, in mm) and medians of ratios (R) of other characters to SVL, followed by ranges in parenthesis. See text for character abbreviations.

|        | 3 males        |               | 4 females      |               |
|--------|----------------|---------------|----------------|---------------|
| SVL    | 22.4 $\pm$ 0.7 | (21.6–23.0)   | 24.3 $\pm$ 1.0 | (23.2–25.4)   |
| RHL    | 36.5           | (36.1–39.2)   | 37.9           | (37.4–38.7)   |
| RHW    | 33.5           | (32.9–33.9)   | 34.8           | (31.9–36.6)   |
| RIND   | 10.4           | (10.2–11.0)   | 10.5           | (9.7–10.6)    |
| RIOD   | 13.9           | (13.5–15.0)   | 14.0           | (13.4–14.7)   |
| RUEW   | 8.7            | (8.3–9.3)     | 9.1            | (8.9–9.4)     |
| RN-EL  | 9.1            | (8.3–9.7)     | 8.5            | (7.9–8.9)     |
| RSL    | 14.8           | (14.4–16.7)   | 15.1           | (14.7–15.4)   |
| REL    | 13.5           | (13.2–14.4)   | 14.7           | (13.0–15.7)   |
| RED    | 11.7           | (11.5–12.0)   | 11.9           | (11.0–12.6)   |
| RTD    | 4.6            | (4.4–4.8)     | 5.2            | (4.3–6.3)     |
| RT-EL  | 1.8            | (1.4–2.2)     | 1.2            | (0.9–1.6)     |
| RFL    | 68.7           | (68.5–72.7)   | 68.6           | (68.1–71.3)   |
| RLAL   | 51.7           | (51.4–52.9)   | 53.5           | (50.9–55.1)   |
| R1FL   | 10.2           | (8.7–10.6)    | 9.6            | (8.6–10.1)    |
| RIPTL  | 4.6            | (3.5–4.8)     | 4.4            | (3.9–5.0)     |
| RHLL   | 165.7          | (161.7–173.5) | 171.9          | (160.3–184.3) |
| RTL    | 52.8           | (52.4–55.7)   | 56.6           | (54.3–61.0)   |
| RFL    | 45.4           | (44.9–45.7)   | 45.6           | (44.5–48.4)   |
| RIMTL  | 3.9            | (3.5–4.2)     | 3.7            | (3.4–4.1)     |
| R1TOEL | 9.7            | (9.6–10.1)    | 9.3            | (9.1–10.2)    |
| R1FD   | 2.9            | (2.8–3.6)     | 3.1            | (2.9–3.7)     |
| R2FD   | 4.3            | (4.2–4.5)     | 4.4            | (4.0–4.5)     |
| R3FD   | 5.3            | (5.3–5.9)     | 5.2            | (4.6–5.9)     |
| R4FD   | 4.9            | (4.7–5.2)     | 5.3            | (4.5–5.5)     |
| R1TOD  | 3.1            | (2.4–3.1)     | 3.1            | (2.4–3.3)     |
| R2TOD  | 3.8            | (2.9–3.9)     | 3.7            | (3.6–4.0)     |
| R3TOD  | 3.9            | (3.8–4.4)     | 4.0            | (3.7–4.2)     |
| R4TOD  | 4.9            | (3.7–5.1)     | 4.9            | (4.4–5.9)     |
| R5TOD  | 4.4            | (4.2–4.8)     | 4.3            | (4.1–5.0)     |

### Comparisons

*Gracixalus seesom* sp. nov. resembles *G. gracilipes* from high elevations of northern Vietnam and southern China in color in preservative (beige dorsum with dark brown upper eyelid), lack of brownish black spots on flank and ventral surface of thigh, and tibiotarsal projection, but differs from it in dorsal color in life and marking on upper lip (dorsum tan in life and lacking white patch under eye vs. dorsum green in life and having white patch under eye to tympanum in *G. gracilipes*). The new species differs from *G. supercornutus* from high elevations of central Vietnam by having tan dorsum lacking large spines, and lacking white patch under eye (vs. dorsum transparent green with distinct brown markings and covered with large spines, and white patch present under eye to tympanum in *G. supercornutus*). *Gracixalus seesom* sp. nov. differs from *G. quangi* from medium to high elevations of north central Vietnam by having tan dorsum lacking small asperities, and lacking brownish black spots on ventral surface of thigh and tibiotarsal projection (vs. dorsum greenish to brownish green scattered with small asperities, dorsum and ventral surface of thigh with brownish black spots, and tibiotarsal projection present in *G. quangi*).

Other species of *Gracixalus* are different from *G. seesom* in coloration and snout shape. The new species having dorsum tan without tubercles, and pointed snout differs from *G.*

*quyeti* from medium to high elevations of central Vietnam (vs. body brownish to moss green with a rounded snout in *G. quyeti*). *Gracixalus seesom* sp. nov. also differs from *G. carinensis* from high elevations of Myanmar through Thailand to northwestern Vietnam, in monotonous tan and nearly smooth dorsum, and triangular snout (vs. dorsum grayish brown with dark brown markings and snout rounded in *G. carinensis*). *Gracixalus seesom* sp. nov. overlaps in male body size with *G. jinxiuensis* from moderate elevations of southeastern China, high elevations of northern Vietnam, and Laos (male SVL 21–24 mm vs. 24 mm in *G. jinxiuensis*), but differs from it in dorsal color, snout shape, and skin structure (dorsum tan without tubercles and snout pointed vs. brown dorsum scattered with tubercles, and snout rounded in *G. jinxiuensis*). The new species (males 21–24 mm, snout pointed, dorsum smooth, tan in color) also differs from *G. medogensis* from high elevation of Xizang, China (vs. 26.5 mm, snout rounded, and dorsum brown scattered with tubercles in *G. medogensis*). *Gracixalus seesom* sp. nov. differs from *G. nonggangensis* from moderate elevation of Vietnamese border of China, and *G. waza* from moderate elevation of northern Vietnam, in body size, skin texture, and coloration (males 21–24 mm, females 23–25 mm SVL, dorsum tan without tubercles, and venter without dark spots vs. male SVL 27–36 mm, females 38 mm, dorsum greyish to moss-green with small tubercles, and venter white with brown spots in *G. nonggangensis* and *G. waza*). Finally, the new species completely differs from *G. lumarius* from high elevation of central Vietnam in body size and dorsal skin texture (male SVL 21–24 mm, dorsum tan and without distinct asperities vs. 39–42 mm, dorsum with distinctive, white conical asperities, and brown diurnally and yellowish brown nocturnally in *G. lumarius*).

## DISCUSSION

Thailand has a long history of amphibian faunal survey, and a monograph was published more than half a century ago (Taylor, 1962). However, *Gracixalus gracilipes* was not listed in Taylor (1962) and its occurrence in Thailand was first recorded by Chan-ard (2003: as *Philautus*) in an illustrated guidebook, where a map and a handwritten illustration are given. However, sources of data or the bases for identification are totally missing. Similarly, Khonsue and Thirakhupt (2001) listed the species in their checklist of Thai amphibians, but no details were given other than its general distribution range (Northern Vietnam, Yunnan P.R.C., and Thailand). Thus, nothing has been known about species of this genus within Thailand. This is probably because few herpetologists paid attention to small rhacophorids, such as this species, in Thailand.

The type locality of the present new species, Pilok in the Thompa Phun National Park, is situated at the Myanmar border of Thailand. Although the region is not poorly surveyed and we attended there in all seasons (Khonsue, unpublished data), we encountered the new species only in early January, when there was little rain and the temperature was low. In contrast, other tree frogs like *Raorchestes parvulus* and *Kurixalus bisacculus* were observed in every occasion. Thus, the new species seems to be less abundant or has a life history different from other small tree frogs.

Our preliminary phylogenetic analyses using only short

sequences of one gene (16S rRNA) showed presence of weakly supported two genetic clades among species of *Gracixalus*. One clade included *G. gracilipes* (the type species of the genus), *G. supercornutus*, *G. quangi*, *G. quyeti*, and the new species, and another clade included *G. carinensis*, *G. jinxiuensis*, *G. nonggangensis*, and *G. waza*. Position of *G. lumarius* was enigmatic. Rowley et al. (2011) similarly used short 16S rRNA sequences and suggested the presence of two clades (Clade I and Clade II) in *Gracixalus*. Contents of these two clades are essentially identical with ours, although their Clade I included *Gracixalus* cf. *jinxiuensis* and *Kurixalus* cf. *ananjevae*. They reported that species in the Clade I have generally small body size (male SVL < 30 mm) and greenish dorsum, whereas those in Clade II have large body size with brownish (never greenish) dorsum and rounded snout (snout shape for Clade I not mentioned). Based on these differences, Rowley et al. (2011) suggested the separation of the genus into two distinct genera in their brief discussion. However, Nguyen et al. (2013) criticized this division by indicating similar dorsal color (yellowish-olive) of *G. quyeti* in Clade I and *G. waza* they recovered in Clade II. *Gracixalus quyeti* actually has a round snout unlike other members in Clade I defined by molecular phylogeny.

Moreover, Rowley et al. (2014) added *G. lumarius* in their Clade I, although the species has the dorsal color and snout shape similar to members of Clade II. This again suggests incongruence between morphological and genetic characteristics in *Gracixalus*. Our result did not support Rowley et al.'s (2014) inclusion of *G. lumarius* in their Clade I. In describing this species, Rowley et al. (2014) showed only result of BI analysis and never made any definite comments. Instead they mentioned that more extensive molecular analysis would be required to resolve the evolutionary relationships within the genus. Thus, inclusion of *G. lumarius* in Clade I is dubious, and from large genetic distances between other species (see Table 2), even inclusion of the species in *Gracixalus* requires confirmation. In contrast, our results conform to Rowley et al. (2014) in recognition of very close relationship of *G. nonggangensis* and *G. waza*. These two species were described nearly simultaneously and have never been compared with each other yet. According to their original descriptions, they are very similar morphologically, and their known localities are very close. Thus, we suspect they represent a single species. If indeed this is the case, and the dates shown in the publications are correct, *G. nonggangensis* (Mo et al., 2013, published on line on 13 February 2013) should be synonymized with *G. waza* (Nguyen et al., 2013, published on line on 14 November 2012).

It should be also noted that the species identity of *G. carinensis* in previous studies (Delorme et al., 2005; Li et al., 2008, 2009) and our own (in this study) is doubtful. At least Vietnamese samples we used are morphologically different from the type series of *Ixalus carinensis* from Myanmar (Matsui, unpublished data). An enigmatic Chinese species *Philautus medogensis* is now placed in *Gracixalus*, but this taxonomic treatment was done simply by its morphological similarity with *G. jinxiuensis* (Li et al., 2009) and no phylogenetic assessment has been made. In view of these facts, further analyses using long sequences for all species are



required to determine the true identity and phylogenetic relationships of species now assigned to *Gracixalus* and related genera.

Mode of egg deposition is an interesting problem in understanding phylogeny and evolution in the genus *Gracixalus*. Except for *G. quyeti*, whose breeding ecology is not reported (Nguyen et al., 2008), species of the Clade I of *Gracixalus* lays small number of eggs in a non-foamy egg mass on leaves (Fei et al., 2009; Orlov and Ho, 2004; Rowley et al., 2014), and it is most probable the new species has a similar breeding habit. Breeding habits of Rowley et al.'s (2011, 2014) Clade II (*G. carinensis*, *G. jinxiuensis*, *G. nonggangensis*, and *G. waza*) are unknown. *Gracixalus lumarius* is reported to breed in the tree-hole like species of *Theloderma* and *Nyctixalus* (Rowley et al., 2014). As discussed above, phylogenetic position of this species requires reexamination and longer sequence data are necessary for assessing its exact position with reference to the two clades of *Gracixalus* and other rhacophorid species.

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