

A new genus and species of treefrog from Medog, southeastern Tibet, China (Anura, Rhacophoridae)

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ABSTRACT

A new genus and species of treefrog is described from Medog, southeastern Tibet, China based on morphological and phylogenetic data. The new genus can be distinguished from other treefrog genera by the following combination of characters: (1) body size moderate, 45.0 mm in male; (2) snout rounded; (3) canthus rostralis obtuse and raised prominently, forming a ridge from nostril to anterior corner of eyes; (4) web rudimentary on fingers; (5) web moderately developed on toes; (6) phalange "Y" shaped, visible from dorsal side of fingers and toes; (7) skin of dorsal surfaces relatively smooth, scatted with small tubercles; (8) iris with a pale yellow, "X" shaped pattern of pigmentation.

Keywords: Taxonomy; New genus; New species; *Theلودerma moloch*; *Nasutixalus medogensis* sp. nov.

INTRODUCTION

The Old World Treefrogs in the family Rhacophoridae are arboreal, occupying different ecological niches from low shrub to tree crown habitats (Wells, 2010). Currently the family consists of 393 recognized species in 17 genera (Frost, 2015), of which 105 species in 11 genera are found in southern and southwestern China (AmphibiaChina, 2015; Frost, 2015). Within China, the Medog (=Motuo) County at the southern slope of the Himalaya in southeastern Tibet harbors 16 known species of Rhacophoridae treefrogs from eight genera, about 15% of the total diversity of the family in China (AmphibiaChina, 2015).

However, despite the rich treefrog diversity of Medog, few detailed surveys have been done in the region, and much is unknown about the treefrog diversity, and the taxonomy of many species of the region remained unclear. For example, the endemic Mossy Treefrog *Theلودerma moloch* (Annandale, 1912)

was described based on two specimens from southern Medog. For nearly a century, there are no further reports or re-description of the species, and its species boundary is solely delimited based on the original description.

During a herpetological survey of southeastern Tibet in 2015, a male treefrog was collected from the tree crown in the tropical rain forest at Medog. Phylogenetic analysis revealed that this specimen shared the same haplotype with a specimen (6255 RAO) also from Medog that was identified as *T. moloch* in Li et al. (2009). However, morphological comparisons reveal that the treefrog we collected from Medog is distinguished readily from the true *T. moloch* by a suit of morphological characters, and both our specimen and the specimen (6255 RAO) in Li et al. (2009) formerly identified *T. moloch* formed a distinct lineage and diverged from the genus *Theلودerma* and all other known genera in the family Rhacophoridae. Therefore, according to the morphological and molecular phylogenetic data of mitochondrial DNA, we describe a new species and a new genus based on our treefrog specimen from Medog. Phylogenetic position of the previously identified *Theلودerma* specimen (6255 RAO) in Li et al. (2009) is also discussed.

MATERIALS AND METHODS

A single male specimen was collected from Gelin, Medog, southeastern Tibet, China. Following euthanasia, liver tissues was taken and preserved in 95% ethanol, and the specimen was fixed in 10% formalin solution and was transferred to 75% ethanol after fieldwork. The male specimen (KIZ 016395) was

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designated as the holotype, and was deposited in Kunming Institute of Zoology, Chinese Academy of Sciences.

Morphological comparisons: All measurements were carried out with slide calipers to the nearest 0.1 mm. Morphological characters used and their measurement methods followed Fei et al. (2009), webbing formula followed Savage & Heyer (1997). The morphological characters and their abbreviations as: SVL, snout-vent length; HL, head length; HW, head width; SL, snout length; INS, internarial distance; IOS, interorbital distance; EHD, eye horizontal diameter; UEW, maximum width of upper eyelid; TD, tympanum diameter; FAHL, forearm and hand length; FAW, maximum width of forearm; HAL, hand length; FML, femur (thigh) length; TBL, tibia (shank) length; TFL, length of tarsus and foot; FOL, foot length.

Morphological data of congeners were obtained from vouchered specimens (Appendix) as well as from literatures (Annandale, 1912; Fei et al., 2009). The following museum abbreviations were used: CIB-Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China. KIZ-Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China.

Molecular analysis: Total DNA of the single treefrog specimen (KIZ016395) from Gelin and two other known species (*Thelederma beibengensis* and *T. moloch*) were extracted with a standard three-step phenol-chloroform extraction method (Sambrook et al., 1989). A 1 999 base pair DNA sequence of mitochondrial gene 12S *rRNA*, *tRNA^{VAL}*, and 16S *rRNA* (12S-16S) was sequenced using primers L2519 and 16Sbr (Table 1). Amplifications were conducted in a 25 μ L volume reaction, involved initial denaturing step at 94 °C for 5 min; then 35 cycles of denaturing at 94 °C for 45 sec, annealing at 55 °C for 45 sec, and extending at 72 °C for 45 sec; and a final extending step of 72 °C for 7 min. The novel sequences were deposited in GenBank (Table 1). The 12S-16S sequences of other 38 specimens were downloaded from GenBank (Table 1).

All dataset were aligned and edited using MEGA 5 (Tamura et al., 2011). The best model of nucleotide substitution was calculated in Modeltest v1.0.1 (Posada, 1998). The phylogenetic relationship was conducted using Bayesian inference (BI) method with software MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003).

RESULTS

Morphological comparison

The male specimen is moderate body size, snout rounded, canthus rostralis obtuse and raised prominently, discs on fingers and toes moderate, rudimentary web on fingers and moderately developed web on toes, skin of dorsal surfaces relatively smooth, scattered some small tubercles, iris with a pale yellow, "X" shaped pattern of pigmentation. The specimen distinguished mainly from the genus *Thelederma* by the absence of large tubercles and jagged skin ridge on dorsum, the presence of prominently raised canthus rostralis, and iris with a pale yellow, "X" shaped pattern of pigmentation.

Phylogenetic analysis

Currently recognized genera of the family Rhacophoridae were

recovered as monophyletic groups in our phylogenetic analysis (Figure 1). However, similar to previous studies (Li et al., 2009), our data cannot resolve phylogenetic relationships among different genera. The Medog treefrog was clustered and share the haplotype with a formerly identified *T. moloch* (specimen voucher number 6255 RAO) in Li et al. (2009), and both of them form a distinct clade from all other species of genus *Thelederma*, including the true *T. moloch*. Such result is similar to the phylogenetic topography recovered in Li et al. (2013) using more datasets.

Therefore, according to a combination of morphological characters and phylogenetic data of mitochondrial genes, we conclude that the male treefrog specimen (KIZ016395) consisted an independent evolutionary lineage and concordant evidence confirm species status (Hou et al., 2014; Wu & Murphy, 2015), which is described as a new species and a new genus in family Rhacophoridae.

Nasutixalus gen. nov. Jiang, Yan, Wang and Che

Type species: *Nasutixalus medogensis* sp. nov.

Diagnosis: (1) Body size moderate (45.0 mm in male); (2) snout rounded; (3) canthus rostralis obtuse and raised prominently, forming a ridge from nostril to anterior corner of eyes; (4) web rudimentary on fingers; (5) web moderately developed on toes; (6) phalange "Y" shaped, visible from dorsal side of fingers and toes; (7) skin of dorsal surfaces relatively smooth, scattered with small tubercles; (8) iris with a pale yellow, "X" shaped pattern of pigmentation, especially distinct in preservative.

Distribution: Currently known only from the type locality, Medog, Tibet, China.

Etymology: The generic nomen *Nasutixalus* is derived from the Latin adjective *nasutus* ("large-nosed" in English), means the prominent ridge from nostril to the anterior corner of eye, and *ixalus*, a common generic root for treefrogs. We suggest the common name of the new genus be "ridged-nose treefrog" in English, and "Leng Bi Shu Wa" (棱鼻树蛙) in Chinese.

Content: The new genus currently contains a single species, *Nasutixalus medogensis* sp. nov. which is described below.

Nasutixalus medogensis sp. nov. Jiang, Wang, Yan and Che (Figures 2-4)

Synonyms

Thermoderma moloch: Li et al., 2009

Holotype: KIZ 016395, adult male, collected from Gelin (N29.21665°, E95.17571°, elevation 1 619 m), Beibeng, Medog, southeastern Tibet, China, on 28 April 2015, by Ke JIANG.

Diagnosis: As for the generic diagnosis.

Description of holotype: Body size moderate, SVL 45.0 mm; body stout, moderately slender at waist. Head width nearly as equal to length (HW/HL=1.04); snout rounded, slightly projecting beyond jaw; canthus rostralis distinct, obtuse, and raised prominently, forming a ridge from nostril to anterior

Table 1 Information of samples used in molecular analysis

Genus	Species	Specimen voucher No.	Locality	GenBank No.
<i>Nyctixalus</i>	<i>spinosus</i>	ACD 1043	Philippine Islands: Mindanao	DQ283114
	<i>pictus</i>	FMNH 231095	Malaysia	DQ283133
<i>Theلودerma</i>	<i>rhododiscus</i>	SCUM 061102L	China: Mt. Dayao, Guangxi	EU215530
	<i>asperum</i>	060821203Rao	China: Jinping, Yunnan	GQ285677
	<i>beibengensis</i>	YPX37270	China: Medog, Tibet	—
	<i>moloch</i>	YPX31941	China: Medog, Tibet	—
	<i>corticale</i>	AMNH A161499	Vietnam	DQ283050
<i>Nasutixalus</i>	<i>medogensis</i> sp. nov.	KIZ016395	China: Medog, Tibet	—
		6255Rao	China: Medog, Tibet	GQ285679
<i>Kurixalus</i>	<i>hainanus</i>	HNNU A1180	China: Mt. Diaoluo, Hainan	EU215548
	<i>odontotarsus</i>	SCUM 060688L	China: Mengyang, Yunnan	EU215549
	<i>idiootocus</i>	SCUM 061107L	China: Lianhuachi, Taiwan	EU215547
	<i>eiffingeri</i>	UMFS 5969	China: Nantou, Taiwan	DQ283122
<i>Pseudophilautus</i>	<i>microtympanum</i>	—	Sri Lanka	DQ346974
	<i>menglaensis</i>	060821286Rao	China: Lvchun, Yunnan	GQ285676
<i>Philautus</i>	<i>acutirostris</i>	—	—	AF458137
	<i>surdus</i>	CAS 219932	Philippine	GQ285676
	<i>abditus</i>	ROM33145	Vietnam: Krong Pa, Gia Lai	GQ285673
<i>Gracixalus</i>	<i>jinxiuensis</i>	KIZ 061210YP	China: Mt. Dayao, Guangxi	EU215525
	<i>carinensis</i>	ROM39660	Vietnam: Sa Pa, Lao Cai	GQ285670
	<i>gracilipes</i>	060821196Rao	China: Mt. Dawei, Yunnan	GQ285668
<i>Rhacophorus</i>	<i>feae</i>	SCUM 050642W	China: Hekou, Yunnan	EU215544
	<i>rhodopus</i>	SCUM 060692L	China: Mengyang, Jinghong	EU215531
	<i>dugritei</i>	SCUM 051001L	China: Baoxing, Sichuan	EU215541
	<i>kio</i>	SCUM 37941C	China: Xishuangbanna, Yunnan	EU215532
<i>Feihyla</i>	<i>palpebralis</i> 1	SCUM 0606132L	China: Mt. Dawei, Yunnan	EU215546
	<i>palpebralis</i> 2	712	Vietnam: Lam Dong	GQ285681
<i>Chiromantis</i>	<i>vittatus</i> 1	KIZ 0001Rao	China: Simao, Yunnan	GQ285684
	<i>vittatus</i> 2	FMNH 254444	Vietnam: Gia Lai	DQ283134
	<i>doriae</i> 1	1056	Vietnam: Binh Thuan	GQ285683
	<i>doriae</i> 2	KIZ 005Rao	China: Simao, Yunnan	GQ285682
<i>Polypedates</i>	<i>leucomystax</i>	CAS 219931	Philippines	AF458140
	<i>megacephalus</i>	SCUM 050508C	China: Mt. Daiyun, Fujian	EU215552
	<i>mutus</i>	SCUM 37940C	China: Xishuangbanna, Yunnan	EU215551
<i>Liuixalus</i>	<i>ocellatus</i>	HN0806045	China: Mt. Wuzhi, Hainan	GQ285672
	<i>romeri</i>	KIZ 061205YP	China: Mt. Shiwan, Guangxi	EU215528
<i>Buergeria</i>	<i>oxycephala</i>	SCUM 050267YJ	China: Hainan	EU215524
<i>Raorchestes</i>	<i>parvulus</i>	KUHE 38322	Thailand: Khao Sabap	LC012865
<i>Ghatixalus</i>	<i>variabilis</i>	VUB0061	India	EU178099
<i>Beddomixalus</i>	<i>bijui</i>	—	India	KC594289, KC594290
<i>Mercurana</i>	<i>myristicapalustris</i>	—	India	KC594294, KC594293
<i>Boophis</i>	<i>tephraeomystax</i>	AMNH A168144	Madagascar	DQ283032
<i>Limnonectes</i>	<i>poilani</i>	AMNH A163717	Vietnam	DQ283378

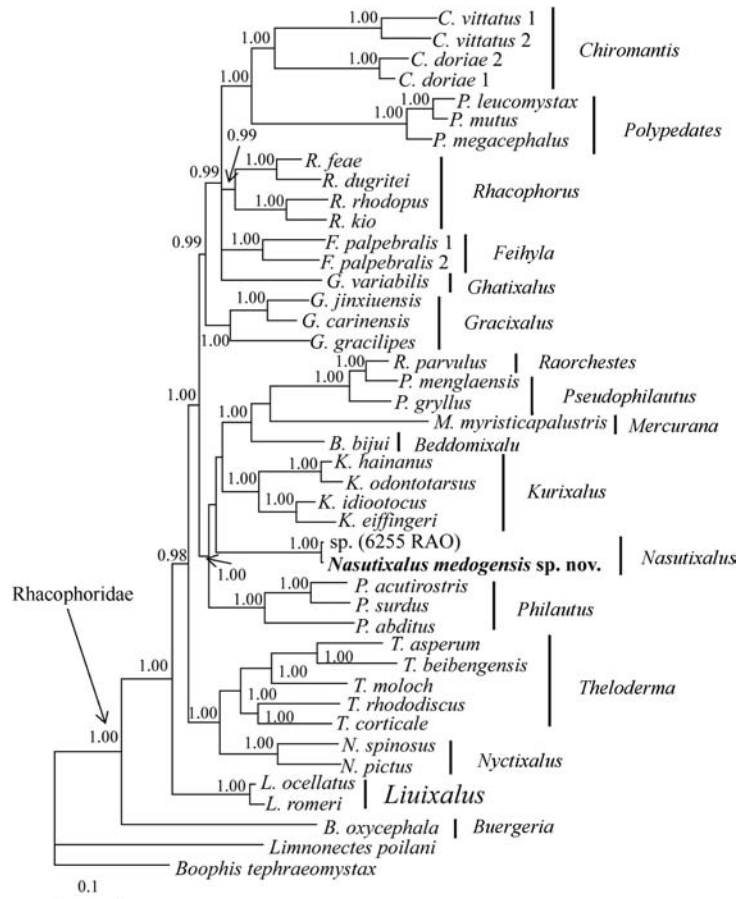


Figure 1 Bayesian inference tree based on molecular data of Rhacophoridae and two outgroups

The characters near branches are Bayesian posterior probabilities (only ≥ 90 were showed).

Table 2 Morphological measurements (mm) of the holotype of *Nasutixalus medogensis* sp. nov.

	SVL	HL	HW	SL	INS	IOS	UEW	EHD	TD	FAHL	FAW	HAL	FML	TBL	TFL	FOL
KIZ 016395	45.0	15.5	16.1	6.8	4.9	4.3	3.9	6.0	2.4	24.4	5.1	14.9	20.8	21.2	32.7	22.1
Ratio to SVL (%)	—	34.3	35.9	15.1	11.0	9.5	8.6	13.2	5.3	54.2	11.3	33.1	46.2	47.1	72.5	49.0

Abbreviations: SVL, snout-vent length; HL, head length; HW, head width; SL, snout length; INS, internarial distance; IOS, interorbital distance; UEW, width of upper eyelid; EHD, eye horizontal diameter; TD, tympanum diameter; FAHL, forearm and hand length; FAW, width of forearm; HAL, hand length; FML, femur length; TBL, tibia length; TFL, length of tarsus and foot; FOL, foot length.

corner of eye; loreal region oblique, concave; internarial distance 1.14 times larger than interorbital distance. Eyes large, prominent, eye diameter 0.39 times of head length; pupil rounded. Interorbital region flat, interorbital distance 1.1 times larger than upper eyelid width. Tympanum distinct, 0.4 times of orbit diameter. Tongue pyriform, deeply notch behind, papillae absent; choanae large, visible viewed from below; a pair of vomerine teeth on inner sides of choanae; single, external, subgular vocal sac present, with a pair of small openings near inner corners of mouth; supratympanic fold from posterior corner of orbit to previous shoulder, distinctly developed but slender.

Fore-limbs long and strong; forearm and hand slightly longer than half of body length; lower arm thick; fingers compressed with discs; circummarginal grooves present; relative length of fingers: I<II<IV<III; nuptial pad present on inner side of first

finger; subarticular tubercles present, distinct; web rudimentary on fingers; fringe distinct; inner metacarpal tubercle elliptical, long, distinct; outer metacarpal elliptical, flat, smaller than inner one; several tubercles scattered on palm, with four tubercles relatively distinct on middle of palm.

Hind limbs relatively long, tibiotarsal articulation reaching the eye when adpressed; heels much overlapped when flexed and held perpendicular to body; shank nearly as equal to thigh (TBL/FML=1.02); foot length nearly as equal to shank (FOL/TBL=1.04); relative toe lengths: I<II<V<III<IV; toe tips with discs; circummarginal grooves present; subarticular tubercles distinct; toe web moderately developed, webbing formula: I 0-1 II 0-1 III 0-1 IV 1-0 V; fringe distinct; small tubercles scattered on undersurfaces of metatarsus; inner metatarsal tubercle elliptical, prominent; outer metatarsal tubercle absent; tarsal fold absent.



Figure 2 Different views of the holotype (KIZ 016395) of *Nasutixalus medogensis* sp. nov. in life (Photos by Ke JIANG)

A: dorsolateral view; B: dorsal view; C: dorsal view of head; D: ventral view; E: ventral view of hand; F: ventral view of foot.



Figure 3 Different views of the holotype (KIZ 016395) of *Nasutixalus medogensis* sp. nov. in preservative (Photos by Ke JIANG)

A: dorsolateral view; B: ventral view.

Skin of dorsal surfaces of head, body and limbs relatively smooth, with small tubercles scattered; loreal and temporal region, and lateral body rough, with distinct tubercles; ventral surface with serried flat tubercles, relatively small on throat, chest and ventral forelimbs, relatively large on belly and ventral thigh; tubercles on basal ventral thigh prominent.



Figure 4 Right eye of the holotype, showing the iris with a pale yellow, "X" shaped pattern of pigmentation, in life (A) and preservative (B) (Photos by Ke JIANG)

Coloration of holotype in life

Coloration of the dorsal and lateral surfaces of head and body are camouflage of pistachio and creamy brown. A distinct, creamy brown, reversed triangular pattern of pigmentation is observed on the dorsal surface of the head, with its base positioned between the orbits. The tip of the triangular pattern of pigmentation extends posteriorly to the pectoral region and connects with the large, creamy brown, "X" shaped pattern of pigmentation that extends further posteriorly and laterally to the pelvis. The coloration of the iris is dark blackish, with a distinct pale yellow, "X" shaped pattern of pigmentation; pupil is jet black. Dorsal surfaces of the limbs are creamy brown, which is more saturated on the disc of fingers and toes. Distinct, pistachio transverse bands were observed on the dorsal surfaces of limbs from the proximal end to the fingers/toes. Lateral surfaces of hind limbs and are yellowish. Coloration of the ventral surfaces of the head, body and limbs are pale flesh color. A single patch of light creamy yellow pigmentations is observed on the chest ventral to axilla on each side. The abdominal region and the ventral surfaces of feet are slightly creamy yellowish.

Coloration in preservative

The patterns of pigmentation in preservative closely resemble the patterns in life. However, the following coloration changes after preservation process: (1) The pistachio and creamy brown coloration of the dorsal and lateral surfaces of head, body, and limbs become light and dark gray respectively; (2) the patches on the chest, ventral surfaces hind limbs, and ventral surfaces of hands and feet are light yellow, and remaining parts of the ventral body and limbs become light gray.

Etymology

The species name “*medogensis*” is named after the type locality, Medog, Tibet, China. According to the Latin name, we suggest the English common name as “Medog Ridged-nose Treefrog”, and the Chinese formal name as “Muō Tuo Leng Bi Shu Wa” (墨脱棱鼻树蛙).

DISCUSSION

In the recent phylogenetic study, Li et al. (2009) showed that a single specimen (6225 RAO) of treefrog from Medog that was formerly identified as *T. moloch*, is remotely related to the genus *Theloderma* and represents a distinct clade. Therefore, Li et al. (2009) concluded *T. moloch* was not a member of the genus *Theloderma*. However, our phylogenetic results show that the true *T. moloch* from Medog (Hou et al., unpublished data) is nested within the genus *Theloderma* and is distantly related to the specimen (6225 RAO) from Li et al. (2009). Furthermore, our results show that the specimen in Li et al. (2009) is clustered and share the haplotype with our new species of the new genus with well-supported, hence it is a conspecific of our new species. Therefore, we recommend further surveys focus on the high tree crown and collect additional specimens of species from the new genus to provide further data to resolve the remaining taxonomic and phylogenetic issues.

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APPENDIX

The following specimens were examined:

Polypedates cf. *braueri*: KIZ 06994, KIZ 0697-98, KIZ 07001, KIZ 010987-89, KIZ 010991, KIZ 010993, KIZ 011034 (10♂♂); KIZ 06993, KIZ 07000, KIZ 07002, KIZ 010990, KIZ 010992, KIZ 011016, KIZ 011029-30 (8♀♀). Medog, Tibet.

Gracixalus medogensis: KIZ 010956 (1♂). Medog, Tibet.

Kurixalus naso: KIZ 010962-64, KIZ 010967, KIZ 010976, KIZ 011003, KIZ 011005, KIZ 011009-10, KIZ 011023 (1010♂♂); KIZ 010966, KIZ 010977, KIZ 011006, KIZ 011028 (4♀♀). Medog, Tibet.

Rhacophorus bipunctatus: KIZ 06999, KIZ 07003-007, KIZ 010979, KIZ 010983, KIZ 011044, KIZ 011047 (10♂♂); KIZ 010960, KIZ 010980

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(2♀♀). Medog, Tibet.

Rhacophorus burmanus: KIZ 016379-80, KIZ 016408-09, KIZ 016441-44, KIZ 016446-47 (10♂♂); KIZ 010957, KIZ 016376-78, KIZ 016410, KIZ 016445 (6♀♀). Medog, Tibet.

Rhacophorus maximus: KIZ 016384 (1♂); KIZ 016385, KIZ 013859 (2♀♀). Medog, Tibet.

Rhacophorus translineatus: KIZ 06648-52, KIZ 011161-62, KIZ 011167-70 (11♂♂); KIZ 012683 (1♀). Medog, Tibet.

Feihyla vittata: KIZ 07352, KIZ 011171-72, KIZ 012708-10 (6♂♂); KIZ 07353-35 (3♀♀). Medog, Tibet.

Theloderma beibengensis: KIZ 020453 (1♂). Medog, Tibet.