大 沙粉罗姆克 70010010 ISSN 2095-8137 CN 53-1229/Q

Volume 37 Issue 1 18 January 2016

## 対約3所定 ZOOLOGICAL RESEARCH

Decoding the diversity of amphibians and reptiles on the Qinghai-Tibetan Plateau



### **ZOOLOGICAL RESEARCH**

### Volume 37, Issue 1 18 January 2016

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Zoological Research Website: http://www.zoores.ac.cn/

## New Year address from Zoological Research

Time is flying. As Chuang Tzu, a Zhou Dynasty Chinese philosopher, said "time goes like a young horse scampering past a narrow cleft". The end of a year is a link between what came before and what follows, and is an opportune time for reflection and anticipation.

In Chinese tradition, 2015 was the Year of the Goat. Just like a goat, a small and hardy animal characterized by endurance, *ZR* is still a small figure compared with many international academic journals, but with endurance and optimism, it continues to promote scientific communication and knowledge dissemination.

In 2015, Zoological Research (ZR) was recognized as a core journal (Level A, top 5%-20%) and ranked 15th among 78 biology-based academic journals by the "RCCSE Chinese Academic Journal Evaluation Report-Ranking List of Authoritative Journals and Core Journals". In this evaluation, which was released by the Research Center of Chinese Science Evaluation (RCCSE), the Library of Wuhan University and the Network of Science & Education Evaluation in China, a total of 6 201 out of 10 000 academic journals were selected, with ZR among the 1 572 core journals. Also in 2015, ZR was honored as one of the "Top 100 Outstanding Science and Technology Journals of China" in recognition for our impact and influence in academic publication. Being honored by one of China's highest awards for an academic journal comes from the constant and enthusiastic support of our diligent authors, readers, and editorial board.

Over the past 35 years, since its founding in 1980, *ZR* has never lost its open-mindedness nor receptiveness to new ideas. In September 2015, to adapt to new digital media, we registered a social WeChat account (ZoolRes) and have posted numerous popular science articles across a wide range of life science interests, with certain articles clicked and browsed nearly 700 times in just a few short months. In regards to this, we would like to extend a special invitation to all our colleagues interested in writing scientific stories that are aimed not only at scholars but also inquisitive members of the public. Please contact us if you want to be involved.

The start of 2016 is, of course, a perfect time to make New Year's Resolutions. Before setting such declarations in stone, however, we should all take time from our hectic schedules to sit with a clear mind and truly contemplate what we could and should be doing in the upcoming year. Promoting an academic journal is never easy, but, with cooperation and combining our efforts, the future of *ZR* is promising. The new year brings hope, challenges and opportunities. In the Chinese culture, 2016 is the Year of the Monkey, an animal that symbolizes thoughtfulness, energy and fearlessness, such as that embodied by the popular Monkey King character. It is our wish that the forthcoming year will allow us to face all difficulties with confidence, wisdom and vibrancy; retain our open-mindedness and consideration; and unceasingly and faithfully fulfil our responsibilities.

We hope that we can continue to work more closely than ever, foster the evolution of young scholars and researchers, and keep advocating for our own improvement. As time continues its inevitable march, the spirit of science and cooperation will ensure the enduring success of *ZR*. As favored Chinese author Mu-Xin says "Time is ruthless, but I ruthlessly fight back by not wasting a single day".

Once again, may the upcoming Year of the Monkey bring you peace, prosperity, and happiness! Your enduring help and support are, as always, deeply appreciated.

Sincerely

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### A new resource for China

David B. WAKE

In my country, as elsewhere in the world, when one wants information about animals, such as amphibians, one typically seeks out a field guide or a monograph. As the amphibian fauna around the world has grown (from about 4 000 species in 1985 to nearly 7 500 today), the books, too, have become thicker, heavier, and more and more unwieldy. These books might contain features to assist identification—written descriptions, keys, photographs and maps of the polygon variety and encompass known sites of occurrence. But the books quickly become out-of-date as knowledge increases and they rarely have information about phylogenetic relationships or display trees. But, we now live in a digital age, and young people, in particular, view books as old-fashioned and inadequate for their needs. The time is ripe for a new approach.

Welcome to the world of AmphibiaChina (2015). This is a new venture, from the Kunming Institute of Zoology, Chinese Academy of Sciences, and it is off to an impressive start. It was inspired by AmphibiaWeb (2015), a website that I initiated with colleagues in 2000 to try to centralize and keep up-to-date information concerning amphibians and their biology and conservation status. In addition, the Amphibian Species of the World (ASW) website (Frost, 2015), operated by Darrel Frost and sponsored by the American Museum of Natural History, provides access to the formal taxonomy of amphibians and additional information (for example, verbal descriptions of species ranges). Both are worldwide in scope and cover all amphibian species.

AmphibiaChina is more restricted in scope, to China, but that country has a very large amphibian fauna (over 400 species, typically with many new species described each year). The new site has some of the features of both of the worldwide sites, but is in a position to go into much more detail. I look forward to seeing not one or a few photos of species, but many. I encourage the use of maps showing specific locations, although these can be challenging to produce and maintain, because they are far superior to the kinds of maps one finds in books. Further, the new site offers novel features, such as an identification system based on DNA barcoding and phylogenetic trees of different clades. AmphibiaChina will be dynamic, constantly undergoing modification in accordance with newly generated knowledge, and improved according to experience and feedback as the site comes into use.

The need for an up-to-date resource, such as AmphibiaChina promises, can be illustrated by our rapidly changing understanding of Chinese amphibians. My own specialty is salamanders, Order Caudata, first considered in depth by the late Chinese biologist Mangven L. Y. CHANG (1936, Contribution à l'étude Morphologique, Biologique et systématique des Amphibiens urodèles de la Chine [in French]. Paris, Librairie Picart) (Chang, 1936), is especially well represented in China, where Chang reported three families and 16 species. Er-Mi ZHAO and Kraig Adler made Chinese herpetology accessible to the world in their grand summary of 1993 (Herpetology of China, Contributions to Herpetology 10: 1-521) (Zhao & Adler, 1993). They recognized 35 species. The time period from the mid-1990s to the present has witnessed a rapid growth of herpetology as a field of science in China, with the development of many active centers of research, and much emphasis was placed on discovery and taxonomy. The most recent published compendium of Chinese amphibians is the massive (629 pp; 260 mm×345 mm; more than 4 kg) book written by Fei et al (2012). This book recognizes 67 salamanders. However, there have been a number of changes since this book appeared and some are no longer recognized and some new ones have been discovered. Thus, at the present time AmphibiaWeb recognizes 73 species of salamanders (including Taiwan, which was also done by Fei et al, 2012). The only effective way to keep track of the rapidly growing knowledge base for Chinese amphibians is though the use of modern digital technology, and that is what AmphibiaChina promises to do.

It takes much effort to maintain a database such as has been established, especially in a period of intense research activity, including controversies over criteria for species recognition. The literature is very dynamic and ever-changing. We are learning more about the biology of these organisms daily and keeping track of the literature is possible only by constantly consulting journals, books and formal and informal reports. The internet/World Wide Web enables tracking knowledge in ways

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DOI:10.13918/j.issn.2095-8137.2016.1.2

unimaginable just a few years ago.

A particular challenge for a website devoted to amphibians is the issue of population declines and disappearances of populations, and even species, around the world. Although this has been well-documented (AmphibiaWeb publishes an extensive list of new literature on this topic every month), despite the intense focus on this general phenomenon, we still lack a full understanding. Certainly the phenomenon is multifactorial, with many immediate causes: habitat modification, invasive species, infectious diseases, human exploitation, and others. However, in the background are more profound and even sinister issues related to global climate change. It will be a continuing challenge to sift through the available information and present a coherent picture of the status of amphibians in the world.

I will follow the development and evolution of AmphibiaChina with great interest. I hope to learn from it, and use it, and I hope that we can develop a good synergy between it and AmphibiaWeb, to our mutual benefit. My very best wishes for success to this new venture, which is off to an impressive start.

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## Advances in herpetological research emanating from China

Robert W. MURPHY

The warming of Earth owing to human activities is resulting in an episode of mass extinction. If global warming is not abated guickly, up to one-fourth of the species on our planet will face extinction in only 35 years. The loss of biodiversity is more than just many species disappearing. It could signal the beginning of the collapse of our ecosystems because the loss of one species can trigger a domino effect. Among vertebrates, the so-called cold bloodied species—fishes, amphibians and reptiles—are the most threatened groups. According to International Union for Conservation of Nature (IUCN), as of 2009, 30% of amphibians are threatened with extinction and another 6% are considered to be Near Threatened. Regarding the world's reptiles, Simon Stuart, Chair of the IUCN's Species Survival Commission, believes their situation "may be much worse than it currently looks". Some 10% of Chinese and South East Asian species of snakes are Threatened with extinction and a whopping 43% are listed in the Endangered and Vulnerable categories. Turtles and tortoises are not doing any better.

The percentages of Threatened species are moving targets because of the discovery of many new cryptic species. Typically, new species await assessments of their status. The number of new species of amphibians and reptiles described each year continues to increase and, unlike for mammals and birds, no end is in sight. Take for example China. Zhao & Adler (1993) summarized China's diversity of amphibians and reptiles. They listed 274 species of amphibians among which 175 species were endemic (occurring only in China). More diverse, the reptiles accounted for 387 species, yet fewer, only 133, were endemic. A quick assessment of the past 22 years indicates we have a long road ahead before documenting the actual number of species, assuming we can reach the end of the road before the species become extinct.

According to AmphibiaWeb (2015), today China has 406 species of amphibians, yet AmphibiaChina (2015) lists 429 species. The latter assessment amounts to an increase of almost 57% since 1993. The vast majority of the increase in amphibian diversity owes to the discovery and description of new species. Based on data from AmphibiaWeb (2015) and Frost (2015), this involves 150 species descriptions and nine new records or resurrections of old species names. Figure 1 shows the number of new species of Chinese amphibians

described since 1994; the number of newly described species tends to increase annually. Every year has witnessed the description of at least one new species. A plot of the total number of amphibian species in China (not shown) shows no asymptote; no data indicate just how many species of amphibians occur in China.

The reptiles show a similar trend to amphibians of increasing diversity, but only by 33%. According to the Reptile Database (Uetz & Hošek, 2015), today China has 514 species of reptiles. which includes lizards, snakes, turtles and one species of alligator. However, unlike amphibians, the increase of 127 species involves the description of only 51 new species (Figure 2). The increase in diversity largely owes to the discovery and resurrection of previously described species. Historically, the diversity of reptiles in China may be better known than amphibians in part due to their broader use in Chinese culture and in traditional medicine. In 1596, the great pharmacologist Shi-Zhen LI of the Ming Dynasty published his book Ben Cao Gang Mu (The Great Pharmacopoeia). In it, LI listed three amphibians (Chinese giant salamander, tadpole and toad) as opposed to five reptiles (turtle, gecko, snake, 100 pace viper and alligator).

The increases in the numbers of species often owe to "less than comfortable" and sometimes outright dangerous fieldwork. Such is the essence of herpetology and the experience with the animals in nature yields invaluable insights into the lives, ecology and vulnerability of each species. The absence of such information hampers efforts to conserve the species. Historically, much of the work was carried out by foreign investigators. Today, Chinese researchers are largely responsible for such initiatives.

Nowadays, phylogenetics forms the philosophical and methodological basis for recognizing species and organizing them into genera and other hierarchical taxonomic categories. The development of affordable molecular genetic approaches

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also catapulted biodiversity assessments. For example, DNA barcoding, a method that uses the nucleotide sequence of a short fragment of mitochondrial DNA, plays a central role in this approach. The comparison of DNA barcodes from widespread taxa allows for the identification of likely cryptic species. Using this extremely fast and efficient approach, Scientists at the Kunming Institute of Zoology have successfully barcoded more

than 80% of all species of Chinese amphibians and from multiple localities. A large proportion of the species have samples from type localities. The approach has been so successful in China that the Kunming Institute of Zoology played the central role in forming ColdCode, the international initiative to DNA barcode all species of amphibians and reptiles (http://coldcode.org/) (Murphy et al., 2013).

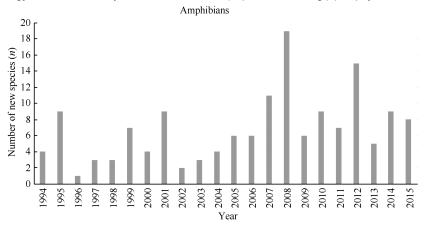


Figure 1 Histogram showing the number of newly described species of amphibians per year occurring in China (data from AmphibiaWeb (2015) and Frost (2015))

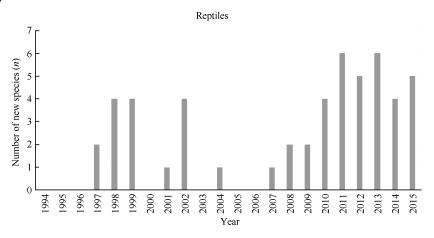


Figure 2 Histogram showing the number of newly described species of reptiles per year occurring in China (data from Reptile Database (Uetz & Hošek, 2015))

Given the biodiversity crisis, conservation is of utmost importance. Conservation usually involves named species and bad taxonomy kills because no species can be protected until it is named (May, 1990). Naming requires discovery and the documentation that a potential new species is on its own evolutionary trajectory. The synergy of fieldwork, documentation, naming and protection requires much effort and commitment. To the surprise of many, *Zoological Research* now documents that the biodiversity of the Qinghai-Tibetan plateau (QTP) is deserving of much further exploration and work. In this special issue, one research team from the Laboratory of Herpetological Diversity and Evolution, KIZ, CAS is invited to report their recent work on the QTP.

Yan et al. (2016) document the occurrence of the family

Ceratobatrachidae in Himalaya region of China and Southeast Asia for the first time. They confirm a new subfamily to accommodate three Chinese species, which they assign to genus *Liurana*. This paper demarks a significant to the origin and diversification of a large radiation of frogs. Jiang et al. (2016c) report the discovery of a new species of treefrog from southeastern Tibet and they erect a new genus for it. This too makes an important contribution to the diversity of frogs within China. Further, Jiang et al. (2016a, b) describe new species of frogs in the genera *Amolops* and *Scutiger*, respectively, also from Tibet. Finally, Wang et al. (2016) describe two new species of lizards in the genus *Japalura* from valley habitats in the Hengduan Mountain Range on eastern Qinghai-Tibetan plateau, highlighting the underestimated diversity of the region and the

importance of valley habitat conservation in the Three-Parallel-Rivers

Overall, one of the most important contributions to this issue of *Zoological Research* is the announcement of AmphibiaChina (Che & Wang, 2016). The project, which is modeled after AmphibiaWeb (2015), will serve to catapult and coordinate amphibian research in China. It will prove to be invaluable for governmental officers, wildlife managers, academics, and the public audience alike. As Wake (2016) notes, it is destined to become THE place for updated information of the diversity of amphibians in China. Surely it will serve to stimulate similar efforts on a global basis. My only question is where is ReptiliaChina?

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# The Australasian frog family Ceratobatrachidae in China, Myanmar and Thailand: discovery of a new Himalayan forest frog clade

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#### **ABSTRACT**

In an effort to study the systematic affinities and specieslevel phylogenetic relationships of the enigmatic anurans variably assigned to the genera Ingerana or Limnonectes (family Dicroglossidae), we collected new molecular sequence data for five species including four Himalayan taxa, Limnonectes xizangensis, Lim. medogensis, Lim. alpine, Ingerana borealis and one southeast Asian species, I. tasanae, and analyzed these together with data from previous studies involving other ostensibly related taxa. Our surprising results demonstrate unequivocally that Lim. xizangensis, Lim. medogensis and Lim. alpine form a strongly supported clade, the sister-group of the family Australasian forest frog family Ceratobatrachidae. This discovery requires an expansion of the definition of Ceratobatrachidae and represents the first record of this family in China. These three species are distinguished from the species of Ingerana and Limnonectes by the: (1) absence of interdigital webbing of the foot, (2) absence of terminal discs on fingers and toes, (3) absence of circumarginal grooves on the fingers and toes, and (4) absence of tarsal folds. Given their phylogenetic and morphological distinctiveness, we assign them to the oldest available generic name for this clade, Liurana Dubois 1987, and transfer Liurana from Dicroglossidae to the family Ceratobatrachidae. In contrast, Ingerana tasanae was found to be clustered with strong support with the recently described genus Alcalus (Ceratobatrachidae), a

small clade of otherwise Sundaic species; this constitutes a new record of the family Ceratobatrachidae for Myanmar and Thailand. Finally, *Ingerana borealis* clustered with the "true" *Ingerana* (family Dicroglossidae), for which the type species is *I. tenasserimensis*.

Keywords: Dicroglossidae; Himalaya; Liurana

#### INTRODUCTION

The frogs of family Ceratobatrachidae (Boulenger, 2009) comprise a morphologically, developmentally, ecologically, and biogeographically greatly variable and, thus, unique clade (Brown et al., 2015). This family is notable for highly variable body size, direct larval development, and the ability to inhabit a wide variety of environments that lack

Received: 27 October 2015; Accepted: 15 December 2015

Foundation items: This study was supported by the Ministry of Science and Technology of China (2014FY210200, 2011FY120200), the program of Chinese Academy of Sciences (2015CASEABRI002), and the Animal Branch of the Germplasm Bank of Wild Species of Chinese Academy of Sciences (the Large Research Infrastructure Funding) to JC; RMB's work on the family Ceratobatrachidae has been supported by the U. S. National Science Foundation (DEB 073199, 0334952, 0743491, 1418895).

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DOI:10.13918/j.issn.2095-8137.2016.1.7

standing water-from small oceanic islands, to high-elevation mossy montane forests (Brown & Alcala, 1982; Brown et al., 2013; Günther, 2015). Currently, 91 species are assigned to three genera: *Platymantis* Günther, 1858, *Cornufer* Tschudi, 1838, and *Alcalus* Brown, Siler, Richards, Diesmos, and Cannatella, 2015 (AmphibiaWeb, 2015; Brown et al., 2015; Frost, 2015). These species are distributed broadly from the South-West Pacific to the island archipelagos of South Asia, with primary centers of species diversity in Philippines and Solomon-Bismarck Archipelago (Brown, 2009; Brown et al., 2013, 2015).

Four species, formerly referred to Southeast Asian frogs *Ingerana* (Dubois, 1987), were recently assigned to the family Ceratobatrachidae based on molecular data (Brown et al., 2015). The four taxa (*I. baluensis, I. mariae, I. rajae, I. sariba*) comprise a monophyletic group now shown to be the sister group of Ceratobatrachinae (genera *Platymantis* and *Cornufer*). However, "true" *Ingerana* (based on the phylogenetic position of the type species, *Ingerana tenasserimensis* [Sclater, 1892]) has been shown in multiple studies to be more closely related to Dicroglossidae (Bossyut et al., 2006; Wiens et al., 2009). Thus, these four species were just recently assigned to the new genus *Alcalus* in the family Ceratobatrachidae (Brown et al., 2015).

The species in genus Ingerana are small, plump frogs with flattened and expanded toe and finger tips (Dubois, 1987). Thirteen species previously have been referred to this genus on the basis of morphological characters and life history traits. However, recently its members have been placed in different genera, and even different families, based on phylogenetic analysis of molecular data analysis, i.e., A. baluensis, A. mariae, A. rajae and I. tenasserimensis (Bossuyt et al., 2006; Frost et al., 2006; Wiens et al., 2009; Brown et al., 2015). The placement of other Ingerana species was controversial, and some species were tentatively placed in different genera, in the absence of accompanying molecular data. For example, Limnonectes xizangensis was variably assigned to the genera Cornufer (Hu, 1977), Ingerana (subgenus Liurana) (Dubois, 1987), Platymantis (Fei et al., 1990), Micrixalus (Zhao & Adler, 1993), and finally to Limnonectes (subgenus Taylorana) (Borah et al., 2013; Frost, 2015). The complex and convoluted taxonomic placement of several of these species has based on morphological or reproductive characters. Because the few key diagnostic characters emphasized by previous worker are variable, and subject to individual interpretation they may have mislead previous attempts to determine systematic affinities of these poorly known frog species.

Here we report the results of a systematic study of five species variably referred to Limnonectes or Ingerana, including Lim. xizangensis, Lim. medogensis, Lim. alpine, I. borealis and I. tasanae. We redistribute them among two families, according to their phylogenetic affinities, as Liurana xizangensis, Liu. medogensis, Liu. alpine, and Alcalus tasanae (family Ceratobatrachidae) and Ingerana borealis (family Dicroglossidae). These discoveries greatly extend the westernmost geographic distribution of the primarily Australasian archipelago family Ceratobatrachidae into

Indochina and China and assign early mainland branching events in this family to lineages now exclusively represented by species with restricted ranges in the high-elevation Himalayan mountains of Tibet.

#### **MATERIALS AND METHODS**

#### Sampling

Four species, Limnonectes xizangensis, Lim. medogensis, Lim. alpine and Ingerana borealis, were sampled from Medog (=Motuo), Tibet (=Xizang), PR China (locality 1 in Figure 1, Table 1). Following the collection of liver tissue samples (preserved in 95% ethanol), the voucher specimens were fixed with 10% formalin and then stored in 70% ethanol. Collection of specimens followed animal-use protocols approved by the Kunming Institute of Zoology Animal Use and Ethics Committee. Two more species, I. tasanae and Occidozyga martensii distributed in Myanmar and Thailand, were also included. We borrowed their tissue samples from the collections of the California Academy of Sciences (CAS), Thailand National History Museum (THNHM), and Field Museum of Natural History (FMNH) (Figure 1, Table 1).

#### DNA extraction and sequencing

Total DNA was extracted using standard phenol-chloroform protocols (Sambrook et al., 1989). One fragment of mitochondrial DNA of 12S rRNA, tRNA-Val, and 16S rRNA (12S-16S) was sequenced for all samples using primers L2519 and 16Sbr (Table 2). Three partial nuclear DNA sequences of recombination activating gene 1 (Rag1), tyrosinase (Tyr) and rhodopsin (Rhod) were sequenced for all samples using primers included in Table 2. Amplifications were conducted in a 25 uL 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 50 °C or 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 products were purified with Gel Extraction Mini Kit (Watson BioTechnologies, Shanghai, China), then sequenced on an ABI 3730×I DNA automated sequencer (Applied Biosystems, UK).

For species not sampled by us, the sequences of 12S-16S, *Rag*1, *Tyr* and *Rhod* were downloaded from GenBank (Table 1). All data were aligned with MUSCLE (Edgar, 2004) and edited using MEGA 5.05 (Tamura et al., 2011).

#### Phylogenetic analysis

We estimated phylogenetic relationships using Bayesian inference (BI) and maximum parimony (MP) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) and PAUP\* 4.0b10a (Swofford, 2003). Mitochondrial and nuclear sequence data were analyzed separately. Then a phylogenetic tree was conducted using the concatenated sequence of all genes. For BI analysis, the best-fitting nucleotide substitution models were selected for 12S-16S and each codon of *Rag1*, *Tyr* and *Rhod* using the Akaike information criterion in MRMODELTEST v2.3 (Nylander, 2004). The BI analysis used four Markov chains, with default heating

Table 1 Samples information used in molecular analysis

		0000	old rodonov domicodo	(20 dec.) (#1000 l	GenBank No			
ı alıllıy	enino	obecies	opeoilleil voucilei IVO.	Eddality (site Hallidel)	12S-16S	Rhod	Rag1	Tyr
Brevicipitidae	Callulina	kreffti	VUB1068 (ES700)	Tanzania	DQ347056	DQ347400	DQ347281	DQ347189
Microhylidae	Elachistocleis	ovalis	TNHC-DCC 3301	South America	DQ347057	DQ347401	DQ347282	DQ347190
Leptodactylidae	Leptodactylus	melanonotus	MVZ 207294 (FC14298)	Costa Rica	DQ347060	ı		DQ347193
Ceratobatrachidae	Alcalus	baluensis	FMNH 44690	Mt. Kinabalu, Borneo, Malaysia	DQ347044	DQ347389	DQ347270	DQ347177
	Alcalus	mariae	KU 309518	Palawan PAIC, Palawan Province, Philippines	KP298038	KP298263	1	1
	Alcalus	tasanae	CAS 232349	Kachin State, Myanmar (2)	KU243087	KU243106	KU243096	KU243116
	Alcalus	tasanae	CAS 247243	Tanintharyi Division, Myanmar (3)	KU243088	KU243107	KU243097	KU243117
	Alcalus	tasanae	THNHM20534	Ranong Province, Thailand (4)	KU243089	KU243108	KU243098	KU243118
	Platymantis	hazelae	CMNH-RSK3918	Negros Isl, Philippines	DQ347019	DQ347369	DQ347248	DQ347153
	Platymantis	sp.	FMNH 259000	Luzon PAIC, Kalinga Province, Philippines	KP298055		KP298265	KP298332
	"Batrachylodes"	sb.	VUB0799	Solomon Islands	DQ346991	DQ347352	DQ347228	DQ347136
	Cornufer	guppyi	UW-JF189	New Britain Island	DQ347043	DQ347388	DQ347269	DQ347176
	Cornufer	guentheri	VUB1017 (SR5543)	Solomon Islands	DQ347046	DQ347391	DQ347272	DQ347179
	Liurana	xizangensis	KIZ06707	Medog Xian, Xizang, China (1)	KU243083	KU243101	KU243092	KU243111
	Liurana	xizangensis	KIZ011107	Medog Xian, Xizang, China (1)	KU243084	KU243102	KU243093	KU243112
	Liurana	medogensis	KIZ010955	Medog Xian, Xizang, China (1)	1	KU243103	1	KU243113
	Liurana	alpine	KIZ011140	Medog Xian, Xizang, China (1)	KU243085	KU243104	KU243094	KU243114
	Liurana	alpine	KIZ011141	Medog Xian, Xizang, China (1)	KU243086	KU243105	KU243095	KU243115
Conrauidae	Conraua	crassipes	ZFMK 75446	Cameroon	DQ347015	DQ347364	DQ347244	DQ347148
Petropedetidae	Arthroleptides	martiensseni	CR 10898	Africa	DQ347064	DQ347410	DQ347289	DQ347197
	Petropedetes	parkeri	VUB0955 (MV)	Africa	DQ347014	ı	ı	DQ347147
Pyxicephalidae	Tomopterna	tandyi	ı	1	DQ347009	DQ347360	DQ347240	DQ347143
	Arthroleptella	villiersi	CR 1070	Africa	DQ347062	DQ347408	DQ347287	DQ347195
	Amietia	fuscigula	CR 1073	Africa	DQ347065	DQ347411	DQ347290	DQ347198
Dicroglossidae	Limnonectes	limborgi	VUB1218	Laos	DQ347061	DQ347407	DQ347286	DQ347194
	Limnonectes	sb.	TNHC 59014	Sulawesi Isl., Indonesia	DQ347045	DQ347390	DQ347271	DQ347178
	Hoplobatrachus	occipitalis	VUB0537	unknown (pet shop)-Africa	DQ346979	DQ347349	DQ347217	DQ347132
	Occidozyga	lima	TNHC 59864-RMB 2134	Java Isl., Indonesia	DQ347025	DQ347375	DQ347255	DQ347159
	Occidozyga	martensi	FMNH 268805	Krabi, Thailand (5)	KU243090	KU243109	KU243099	KU243119
	Ingerana	tenasserimensis	CAS 205064	Myanmar	DQ347030	AY322236	DQ347258	AY322344
	Ingerana	borealis	KIZ020455	Medog Xian, Xizang, China (1)	KU243091	KU243110	KU243100	KU243120
Phrynobatrachidae	Phrynobatrachus	krefftii	VUB1068 (ES700)	Tanzania	DQ347059	DQ347403	DQ347284	DQ347192
	Phrynobatrachus	africanus	CAS 207779	Equatorial Guinea	DQ347031	DQ347378	DQ347259	DQ347164
Ranidae	Amolops	larutensis	TNHC 57944-JAM 1371	Thailand	DQ347053	DQ347398	DQ347279	DQ347186
	Staurois	natator	CMNH-H1626	Mindanao Isl., Philippines	DQ347020	DQ347371	DQ347250	DQ347155
	Rana	sylvatica	MVZ 137426	New York, USA	DQ347052	DQ347397	DQ347278	DQ347185

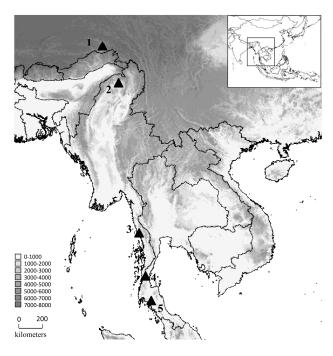


Figure 1 Map of sampling sites

Numbers correspond to localities in Table 1.

values, and run for 5 million generations while sampling trees every 1 000 generations. The first 25% sampled trees were discarded as burn-in, and log-likelihood scores were examined using Tracer v 1.4 (Rambaut & Drummond 2007) to assure convergence (effective sample size [ESS] values >200). For the MP analysis, full heuristic tree searches were used, with 1 000 replications, random addition of sequences and tree-bisection-reconnection (TBR) branch swapping. Non-parametric bootstrap support was estimated using 1 000 replicates of full heuristic searches.

#### **RESULTS**

#### Sequence information

Sequencing generated a total of 1 371 base pairs (bp) of 12S-16S data for *Limnonectes alpine* and *Ingereana tasanae*. Additionally, a part of fragment of 12S-16S was successfully sequenced for *Occidozyga martensii*, *Lim. xizangensis* and *I. borealis*. We were unable to collect 12S-16S for *Lim. medogensis*. For nuclear sequences of *Rag*1, 1 100 bp was successfully sequenced for all samples except for *Lim. medogensis*, but we only included 553 bp in subsequent analyses so as to match *Rag*1 data sequences available on GenBank. Sequences of 553 bp *Tyr* and 316 bp *Rhod* were successfully sequenced for all samples. All new generated sequences were submitted to GenBank (Accession numbers KU243083-KU243120, Table 1).

Table 2 Primers information used for four DNA fragments sequencing

Locus	Primer name	Sequence (5'-3')	Tm	Citation
12S-16S	L2519	AAACTGGGATTAGATACCCCACTAT	55	Richards & Moore, 1996
	H3296	GCTAGACCATKATGCAAAAGGTA		
	16Sbr	CCGGTYTGAACTCAGATCAYGT		Palumbi et al., 1991
	16Sbr	CCGGTYTGAACTCAGATCAYGT		
	12/16S-696F	TATARCAATAGTACCGCAAG		This study
	STW-NA1	GGGTGACGGGCGGTTTGT		
Rag1	L-RAG1RAn	CTGGTCGTCAGATCTTTCAGC	50	Stuart, 2008
	H-RAG1RAn	GCAAAACGTTGAGAGTGATAAC		
	L-RAG1RAninT	GGAAATTGGTGGAATCCTCAG		
	H-RAG1RAninT	ATATAGATAGAGCCTGAGGC		
Tyr	TYR 1 G	TGCTGGGCRTCTCTCCARTCCCA	50	Bossuyt & Milinkovitch, 2000
	TYR 1 B	AGGTCCTCYTRAGGAAGGAATG		
Rhod	RhoG 1 A	ACCATGAACGGAACAGAAGGYCC	50	
	RhoG 1 G	GTAGCGAAGAARCCTTCAAMGTA		

#### Phylogenetic relationships

The best-fitting model were TVM+I+G for mitochondrial 12S-16S, K80+I, TIMef+I and TIMef+I for three codon positions of *Rag*1, TVM+I+G, K81+I and GTR+G for three codon positions of *Tyr*, SYM+G, TVM+I+G and TIM+G for three codon positions of *Rhod*. The phylogenetic analyses based on nuclear DNA and mtDNA showed similar topologies. Most recognized families formed monophyletic groups; however, the monophyly of Dicroglossidae was not recovered using mtDNA, but highly supported by nuclear DNA. This possibly is due to the inability of mtDNA

sequence to resolve phylogenetic relationship at deeper levels (i.e., Kingston et al., 2009), or sparse taxon sampling in our analysis. The five focal species were yielded the same topology in both phylogenetic analyses, so the difference between mtDNA and nuclear DNA topologies do not affect our taxonomy. The Bayesian tree resulting from based on concatenated sequence of all genes is shown in Figure 2. Limnonectes xizangensis, Lim. medogensis, Lim. alpine and Ingerana tasanae clustered with species of family Ceratobatrachidae. Three primary lineages were identified in this family, corresponding to two

known subfamilies Alcalinae (Clade A) and Ceratobatrachinae (Calde B), and a new lineage (Clade C), unsampled in previous phylogenetic estimates (Brown et al., 2015). Samples of *Ingerana tasanae* from Thailand and Myanmar grouped together, and this clade formed a strongly supported group with *Alcalus baluensis* and *A. mariae* (Clade A). *Limnonectes xizangensis*, *Lim*.

medogensis and Lim. alpine formed a monophyletic group (Clade C), which is strongly supported as related to the family Ceratobatrachidae. Finally, Ingerana borealis samples clustered with species in the subfamily Occidozyginae (Dicroglossidae). This species formed a clade with I. tenasserimensis (type species of Ingerana), as the sister group to Occidozyga.

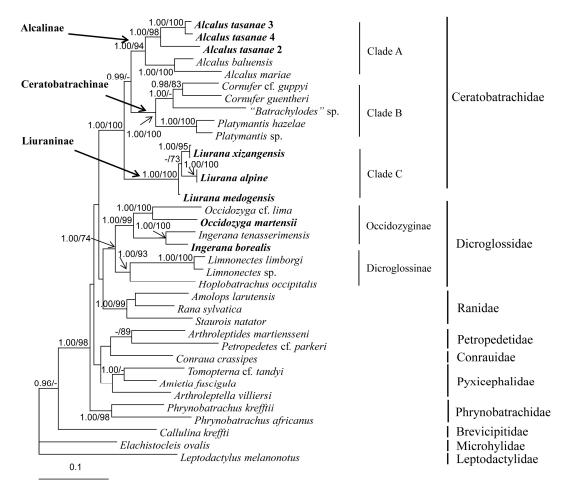


Figure 2 Bayesian inference tree based on concatenated analysis of all genes

Nodal support values are Bayesian posterior probabilities (only ≥90 are shown) and bootstrap proportions from maximum parsimony analysis (only ≥70 are shown). Newly sequenced samples are emphasized with bold text.

#### DISCUSSION

## Taxonomy of species of *Limnonectes* and *Ingerana*, and a record of a new family for China, Myanmar and Thailand

The three poorly understood species, formerly referred to *Limnonectes* and *Ingerana* from the largely unexplored area of Himalayan Tibet (*Lim. xizangensis, Lim. medogensis* and *Lim. alpine*), have had unstable taxonomic histories (Frost, 2015) and, until now, unclear systematic affinities. Dubois (1987) established the genus *Ingerana*, in which there are two

subgenera Ingerana (Ingerana) and Ingerana (Liurana). Ingerana xizangensis (formerly Cornufer xizangensis, Hu, 1977) was included in subgenus Ingerana (Liurana) by Dubois (1987). Fei et al. (1997) identified significant morphological differences between these two subgenera, including the presence of lingual papilla on the tongue, the absence of terminal discs on fingers and toes, and the absence of circumarginal grooves on fingers and toes in Ingerana (Liurana), (Figures 3-4). Thus, Liurana was elevated to the level of genus to include the species Liu. xizangensis, Liu.



Figure 3 Photos of *Liurana alpine* and *Liurana xizangensis* in life (Photos by Kai WANG)

A-D: dorsolateral view; ventral view; ventral view of hand; and ventral view of foot of *Liu. Alpine*, respectively; E-H: dorsolateral view; ventral view; ventral view of hand, and ventral view of foot of *Liu. Xizangensis*, respectively.



Figure 4 Photos of *Liurana medogensis* (Photos by Kai WANG) A-C: dorsal view, dorsolateral view, and ventral view (C) in life, respectively; D: ventral view of hand (above) and foot (below) in drawing (from Fei et al., 2009).

medogensis, Liu. alpine and Liu. liui (Fei et al., 1997, 2009, 2012; Huang & Ye, 1997). Fei et al. (2009) considered Liurana to be part of the family Occidozygidae. Subsequently, Fei et al. (2010) established a new subfamily Liuraninae in the family Occidozygidae based on morphological data. Frost et al. (2006) considered Liurana to be a junior synonym of Ingerana on the basis of the original description and overlapping character states. Based on available morphological characters, Borah et al. (2013) placed Liurana in synonymy with Taylorana (now considered to be a subgenus of

Limnonectes, [Frost, 2015]). Thus, for the last several years, these species have resided in Limnonectes (Frost, 2015) pending appropriate phylogenetic analysis to determine of their systematic affinities.

Based on analysis of multilocus DNA sequence data, Liu. xizangensis, Liu. medogensis and Liu. alpine are herein assigned to the family Ceratobatrachidae and represent the first record of this family in China. In our analysis these species formed strongly supported monophyletic group. clustering with members of the Ceratobatrachidae (sensu Brown et al., 2015). In contrast, species of genus Ingerana (I. tenasserimensis and I. borealis) and Limnonectes (Lim. limborgi, Lim. sp.) formed the strongly supported clades in subfamily Occidozyginae, as showed in previous studies (i.e. Bossuyt et al., 2006; Wiens et al., 2009; Pyron & Wiens, 2011). Based on our observations of morphological variation, these three species likewise are distinguished from the species of Ingerana and Limnonectes by the: (1) absence of interdigital webbing of the feet, (2) absence of terminal discs on fingers and toes, (3) absence of circumarginal grooves on the fingers and toes, and (4) absence of tarsal folds. All available evidence supports the recognition of Liu. xizangensis, Liu. medogensis and Liu. alpine as single taxon, for which Liurana is the available generic name with priority. We assign Liurana to the family Ceratobatrachidae. Within Ceratobatrachidae, three lineages are recognized: Clade A and Clade B (Figure 2) correspond to previously recognized subfamilies Alcalinae and Ceratobatrachinae, respectively. The genus Liurana (Clade C) is equivalent in species content to the subfamily Liuraninae Fei, Ye and Jiang, 2010, now transfered to the family Ceratobatrachidae.

Ingerana tasanae is distributed in western and central peninsular Thailand, and its range possibly extends into adjacent Tenaserim and Myanmar (Stuart et al., 2008). Our molecular data clearly place all Ingerana tasanae samples in the same clade as other members of the genus Alcalus (Ceratobatrachidae). This constitutes a new record of family Ceratobatrachidae for Myanmar and Thailand. Our northern Myanmar samples of A. tasanae is highly divergent from individuals from southern Myanmar and southern Thailand. It remains possible that additional taxonomic diversity will be revealed in the genus Alcalus with accumulation of data and field studies of these populations.

Previous studies placed *I. borealis* in the genus *Phrynoglossus* (Fei et al., 2009, 2010, 2012), *Occidozyga* (Ahmed et al., 2009; Mathew & Sen, 2010), and *Ingerana* (Sailo et al., 2009). Based on our molecular data, *I. borealis* falls into a strongly supported clade with *I. tenasserimensis*, the type species of *Ingerana*. Thus, our molecular data support its systematic position within genus *Ingerana* based on morphological comparison by Sailo et al. (2009).

### New insight from the phylogeny and distribution of Ceratobatrachidae

Brown et al. (2015) developed a stable taxonomy for the family Ceratobatrachidae. Two subfamilies were identified: Ceratobatrachinae and Alcalinae. Ceratobatrachinae includes

two large monophyletic radiations, *Cornufer* and *Platymantis*. The species belonging to the subfamily Ceratobatrachinae have a broad distribution in the south-west Pacific, including Philippines, Borneo, New Guinea, Admiralty and Bismarck archipelagos, Solomon Islands, and Fiji. Alcalinae includes only four species of *Alcalus*, which are distributed only on the island archipelagos of Southeast Asia (Sundaland).

Our research identified other four species which we now transfer to Ceratobatrachidae; this greatly increases the distribution of the family to the mainland of Southeast Asia and the Himalayan region (Figure 1). Given our experience with the unexpected phylogenetic affinities of the species studied here, we would not be surprised if additional phenotypically similar taxa are found to belong in Ceratobatrachidae in the near future. Of particular note, *Ingerana charlesdarwini* (Das, 1998), distributed in the Andaman Islands (India), could very well be the sister lineage to the remaining lineages in this large and spectacularly diverse anuran family.

The surprising discovery that the clade Ceratobatrachidae is broadly distributed from the Himalayas, mainland and peninsular southeastern Asia, to the southwest Pacific, will help us to understand the biogeography in this region. The sister-group relationship of Ceratobatrachinae and Alcalinae, although not unequivocally supported mirrors the geographic distribution of these clades. This relationship between mainland and archipelago species is also seen in the divergence between the mainland species *Alcalus tasanae* and the archipelago species *A. mariae* and *A. baluensis*. Additional, unexpected patterns between mainland and island taxa may be found with more complete taxon sampling, which emphasizes the need for additional fieldwork in mainland southeastern Asia.

#### **ACKNOWLEDGEMENTS**

We would like to thank Dr. Pi-Peng LI (Shenyang Normal University), Mr. Tao LIANG, Mr. Duan YOU, and Mr. Ya-Di HUANG, who helped with fieldwork in Tibet, Prof. Yue-Zhao WANG (CIB), Prof. Yue-Ying CHEN (CIB) and Prof. Sheng-Quan LI (CIB) for kindly letting us examine specimens under their care. We also thank the California Academy of Sciences (CAS), Thailand National History Museum (THNHM), and Field Museum of Natural History (FMNH) for loan of tissue samples.

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## 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 threefrog 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; Theloderma 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 clown 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 *Theloderma 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. Phylogentic 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 Theloderma 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 *Theloderma* 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

Received: 27 October 2015; Accepted: 15 December 2015 Foundation items: This study was supported by the Ministry of Science and Technology of China (2014FY210200, 2011FY120200) and the Animal Branch of the Germplasm Bank of Wild Species of Chinese Academy of Sciences (the Large Research Infrastructure Funding).

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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 (*Theloderma 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*, *tRNAVAL*, and 16S *rRNA* (12S-16S) was sequenced using primers L2519 and 16Sbr (Table 1). Amplifications were conducted in a 25 uL 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, scatted some small tubercles, iris with a pale yellow, "X" shaped pattern of pigmentation. The specimen distinguished mainly from the genus *Theloderma* 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 *Theloderma*, 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, scatted 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" (核鼻树蛙) 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.
Vyctixalus	spinosus	ACD 1043	Philippine Islands: Mindanao	DQ283114
	pictus	FMNH 231095	Malaysia	DQ283133
Theloderma	rhododiscus	SCUM 061102L	China: Mt. Dayao, Guangxi	EU215530
	asperum	060821203Rao	China: Jinping, Yunnan	GQ285677
	beibengensis	YPX37270	China: Medog, Tibet	_
	moloch	YPX31941	China: Medog, Tibet	_
	corticale	AMNH A161499	Vietnam	DQ283050
Nasutixalus	medogensis sp. nov.	KIZ016395	China: Medog, Tibet	_
		6255Rao	China: Medog, Tibet	GQ285679
Kurixalus	hainanus	HNNU A1180	China: Mt. Diaoluo, Hainan	EU215548
	odontotarsus	SCUM 060688L	China: Mengyang, Yunnan	EU215549
	idiootocus	SCUM 061107L	China: Lianhuachi, Taiwan	EU215547
	eiffingeri	UMFS 5969	China: Nantou, Taiwan	DQ283122
Pseudophilautus	microtympanum	_	Sri Lanka	DQ346974
	menglaensis	060821286Rao	China: Lvchun, Yunnan	GQ285676
Philautus	acutirostris	_	_	AF458137
	surdus	CAS 219932	Philippine	GQ285676
	abditus	ROM33145	Vietnam: Krong Pa, Gia Lai	GQ285673
Gracixalus	jinxiuensis	KIZ 061210YP	China: Mt. Dayao, Guangxi	EU215525
	carinensis	ROM39660	Vietnam: Sa Pa, Lao Cai	GQ285670
	gracilipes	060821196Rao	China: Mt. Dawei, Yunnan	GQ285668
Rhacophorus	feae	SCUM 050642W	China: Hekou, Yunnnan	EU215544
	rhodopus	SCUM 060692L	China: Mengyang, Jinghong	EU215531
	dugritei	SCUM 051001L	China: Baoxing, Sichuan	EU215541
	kio	SCUM 37941C	China: Xishuangbanna, Yunnan	EU215532
Feihyla	palpebralis 1	SCUM 0606132L	China: Mt. Dawei, Yunnan	EU215546
	palpebralis 2	712	Vietnam: Lam Dong	GQ285681
Chiromantis	vittatus 1	KIZ 0001Rao	China: Simao, Yunnan	GQ285684
	vittatus 2	FMNH 254444	Vietnam: Gia Lai	DQ283134
	doriae 1	1056	Vietnam: Binh Thuan	GQ285683
	doriae 2	KIZ 005Rao	China: Simao, Yunnan	GQ285682
Polypedates	leucomystax	CAS 219931	Philippines	AF458140
	megacephalus	SCUM 050508C	China: Mt. Daiyun, Fujian	EU215552
	mutus	SCUM 37940C	China: Xishuangbanna, Yunnan	EU215551
Liuixalus	ocellatus	HN0806045	China: Mt. Wuzhi, Hainan	GQ285672
	romeri	KIZ 061205YP	China: Mt. Shiwan, Guangxi	EU215528
Buergeria	oxycephala	SCUM 050267YJ	China: Hainan	EU215524
Raorchestes	parvulus	KUHE 38322	Thailand: Khao Sabap	LC012865
Ghatixalus	variabilis	VUB0061	India	EU178099
Beddomixalus	bijui	_	India	KC594289, KC594290
Mercurana	myristicapalustris	_	India	KC594294, KC594293
Boophis	tephraeomystax	AMNH A168144	Madagascar	DQ283032
Limnonectes	poilani	AMNH A163717	Vietnam	DQ283378

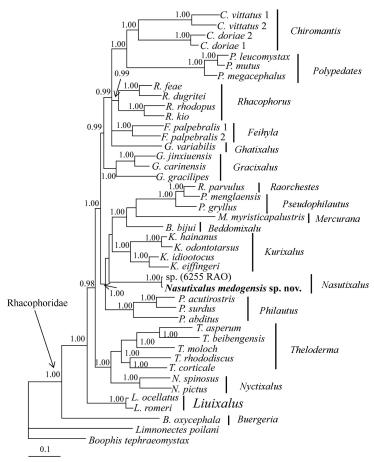


Figure 1 Bayesian inference tree based on molecular data of Rhacophoridae and two outgroups

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 scatted 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<sup>-</sup>-1 III 0-1 IV 1-0 V; fringe distinct; small tubercles scatted 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 "Muo 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.

#### **ACKNOWLEDGEMENTS**

We thank to Dr. Pi-Peng LI (Shenyang Normal University), Dr. Guang-Pu GUO (Tongji University), and the volunteers, Mr. Tao LIANG, Mr. Duan YOU, and Mr. Ya-Di HUANG, who helped with fieldwork in Tibet.

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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 (1010β3); 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 (1033); KIZ 010960, KIZ 010980

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(2♀♀). Medog, Tibet.

Rhacophorus burmanus: KIZ 016379-80, KIZ 016408-09, KIZ 01641-44, KIZ 016446-47 (10♂♂); KIZ 010957, KIZ 016376-78, KIZ 016410, KIZ 016445 (6♀♀). Medog, Tibet.

Rhacophorus maximus: KIZ 016384 (1 $\circlearrowleft$ ); KIZ 016385, KIZ 013859 (2 $\updownarrow$  $\updownarrow$ ). Medog, Tibet.

Rhacophorus translineatus: KIZ 06648-52, KIZ 011161-62, KIZ 011167-70 (113); KIZ 012683 (1 $\mathfrak P$ ). Medog, Tibet.

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

Theloderma beibengensis: KIZ 020453 (13). Medog, Tibet.

## A new species of the genus *Scutiger* (Anura: Megophryidae) from Medog of southeastern Tibet, China

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#### **ABSTRACT**

A new species of Scutiger Theobald, 1868 is described from Medog, southeastern Tibet, China, based on morphological and molecular data. The new species was previously identified as Scutiger nyingchiensis, but it can be differentiated from the latter and all other congeners by the following combination of characters: (1) medium adult body size, SVL 50.5-55.6 mm in males and 53.8-57.2 mm in females; (2) maxillary teeth absent; (3) web rudimentary between toes; (4) prominent, conical-shaped tubercles on dorsal and lateral surfaces of body and limbs; (5) tubercles covered by black spines in both sexes in breeding condition; (6) a pair of pectoral glands and a pair of axillary glands present and covered by black spines in males in breeding condition, width of axillary gland less than 50% of pectoral gland; (7) nuptial spines present on dorsal surface of first and second fingers, and inner side of third finger in males in breeding condition; (8) spines absent on the abdominal region; (9) vocal sac absent. In addition, the distribution and conservation status of the new species are also discussed.

**Keywords:** Eastern Himalayas; Tibet; Scutiger nyingchiensis; Scutiger spinosus sp. nov.; DNA barcoding

#### INTRODUCTION

The Tibet-Hengduan Mountain area is one of the 35 biodiversity hotspots of the world (Mittermeier, 2004) and supports an abundance of organisms, many of which are endemic to the region. Among the endemic fauna, the majority of species in the megophryid frog genus *Scutiger* Theobald, 1868 are known only from the montane habitats at high elevations between 1 900 m and 5 100 m in this region. Currently, there are 20

species recognized in the genus (Frost, 2015), of which six species are known from Tibet (Jiang et al., 2012), including *S. boulengeri* (Bedriaga, 1898), *S. maculatus* (Liu, 1950), *S. mammatus* (Güther, 1896), *S. nyingchiensis* Fei, 1977, *S. wuguanfui* Jiang et al., 2012, and *S. sikkimensis* (Blyth, 1854).

During our fieldwork from 2011 to 2013 in southeastern Tibet, we collected 16 specimens of megophryid frogs that were initially identified as *Scutiger nyingchiensis* from 62K, Medog (=Motuo). After morphological comparisons and genetic analysis with respect to topotype specimens of *S. nyingchiensis*, we were able to distinguish however, the *Scutiger* specimens from 62K, Medog can be readily distinguished from *S. nyingchiensis* by a suit of morphological characters and a significant genetic distance. Therefore, we describe the 62K population of *Scutiger* as a new species.

#### **MATERIALS AND METHODS**

From June 2011 to June 2013, a total of 16 individuals (12 males and four females) were collected from 62K, Medog, southeastern Tibet, China. Following euthanasia, liver tissues were taken from each individual and preserved in 95% ethanol, and all specimens were then fixed in 10% formalin solution and transferred to 75% ethanol after returning from the field. All specimens were designated as the type series, and

Received: 17 October 2015; Accepted: 20 December 2015

Foundation items: This study was supported by the "Light of West China" Program of the Chinese Academy of sciences (CAS), the Ministry of Science and Technology of China (2014FY210200, 2011FY120200), and the Animal Branch of the Germplasm Bank of Wild Species of Chinese Academy of Sciences (the Large Research Infrastructure Funding)

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were deposited in Kunming Institute of Zoology, Chinese Academy of Sciences.

#### Morphological analysis

Morphological characters used and their measurement methods followed Fei et al. (2009). Measurements were taken with dial calipers and recorded to the nearest 0.1 mm.

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; 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.

The new species was compared to museum specimens (Appendix) as well as descriptions in the literature: *Scutiger adungenis* (Dubois, 1979), *S. bhutanensis* (Delorme & Dubois, 2001), *S. brevipes* (Liu, 1950), *S. nepalensis* (Dubois, 1973), and *S. chintingensis*, *S. glandulatus*, *S. gongshanensis*, *S. jiulongensis*,

S. liupanensis, S. maculatus, S. muliensis, S. ningshanensis, S. pingwuensis, S. tuberculatus and S. wanglangensis from 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 was extracted from 15 specimens of seven Tibetan *Scutiger* species (Table 1), using a standard three-step phenol-chloroform method (Sambrook et al., 1989). A 561 base pair DNA fragment of the mitochondrial gene Cytochrome Oxidase Subunit I (COI) was amplified and sequenced for all samples using primers Chmf4 and Chmr4 (Che et al., 2012). Protocols for PCR and sequencing followed Che et al. (2012). All novel sequences were deposited in GenBank (KU243053-KU243067, Table 1). Sequences for three additional species of Tibetan *Scutiger* were downloaded from Genbank as well outgroup taxa including *Xenophrys*, *Brachytarsophrys* and *Leptolalax* (Table 1).

Table 1 Information of samples used in molecular analysis

Genus	Species	Locality	Specimen voucher No.	GenBank No.
Scutiger	spinosus	China: Medog, Tibet	KIZ011114	KU243053
			KIZ011100	KU243054
			KIZ012645	KU243055
	nyingchiensis	China: Nyingchi (=Linzhi), Tibet	KIZ017459	KU243056
			KIZ017460	KU243057
	sikimmensis	China: Yadong, Tibet	KIZ011127	KU243058
			KIZ07371	KU243059
	wuguanfui	China: Medog, Tibet	KIZ011101	KU243060
			KIZ011102	KU243061
	gongshanensis	China: Gongshan, Yunnan	CIB20070717001	KU243062
			CIB20070717002	KU243063
	boulengeri	China: Bom (=Bomi), Tibet	KIZ06712	KU243064
			KIZ06713	KU243065
	jiulongensis	China: Ganzi, Sichuan	KIZ045055	KU243066
			KIZ045056	KU243067
	boulengeri	China: Gansu	_	KJ082074
			_	KJ082073
	ningshanensis	China: Shaanxi	_	KF757393
			_	KF757392
	liupanensis	China: Ningxia	_	JN700835
			_	JN700834
Xenophrys	maosonensis	<del>_</del>		KR087950
	major	<del>_</del>		KR087949
	jingdongensis	<u> </u>	_	KR087947
Leptolalax	minimus	<del>_</del>		KR087759
Brachytarsophrys	feae	_		JN700843

All sequences were aligned and edited in MEGA 5 (Tamura et al., 2011). The best model of nucleotide substitution for each codon position was SYM+I, F81 and GTR+G determined using

jModeltest v1.0.1 (Posada, 1998). Bayesian inference (BI) was used to generate a phylogenetic relationship using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Using different model

for each codon position, the Markov chains were estimated for 10 million generations, and every 100 generations were sampled. The genetic distance between species was calculated using MEGA 5 with Kimura 2-parameter model (Che et al., 2012).

#### **RESULTS**

#### Morphological comparison

The results of the morphological comparisons between the *Scutiger* population from 62K, Medog and its congeners are summarized in Table 2. The morphological comparisons reveal that the *Scutiger* population at 62K, Medog can be readily distinguished from morphologically similar *S. nyingchiensis* and other congeners by a suite of diagnostic characters, including a light brown triangular pattern of pigmentation on the dorsal surface of the head, rudimentary webbing between toes, and prominent and conical shaped tubercles on the dorsal and lateral surfaces of the body and limbs.

#### Phylogenetic relationships

The 561 base pairs (bp) of COI sequences for 15 individuals were sequenced (Table 1). There are 155 and 202 potentially parsimony informative sites within genus *Scutiger* and between the ingroup and outgroup, respectively. The BI analysis supports the genus *Scutiger* as a monophyletic group. Five lineages are identified based on available data for *Scituger* (Clade A-E, Figure 1). Species distributed in Tibet are recovered in Clade A, C, D, and E. The 62K population of *Scutiger*, along with *S nyingchiensis* from Nyingchi, Tibet and *S. gongshanensis* from Gongshan, Yunnan, forms the linage D. The 62K population of *Scutiger* differs from the phenotypically similar *S. nyingchiensis* and from *S. gongshanensis* by a genetic distance of 10.6% and 10.9% respectively. *Scutiger nyingchiensis* and *S. gongshanensis* are sister species, with a genetic distance of 8.5%.

Furthermore, the populations of *S. boulengeri* from Gansu and Tibet do not form a monophyletic lineage, and the genetic distance between the two populations is 8.1%.

Given that both morphological and molecular results support the *Scutiger* population from 62K, Medog as a distinct evolutionary lineage distinct from all other Tibetan *Scutiger*, therefore, following the species concept by and concordant evidence confirm species status (Wu & Murphy, 2015), herein we describe the 62K population of *Scutiger* as a new species.

### Scutiger spinosus sp. nov. Jiang, Wang, Li and Che (Figures 2-4)

Synonyms

Scutiger nynchiensis: Li et al., 2010; Jiang et al., 2012

**Holotype:** KIZ 011114 (Figure 2), an adult male from 62K, Medog, Tibet, China (N29°42'33.6", E95°34'56.0", elevation 2 705 m). Collected by Ke JIANG on 07 June 2011.

Paratypes: a total of 15 specimens (11 males and four females) from the same locality as holotype, including one adult male

(KIZ 011113) and two adult females (KIZ 011093, KIZ 011100), collected by Ke JIANG and Pi-Peng LI as same date as holotype; three adult males (KIZ 012645, 012647-48) and one adult female (KIZ 012646) collected by Ke JIANG, Kai WANG and Jiang XIE on 15 July 2012; and seven adult males (KIZ 013862-64, 013866-69) and one adult female (KIZ 013865), collected by Ke JIANG and Kai WANG on 17 June 2013.

**Diagnosis:** Scutiger spinosus sp. nov. is identified to the genus Scutiger by its molecular phylogenetic position and the following morphological characters: (1) maxillary teeth absent or indistinct; (2) vomerine teeth absent; (3) tympanum and tympanic ring entirely absent; (4) pupil vertical, elliptical; (5) pectoral and axillary gland present in males, and covered by black spines in breeding condition; (6) inner three fingers of males with black nuptial spines in breeding condition.

Scutiger spinosus sp. nov. is distinguished from all other congeners by the following combination of morphological characters: (1) medium adult body size, SVL 50.5-55.6 mm in males and 53.8-57.2 mm in females; (2) maxillary teeth absent; (3) web rudimentary between toes; (4) prominent, conical-shaped tubercles on dorsal and lateral surfaces of body and limbs; (5) tubercles covered by black spines in both sexes in breeding condition; (6) a pair of pectoral glands and a pair of axillary glands present and covered by black spines in males in breeding condition, width of axillary gland less than 50% of pectoral gland; (7) nuptial spines on dorsal surface of first and second fingers, and inner side of third finger in males in breeding condition; (8) spines absent on the abdominal region; and (9) vocal sac absent.

Description of the holotype: SVL 51.7 mm; body slightly compressed, moderately slender at waist; head large and flat, width nearly equal to length (HW/HL=1.04); snout rounded, slightly projecting beyond jaw; canthus rostralis obtuse, loreal region oblique and slightly concave; nostril midway between the tip of snout and eye; eye relatively large (EHD/HL=0.35); pupil vertical; interorbital space flat, interorbital distance less than upper eyelid width (IOS/UEW=0.90); tympanum and tympanic rim absent; a small tooth-like projection on anteromedial edge of mandible; jaws without teeth; tongue oval, slightly emarginate behind, papillae absent; choanae located against anterior border of palate, visible when viewed from below; vomerine teeth absent; vocal sac absent; supratympanic fold from posterior corner of orbit to shoulder, distinctly developed.

Forelimbs long; forearm enlarged; fingers slender, free of web or dermal fringes; relative length of fingers: I=II<IV<III; tips of fingers rounded, not dilated; subarticular tubercles absent; inner metacarpal tubercle almost equal to outer metacarpal tubercle, both large and flat; nuptial spines on dorsal surface of first and second fingers, and on inner side of third finger.

Hindlimbs moderately short, tibiotarsal articulation reaching the corner of mouth when adpressed, heels do not touch when folded at right angles to the body (FML/TBL=1.07); foot longer than shank (TFL/TBL=1.26); tips of toes round; dermal fringes

Table 2 Morphological comparisons of Scutiger spinosus sp. nov. and it congeners

Species	Male SVL	Female SVL	Maxillary teeth or tooth	Toes webbing	Spine patches on belly in males	Spine patches on breast, and the relative size	Nuptial spines on the Vocal sac number of fingers	Vocal sac
spinosus	50.5-55.6 n=12	53.8-57.2 n=4	Absent	Feeble	Absent	Two pairs, the inner one larger than the outer one	І, ІІ, ІІІ	Absent
adungensis	71.0-73.0 n=2	1	Present	Feeble	Absent	One pair	Ι, ΙΙ	Present
bhutanensis	53.0-53.4 n=2	I	Absent	Feeble	Absent	Two pairs, the inner one similar to the outer one	І, ІІ	Absent
boulengeri	44.9-53.7 n=20	40.2-58.2 n=8	Absent or Present	Developed	Present	Two pairs, the inner one similar to the outer one	І, ІІ, ІІІ	Absent
brevipes	68.0-80.0 <i>n</i> =10	58.0–68.0 <i>n</i> =9	Absent	Feeble	Absent	Two pairs, the inner one much larger than the outer one	Ι, Π	Absent
chintingensis	42.0-50.3 n=22	48.0-52.8 <i>n</i> =6	Present	Developed	Absent	Two pairs, the inner one slightly larger than $\ \ I,II,III$ the outer one	І, ІІ, ІІІ	Absent
glandulatus	68.0-90.0 <i>n</i> =17	58.0-83.7 <i>n</i> =14	Absent	Feeble	Absent	Two pairs, the inner one much larger than the outer one	І, ІІ	Absent
gongshanensis	47.0-57.0 <i>n</i> =19	49.0-60.0 <i>n</i> =2	Present	Absent	Absent	One pair	Ι, ΙΙ	Present
jiulongensis	67.4-81.5 n=20	1	Absent	Feeble	Absent	Two pairs, the inner one much larger than the outer one	Ι, ΙΙ	Absent
liupanensis	40.6-48.0 n=20	52.0-59.5 n=3	Present	Feeble	Present	Two pairs, the inner one similar to the outer one	І, ІІ, ІІІ	Absent
maculatus	65.4 n=1	69.0 <i>n</i> =1	Present	Developed	Absent	Two pairs, the inner one slightly larger than the outer one	П, П, Ш	Absent
mammatus	58.8-71.7 n=11	63.0-77.3 <i>n</i> =6	Absent or Present	Developed	Absent	One pair	Ι, ΙΙ	Absent
muliensis	68.2-80.0 <i>n</i> =10	60.1-67.5 <i>n</i> =10	Absent	Feeble	Absent	One pair	І, ІІ	Absent
nepalensis	68.0-73.5 n=4	59.5-64.5 n=2	Unknown	Unknown	Absent	Two pairs, the inner one similar to the outer one	І, ІІ, ІІІ	Absent

							0	Continued
Species	Male SVL	Female SVL	Maxillary teeth or tooth	eeth or Toes webbing		Spine patches on Spine patches on breast, belly in males and the relative size	Nuptial spines on the number of fingers	Vocal sac
ningshanensis	51.0 <i>n</i> =1	41.0 <i>n</i> =1	Present	Feeble	Present	Two pairs, the inner one similar to the outer one	І, ІІ, ІІ	Absent
nyingchiensis	52.8-67.6 n=5	70.0 <i>n</i> =1	Present	Developed	Absent	Two pairs, the inner one slightly larger than $\ \ I$ , $II$ , $III$ , $III$ , the outer one	І, ІІ, П	Absent
pingwuensis	60.7-75.8 n=20	77.5 n=1	Absent	Feeble	Present	Two pairs, the inner one much larger than $\ \ I\ ,\ II\ ,\ III$ the outer one	І, ІІ, П	Absent
sikimmensis	46.9-51.3 n=8	50.8-53.9 n=2	Present	Feeble	Absent	Two pairs, the inner one slightly larger than $\ \ I\ ,\ II\ ,\ III$ the outer one	І, ІІ, ПІ	Absent
tuberculatus	68.0-76.0 <i>n</i> =16	63.6-79.0 n=7	Absent	Feeble	Absent	Two pairs, the inner one much larger than $\ \ I$ , $II$ the outer one	П, П	Absent
wanglangensis	52.7-58.2 n=6	64.3 <i>n</i> =1	Present	Feeble	Present	Two pairs, the inner one similar to the outer one	І, ІІ, ПІ	Absent
wuguanfui	77.5-83.8 n=6	107.4-116.7 n=2	Absent	Feeble	Absent	Two pairs, the inner one similar to the outer one	І, ІІ, ІІІ	Present

Information of S. spinosus sp. nov., S. boulengeri, S. mammatus, S. nyingchiensis, S. sikimmensis, and S. wuguanfui are obtained by this study.

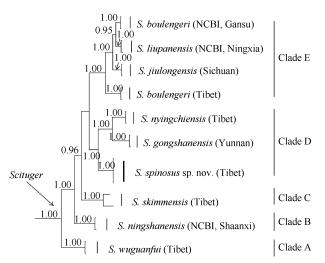


Figure 1 Bayesian inference tree based on barcoding COI data of the Tibetan congeners of the genus *Scutiger* 

The numbers represent Bayesian posterior probabilities (only  $\geqslant$ 90 were showed).



Figure 2 Scutiger spinosus sp. nov. holotype (KIZ 011114) in preservative (Photos by Ke JIANG)

A: Dorsolateral view; B: Dorsal view of body; C: Ventral view; D: Dorsolateral view of body, showing tubercles and spines; E: Dorsal view of hindlimbs, showing tubercles and spines on thigh; F: Ventral view of hand; G: Ventral view of foot.

feeble; relative length of toes: I < II < III < V < IV; subarticular tubercles absent; ridges on undersurfaces of toes absent; inner metatarsal tubercle elliptical and prominent, outer metatarsal tubercle absent.

Skin of dorsal side extremely rough. Forehead and upper lip relatively smooth, scatted small tubercles without spine; small



Figure 3 Scutiger spinosus sp. nov. paratype (KIZ 013867), adult male in life (Photos by Kai WANG)

A: Dorsolateral view; B: Dorsal view; C: Ventral view; D: Ventral view of hand; E: Ventral view of foot.



Figure 4 Scutiger spinosus sp. nov. paratype (KIZ 013865) adult female in life (Photos by Kai WANG)

A: Dorsolateral view; B: Ventral view; C: Ventral view of hand; D: Ventral view of foot.

tubercles present below supratympanic fold, each tubercle bearing one black spine; supratympanic fold, top of head, dorsal sides of body and limbs, and flanks covered with large, prominent, conical-shaped tubercles, all bearing black spines except on dorsal side of forearm; small tubercles scatted among the larger tubercles, mostly bearing black spine. Skin on ventral surface of body mostly smooth; numerous small black spines present on margin of lower jaw, small patches of black spines present near armpit. A pair of pectoral glands and a pair of axillary glands present on chest, pectoral twice longer than axillary, both covered by dense black spines.

Coloration in life: A light brown, triangular pattern of pig-

mentation is observed on the dorsal surface of the anterior portion of the head, the apex is at the tip of snout and the base is between eyes. Several dark spots overlay the triangular pattern. Lateral surfaces of the head are dark brown; upper lip is creamy white, with irregular shaped brown mottling. The coloration of the remaining dorsal surfaces of the head, body, lower forelimbs, and hind limbs are dark brownish gray. The flanks are light brown and gradually fade into light brownish yellow ventrally. Dorsal surfaces of the upper forelimbs are light brownish yellow, with few transverse stripes of darker brown; dorsal surfaces of the lower forelimbs are dark gray, with few

light gray transverse bands. Light mottling patterns of pigmentations are also observed on the dorsal surfaces of the hind limbs. Ventral surfaces of the head, body, and limbs are uniform light yellow.

The coloration in preservative closely resembles the coloration in life, except that the off-white coloration of the lips and the light brownish yellow of the venter fade and become light gray.

**Variation:** Measurements of type series are shown in Table 3. Individuals of type series are generally uniform in external characters, except for the characters related to sexual dimorphism in the males.

Table 3 Morphological measurements (mm) of the type series of Scutiger spinosus sp. nov.

Number	Status	Sex	SVL	HL	HW	SL	INS	IOS	UEW
KIZ011114	Holotype	Male	51.7	18.7	19.4	7.5	4.3	4.5	5.0
KIZ013868	Paratype	Male	53.6	18.4	18.4	6.8	4.0	3.8	5.1
KIZ013866	Paratype	Male	55.3	18.0	18.9	7.0	4.3	3.8	5.1
KIZ013863	Paratype	Male	54.4	17.7	18.6	6.6	4.4	3.8	5.0
KIZ013867	Paratype	Male	51.8	18.3	18.2	6.9	4.2	3.6	4.8
KIZ013862	Paratype	Male	54.3	18.2	17.8	6.7	4.6	4.1	4.7
KIZ013869	Paratype	Male	54.5	18.0	18.2	6.1	4.4	4.5	5.2
KIZ013864	Paratype	Male	52.1	17.7	17.3	6.9	4.3	3.7	5.1
KIZ011113	Paratype	Male	50.5	18.0	18.0	7.3	4.1	4.4	4.4
KIZ011099	Paratype	Male	55.5	19.1	19.8	7.9	4.6	4.6	4.7
KIZ012645	Paratype	Male	52.7	19.3	19.8	7.0	4.2	4.4	4.7
KIZ012648	Paratype	Male	55.3	18.8	18.9	7.2	4.2	3.7	5.1
KIZ012647	Paratype	Male	55.6	19.8	19.8	7.3	4.5	4.1	5.4
		Range	50.5-55.6	17.7-19.8	17.3-19.8	6.1-7.9	4.0-4.6	3.6-4.6	4.4-5.4
		Average	53.6	18.5	18.7	7.0	4.3	4.1	4.9
	Ratio to	o SVL (%)		34.5	34.9	13.1	8.0	7.6	9.1
KIZ013865	Paratype	Female	54.2	17.4	17.9	6.3	4.5	3.5	4.7
KIZ011093	Paratype	Female	57.2	19.3	19.5	7.5	4.8	4.4	5.8
KIZ011100	Paratype	Female	56.9	18.3	18.4	7.1	4.6	4.1	5.0
KIZ012646	Paratype	Female	53.8	18.4	18.6	7.0	4.7	4.4	5.5
		Range	53.8-57.2	17.4-19.3	17.9-19.5	3.9-7.5	4.5-4.8	3.5-4.4	4.7-5.8
		Average	55.5	18.4	18.6	6.4	4.7	4.1	5.3
	Ratio to	o SVL (%)		33.2	33.5	11.5	8.5	7.4	9.5
Number	EH	lD	FAHL	FAW	HAL	FML	TBL	TFL	FOL
KIZ011114	6.5	5	26.6	6.9	14.2	22.3	20.9	37.4	26.4
KIZ013868	6.4	1	27.2	7.3	13.4	23.6	21.6	37.3	25.2
KIZ013866	6.6	3	29.4	6.8	13.3	23.6	21.6	39.0	26.7
KIZ013863	6.8	3	29.3	7.0	14.2	23.3	21.6	36.1	25.4
KIZ013867	6.4	1	27.9	6.5	13.9	22.5	20.6	35.2	24.6
KIZ013862	6.6	3	28.6	6.5	14.1	22.8	22.3	34.7	26.5
KIZ013869	6.9	9	28.6	7.7	13.5	23.0	21.4	37.7	26.3
KIZ013864	6.2	2	27.3	6.9	14.1	21.1	21.3	37.5	26.5
KIZ011113	6.4	1	25.5	5.5	13.6	22.7	20.9	36.6	24.2
KIZ011099	6.6	3	30.1	6.9	16.1	26.6	24.3	42.0	29.2
KIZ012645	6.0	)	27.6	6.0	13.2	23.0	20.9	37.7	25.8

								Continued
Number	EHD	FAHL	FAW	HAL	FML	TBL	TFL	FOL
KIZ012648	6.5	25.6	6.5	14.4	23.5	21.0	38.0	27.1
KIZ012647	6.3	26.1	6.7	14.1	23.2	21.7	26.3	25.3
Range	6.0-6.8	25.5-30.1	5.5-7.7	13.2-16.1	22.3-26.6	20.6-24.3	26.3-42.0	24.2-29.2
Average	6.5	27.7	6.7	14.0	23.2	21.5	36.6	26.1
Ratio to SVL (%)	12.1	51.7	12.5	26.1	43.3	40.1	68.3	48.7
KIZ013865	6.5	26.4	3.9	13.5	19.6	19.6	32.0	21.6
KIZ011093	6.3	29.1	5.2	15.3	22.8	20.9	37.3	24.2
KIZ011100	6.4	28.5	4.6	15.8	23.3	20.7	37.9	26.3
KIZ012646	7.0	27.4	4.0	15.0	21.7	19.9	34.5	24.0
Range	6.3-7.0	26.4-29.1	3.9-5.2	13.5-15.8	19.6-23.3	19.6-20.9	32.0-37.9	21.6-26.3
Average	6.6	27.9	4.4	14.9	21.9	20.3	35.4	24.0
Ratio to SVL (%)	11.9	50.3	7.9	26.8	39.5	36.6	63.8	43.2

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; 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.

Sexual dimorphism: In males, small patches of black spines are present on the armpit, and a pair of pectoral glands and a pair of axillary glands are present on the chest, with the former ones much larger than the latter ones. Both pectoral and axillary glands are covered by black spines in breeding condition. Nuptial black spines are present on the dorsal surfaces of the first and second finger and the inner side of the third finger with in breeding condition. Forearms of males are much stronger than females (FAW male/female=1.5). In breeding condition, spines on the dorsal and lateral surfaces of males are more distinct and denser than females. Vocal sac and lineae masculinae are absent in males.

**Ecological notes:** The new species inhabits the mixed forest at high elevation, sympatric with *Scutiger wuguanfui*, *Nanorana medogensis* (Fei and Ye, 1999), and *Liurana xizangensis* (Hu, 1977). Breeding pairs were found under logs during the day and in the streams and ponds at night from the beginning to the end of June. The breeding habitats include slow-flowing streams and small to medium permanent ponds, with brownish, slightly acidic water. Males display inguinal amplexus, where the males grasp the females at the waist (Figure 5). Eggs are laid in donut-shaped masses and are attached to the under surfaces of logs and rocks (Figure 6). There is no evidence of



Figure 5 Breeding pair of the *Scutiger spinosus* sp. nov., showing the inguinal amplexus (Photos by Kai WANG)
A: lateral view: B: ventral view.





Figure 6 Breeding habitat (A) and eggs (B) of *Scutiger spinosus* sp. nov. at 62K of Medog, southeastern Tibet, China (Photos by Kai WANG and Pi-Peng LI)

nuptial spines on the fingers and pectoral and axillary glands in mid July. Tadpoles overwinter and take more than one year to develop into metamorphic juveniles, which is a common adaptation for high-elevation amphibians.

**Etymology:** The specific epithet *spinosus*, is derived from the Latin word *spina*, meaning spine and combined with <u>ō</u>sus to form an adjective indicating an abundance of the noun, spina. *Spinosus* is in reference to the conical-shaped tubercles and the numerous spines on the dorsal surfaces of this species.

Based on the Latin name we suggest the English common name to be "Spiny Lazy Toad", the Chinese name as "Ci You Chi Tu Chan" (刺疣齿突蟾).

Morphological Comparisons: Scutiger spinosus sp. nov. is morphologically most similar to S. nyingchiensis and S. boulengeri, in which both species have similar body size, brownish gray dorsal coloration, and distinct tubercles on the dorsal surface of the body. However, the new species can be distinguished readily from S. nyingchiensis (Figure 7) by having rudimentary webbings on toes (v.s. well developed), prominent, conical-shaped tubercles on the dorsal and lateral surfaces of the body and limbs (v.s. tubercles elongated, relatively low, and not conical in shape), as well as by the presence of a distinct, light brown, triangular pattern on the dorsal surface of the head (v.s. absent), presence of large tubercles on the dorsal surfaces of thigh (v.s. absent), presence of spines on the upper arm (v.s. absent), presence of numerous, spine-bearing, small tubercles scatted among the large tubercles on the dorsal and lateral surfaces of the body and limbs (v.s. absent), and the presence of spines on the tubercles on the dorsal surface of the body and limbs in females (v.s. absent); and from S. boulengeri by having rudimentary webbings on toes (v.s. well developed), prominent, conical shaped, spine-bearing tubercles on the dorsal and lateral surfaces of the body (v.s. relatively flat, not conical shaped, with smaller spines), and by the absence of spines on the abdominal region in breeding condition in males (v.s. present).



Figure 7 Male and female of *Scutiger nyingchiensis* (Photos by Da-Hu ZOU, Bao-Lin ZHANG and Ke JIANG).

Dorsolateral view (A), ventral view (B) and ventral view of foot (C) in life, and dorsal view in preservative (D) of the adult topotypic male (KIZ 017460) from Bujiu, Nyingchi, Tibet, China. Dorsolateral view in life (E) and in preservative (F) of the adult female (KIZ 016237), showing the absence of spines on the dorsal surface of the body, from Gongbo'gyamda (=Gongbu'jiangda), Tibet, China.

Scutiger spinosus sp. nov. is closely related to *S. gong-shanensis* based on the molecular phylogeny, but the new species differs from the latter by the absence of vocal sacs in males (*v.s.* presence), presence of axillary glands (*v.s.* absent) and presence of black spines on dorsal tubercles (*v.s.* absent). Additionally, the new species differs from the sympatric *S. wuguanfui*, by having a distinct dorsal coloration (brownish gray *v.s.* dark purplish brown), a smaller adult body size (SVL 50.5-55.6 mm in males, 53.8-57.2 mm in females *v.s.* 77.5-83.8 mm in males, 107.4-116.7 mm in females), prominent, conical, spine-bearing tubercles on the dorsal and lateral surfaces of body and limbs (*v.s.* flat protuberances that are relatively smooth), as well as by the absence of vocal sacs in males (*v.s.* presence).

#### **DISCUSSION**

Our molecular data shows that all known *Scutiger* species form a monophyletic group (Figure 1). Surprisingly, despite the closer geographic proximity and similar external morphology between *S. nyingchiensis* and *S. spinosus*, *S. nyingchiensis* is more distantly related to *S. spinosus* than *S. gongshanensis*. *Scutiger gongshanesis* is distributed much farther away in the Hengduan Mountain Range on the east and has quite different external morphology. Additionally, *Scutiger boulengeri* is paraphyletic based on our molecular phylogeny, which might be the results of mitochondrial gene introgression with other sympatric *Scutiger* species (Chen et al., 2009), existence of cryptic species, or mis-identification of congeners. Further phylogenetic and phylogeographic studies are needed to gain a better understanding of this interesting relationship.

Recently, Sarania et al. (2015) reported *S. nyingchiensis* from the China-India Disputed Region based on photographic evidence without vouchered specimens. However, based on the morphological characters described and shown in their photos, such as the rudimentary webbings on toes, the presence of spines on tubercles in females, and an inverted triangular mark of pigmentation from the upper eyelid to the snout, these individuals possess diagnostic characters of the new species, and thus are likely to be *S. spinosus* instead of *S. nyingchiensis*. Further examinations of vouchered specimens from the region are needed to ensure the taxonomic status of this population.

Although the new species is locally abundant, its pristine habitats are currently under great threats from rapid developments of roads in southeastern Tibet. Several breeding pools of the new species were eliminated entirely during the sampling period of our study, and significant deforestations were observed along the roads. Because the new species and other amphibians from the same habitats, namely *S. wuguanfui*, *Nanorana medogensis* and *Liurana xizangensis* are believed to be restricted to the mixed forests in this region, such deforestation and destructions of breeding habitat could be devastating to the long-term survival of these endemic amphibians. We recommend immediate attention to the conservation and protection of habitats in this area including the type locality of the new species. Given the recent human incroachment into these areas, in-depth assessments of the ecology and population

status of the endemic amphibians including the new species *Scutiger spinosus* are needed now more than ever.

#### **ACKNOWLEDGEMENTS**

We thank to Mr. Bao-Lin ZHANG (KIZ) and Mr. Jiang XIE (North China Electric Power University Science and Technology College), and the volunteers, Mr. Tao LIANG, Mr. Duan YOU, Mr. Ya-Di HUANG and Mr. Ya-Qiang SUN, who helped with fieldwork in Tibet, Dr. Yun-Ke WU (Cornell University) and Dr. Yu ZENG (University of California, Berkeley) for providing some literatures.

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#### **APPENDIX**

The following specimens were examined:

Scutiger boulengeri (n=28): KIZ 012573, KIZ 012581, KIZ 012583-84 (4ぷ♂), Nyalam (=Nielamu), Tibet; KIZ 012605 (1♂), KIZ 012602-04 (3♀♀), Tingri (=Dingri), Tibet; KIZ 014935-39, KIZ 014941-42, KIZ 014944-46 (9♂♂), KIZ 014948, KIZ 014950 (2♀♀), Qamdo (=Changdu), Tibet; KIZ 012606-07, KIZ 012609-11, KIZ 012614 (6♂♂), KIZ 012616-18 (3♀♀), Ndong (=Naidong), Tibet.

Scutiger glandulatus (n=7, unmeasured): KIZ 016477-80, KIZ 016482 (5%%), KIZ 016481, KIZ 016483 (2 $\mathbb{Q}$  $\mathbb{Q}$ ), Ganzi, Sichuan.

Scutiger mammatus (n=17): KIZ 016469-70, KIZ 017293-95, KIZ 017447-48, KIZ 017454-56, KIZ 017458 (11♂♂), KIZ 016471, KIZ 017288-89, KIZ 017449, KIZ 017457 (5♀♀), Jomda (=Jiangda), Tibet; KIZ 014035 (1♀), Baxoi (=Basu), Tibet.

Scutiger nyingchiensis (n=6): KIZ 017459-60 (2♂♂, Nyingchi); KIZ 09416, KIZ 019415-16 (3♂♂), KIZ 016217 (1♀), Gongboʻgyamda (=Gongbuʻjiangda), Tibet.

Scutiger sikimmensis (n=10): KIZ 011127, KIZ 013983-88, KIZ 013990 (8♂♂), KIZ 013982, KIZ 013989 (2♀♀), Yadong, Tibet.

Scutiger wuguanfui (n=8): KIZ 01101-02, KIZ 011109-11, KIZ 012649 (6♂♂), KIZ 011116, KIZ 012650 (2♀♀), Medog, Tibet.

## A new species of the genus *Amolops* (Amphibia: Ranidae) from southeastern Tibet, China

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#### **ABSTRACT**

A new species of the genus Amolops Cope. 1865 is described from Nyingchi, southeastern Tibet, China, based on morphological and molecular data. The new species, Amolops nyingchiensis sp. nov. is assigned to the Amolops monticola group based on its skin smooth, dorsolateral fold distinct, lateral side of head black, upper lip stripe white extending to the shoulder. Amolops nyingchiensis sp. nov. is distinguished from all other species of Amolops by the following combination of characters: (1) medium body size, SVL 48.5-58.3 mm in males, and 57.6-70.7 mm in females; (2) tympanum distinct, slightly larger than one third of the eye diameter; (3) a small tooth-like projection on anteromedial edge of mandible; (4) the absence of white spine on dorsal surface of body; (5) the presence of circummarginal groove on all fingers; (6) the presence of vomerine teeth; (7) background coloration of dorsal surface brown, lateral body gray with yellow; (8) the presence of transverse bands on the dorsal limbs; (9) the presence of nuptial pad on the first finger in males; (10) the absence of vocal sac in males. Taxonomic status of the populations that were previously identified to A. monticola from Tibet is also discussed.

**Keywords:** Amolops monticola group; Amolops nyingchiensis sp. nov.; DNA barcoding; Tibet

#### INTRODUCTION

The cascade frogs of the genus *Amolops* Cope, 1865 inhabits rocky, fast-running streams and small rivers. Currently, the genus contains 49 recognized species (Frost, 2015), distributing from Nepal eastwards to southern China, and

southeastwards to Malaysia. Previous phylogenetic studies of the genus *Amolops* all supported the monophyly of this genus (Cai et al., 2007; Matsui et al., 2006; Stuart, 2008). Based on morphological data, both Fei et al. (2005) and Stuart et al. (2010) recognized the *A. monticola* group.

Firstly described by Fei et al. (2005) based on morphological character and later confirmed by phylogenetic data, the *A. monticola* group includes species that possess smooth skin, lateral side of head dark, with a light-colored upper lip-stripe extending to the shoulder, and distinct dorsolateral folds (Stuart et al., 2010). Currently, the *A. monticola* species group includes 14 species distributed in southern China, southern and southeastern Asia (Stuart et al., 2010; Frost, 2015). Six species of the *A. monticola* group are known in China, four of which are found in Tibet, including *A. aniqiaoensis* Dong, Rao and Lü, 2005, *A. chayuensis* Sun, Luo, Sun and Zhang, 2013, *A. gerbillus* (Annandale, 1912), and *A. monticola* (Anderson, 1871) (Fei et al., 2009b; Sun et al., 2013).

During our herpetological surveys in southeastern Tibet in July 2012, April 2014 and May 2015, a total of 23 specimens of *Amolops* were collected from two localities of Nyingchi (=Linzhi) Prefecture. These specimens are referred to the *A. monticola* 

Received: 20 October 2015; Accepted: 18 December 2015

Foundation items: This study was supported by the Ministry of Science and Technology of China (2014FY210200 and 2011FY120200), the Animal Branch of the Germplasm Bank of Wild Species of Chinese Academy of Sciences (the Large Research Infrastructure Funding), and partially supported by the project "National Second Survey of Wild-animals in Xizang Autonomous Region of China"

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group based on morphological and molecular data, and they cannot be assigned to any known congeners. Therefore, we describe it as a new species. Taxonomic status of the populations from Tibet that were previously identified to *A. monticola* is also discussed.

#### **MATERIALS AND METHODS**

#### Sampling

A total of 23 individuals of new species were collected from southeastern Tibet, China, including 13 adults and five subadults from Medog (=Motuo), and five adults from Mainling (=Milin). Following euthanasia, all specimens were fixed in 10% formalin solution after sampling of liver tissues (in 95% ethanol), and transferred to 75% ethanol after fieldwork. All specimens were designated as the type series.

#### Morphological analysis

All the 18 adult specimens of new species were measured, and five other species were examined (Appendix). 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. (2009a), 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 (Anderson, 1871; Fei et al., 2009b; Liu et al., 2000). 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

The genome DNA of four individuals of the new species of *Amolops* (KIZ 016415 and 016416 from Medog, 012632 and 012633 from Mainling) and other three known species from Tibet, China (*A. aniqiaoensis*, *A. chayuensis* and *A. medogensis*, in Table 1) was extracted from liver tissues with a standard three-step phenol-chloroform extraction method (Sambrook et al., 1989). A 600 base pair DNA barcoding sequence of mitochondrial gene cytochrome oxidase subunit I (COI) was sequenced using primers Chmf4 and Chmr4 (Che et al., 2012). Protocols for PCR and sequencing followed Che et al. (2012). All newly generated sequences were deposited in GenBank (Table 1). The COI sequences of the available 14 species of the genus *Amolops* and seven outgroup species were downloaded from GenBank (Table 1).

All dataset were aligned and edited using MEGA 5 (Tamura et al., 2011). For each codon position, the best model of

nucleotide substitution was calculated in Modeltest v1.0.1 (Posada, 1998). Bayesian inference (BI) was used to generate a phylogenetic relationship using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). With different four runs, the Markov chains were estimated for 10 million generations, and every 100 generations were sampled. The pairwise comparisons for genetic distance among species was calculated using MEGA 5 with Kimura 2-parameter model (Che et al., 2012).

#### **RESULTS**

#### Genetic analysis

Our results show that all Amolops species used in the current study form a monophyletic group using BI analysis. Given the limited genetic data used, our results did not provide enough resolution regarding phylogenetic relationships among different species groups of the genus Amolops. However, the data does support all four individuals of the new species as distinct mitochondrial lineage with high support values. The lineage of new species is clustered with A. bellulus (Yunnan, China), A. mengyangens (Yunnan, China) and A. anigiaoensis (Tibet, China) (Figure 1). For Amolops species used in our study, the average genetic distance between congeners is between 3.7% and 26.3% (Table 2), while the new species possesses a 5.5% genetic divergence from its sister species A. bellulus.

#### Morphological comparison

Our morphological comparisons support that the new species of *Amolops* as a member of the *A. monticola* group, because the new species possesses diagnostic characters of the species group, including light lip stripes and distinct dorsolateral folds. However, the new species differ from all congeners of the *A. monticola* species group by distinct coloration, the presence of circummarginal grooves on all fingers, presence of vomerine teeth, absence of white spine on the dorsal surfaces of head and body, and absence of vocal sacs in males.

#### **Taxonomic conclusion**

Because morphological and phylogenetic data support that the Nyingchi population of *Amolops* represent a distinct and independent evolutionary lineage and concordance among independent evidence confirms species status (Hou et al., 2014; Wu & Murphy, 2015), we describe it herein as a new species.

#### Taxonomic account

Amolops nyingchiensis sp. nov. Jiang, Wang, Xie, Jiang, and Che (Figure 2-3)

**Holotype:** KIZ 016432 (Figure 2), an adult male, from Gedang (N29.43871°, E95.66502°, elevation 1 887 m), Medog, Nyingchi Prefecture, Tibet, PR China, collected by Ke JIANG on 02 May 2015, and deposited in KIZ.

**Allotype:** KIZ 016418, an adult female, specimen shares the same locality and collection information as the holotype.

**Paratypes:** two adult males, KIZ 016415 and KIZ 016433, and nine adult females KIZ 016416-17, KIZ016419-24, KIZ 016435, all share the same locality and date as the holotype, collected by Ke JIANG, Fang YAN and Da-Hu ZOU. Three adult males, KIZ 012633-35, and two adult females, KIZ 012632 and KIZ 012646, from Zhibai (N29.62078°, E94.93358°; elevation 2 941 m),

Paizhen, Mainling (=Milin), Nyingchi Prefecture, Tibet, PR China, collected by Ke JIANG, Kai WANG and Jiang XIE on 05 July 2012. Above specimens are deposited in KIZ. Five sub-adults, CIB 0140506-10, collected by Jian-Ping JIANG and Wu-Lin LIU from the same locality as the holotype on 24 April 2014, and deposited in CIB.

Table 1 Information of samples used in molecular analysis

Genus	Species	Locality	Specimen voucher No.	GenBank No.
Amolops	nyingchiensis	China: Gedang, Medog, Tibet	KIZ016416	KU243068
			KIZ016415	KU243069
		China: Paizhen, Mainling, Tibet	KIZ012633	KU243070
			KIZ012632	KU243071
	aniqiaoensis	China: Medog, Tibet	KIZ07364	KU243072
			KIZ011138	KU243073
	chayuensis	China: Baxoi (=Basu), Tibet	KIZ014016	KU243074
			KIZ014022	KU243075
	medogensis	China: Medog, Tibet	KIZ06635	KU243076
			KIZ06638	KU243077
	bellulus	China: Tengchong, Yunnan	KIZYPX9037	KU243078
			KIZYPX9038	KU243079
	lifanensis			JN700797
	mantzorum			JN700798
				JN700799
	loloensis			JN700801
				JN700802
	jinjiangensis			JN700803
	granulosus			JN700804
	tuberodepressus			JN700805
	wuyiensis			JN700806
				JN700807
	hainanensis			JN700808
				JN700809
	hongkongensis			JN700810
				JN700811
	daiyunensis			JN700812
	marmoratus			KR087617
	mengyangensis			KR087618
				KR087619
	panhai			KR087620
				KR087621
	ricketti			KR087623
				KR087622
Odorrana	tiannanensis			KR087851
	livida			KR087842
	tormotus			DQ835616
Rana	warszewitschii			KR863028
Hylarana	taipehensis			KR087734
ndosylvirana	milleti			KR087729
Sylvirana	menglaensis			KR087719

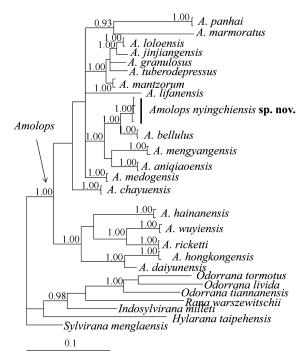


Figure 1 Bayesian inference tree based on barcoding COI data of the Tibetan congeners of the genus *Amolops* and selected outgroups The characters near branches are Bayesian posterior probabilities (only ≥90 were showed).

**Diagnosis:** According to morphological character for the *Amolops monticola* group by Stuart et al. (2010), *Amolops nyingchiensis* sp. nov. is placed in the *Amolops monticola* group by following diagnosis: (1) skin smooth; (2) dorsolateral fold distinct; (3) lateral side of head black, upper lip stripe white extending to the shoulder.

Amolops nyingchiensis sp. nov. is further distinguished from all other congeners in the Amolops monticola group by the following combination of characters: (1) medium body size, SVL 48.5-58.3 mm in males, and 57.6-70.7 mm in females; (2) tympanum distinct, slightly larger than one third of the eye diameter; (3) a small tooth-like projection on anteromedial edge of mandible; (4) the absence of white spine on dorsal surface of body; (5) the presence of circummarginal groove present on all fingers; (6) the presence of vomerine teeth; (7) background coloration of dorsal surface brown, lateral body gray with yellow; (8) the presence of transverse bands on dorsal limbs; (9) the presence of nuptial pad on the first finger in males; (10) the absence of vocal sac in males.

**Description of the holotype:** Body size moderate, SVL 58.3 mm, slightly compressed vertically. Head slightly longer than wide (HL/HW=1.05); snout projecting forward and depressed, somewhat pointed at tip; nostril lateral, closer to eye than tip of snout; canthus rostralis distinct, slightly constricted behind nostrils; loreal region concave and oblique; eye relatively large (EHD/HL=0.40); interorbital distance same as width of upper eyelid; tympanum distinct, about one third of eye diameter (THD/EHD=0.34), tympanic rim not elevated; ridge of upper lip

distinct. Vomerine teeth weakly developed, on two short oblique between choanae; tongue pyriform, deeply notched posteriorly, free for approximately two third of its length; a small tooth-like projection on anteromedial edge of mandible; vocal sac and vocal sac opening absent.

Fore-limb robust; tips of all four fingers expended into discs, disc on finger III approximately equal to diameter of tympanum; circummarginal grooves present on tips of all fingers, relatively feeble on first finger; relative finger length: I<II<IV<III; single subarticular tubercle on finger I and II, two on finger III and IV; palmar tubercles absent; fringe absent; nuptial pad well developed, distinct, feebly granular, relatively smooth.

Hind limb slender, tibiotarsalis beyond the snout when adpressed, heels overlap when flexed and held perpendicular to body. Tips of all five toes expanded into discs, width of toe IV disc less than finger III disc; relative toe length: I<II<VIV; toe web developed, webbing formula: I 0-0 II 0-0 III 0-1 IV 1-0 V; elongate, oval inner metatarsal tubercle present; outer metatarsal tubercle absent.

Dorsal and lateral head and body smooth, except few indistinct tubercles present on temporal head and above vent; supratympanic fold indistinct; dorsolateral fold distinct, from rear of upper eyelid to near vent; ventral surfaces smooth except lightly flat tubercles on basal ventral surface of thigh; one low rictal gland, continuous with upper lip.

Coloration of holotype in life: Dorsal surfaces of the head and body and lateral surfaces of the snout are flesh ocher. Coloration is much lighter along the upper margins of the dorsolateral fold. Small black spots are randomly scattered on the dorsal surfaces of the head and body. A white lip-stripe is present from the tip of the snout to the anterior joint of the shoulder on each side. The upper one fourth of the iris is golden yellow with small brown spots, while the remaining lower part of the iris is reddish brown. The tympanic region is dusky brown, with some lime-green mottling patterns of pigmentations scattered. A black stripe runs from the tip of snout to the anterior corner of the eye along the lower edge of canthus rostralis, and the stripe continues from the posterior corner of the eye along the dorsolateral fold to the pelvis. Lateral surfaces of the body are gray with olive yellow, scattered with some darker mottling. Dorsal surfaces of the limbs are rufous, darker on the hind limbs. Lateral surfaces of the hind limbs are trogon yellow, and the coloration is more obvious closer to the bases of the limbs. Irregular black, transverse bands are observed on the dorsal surfaces of limbs, and the bands are much more distinct on the hind limbs. Dark marbled patterns of pigmentation are observed on the dorsal surfaces of the fingers and toes.

The throat and chest are pinkish white, with blackish vermiculate patterns of pigmentation. Abdominal region is off-white, and faded dark gray blotches of pigmentation are observed on the anterior part of the region. The ventral surfaces of the fore-limbs are light orange yellow. The ventral surfaces of the thigh and shank are pinkish-yellow and light orange yellow respectively.

Coloration of holotype in preservative: Dorsal surfaces of the head and body are dark gray, with scattered small, indistinct,

Table 2 The interspecific distances within Amolops based on COI dataset

	_	2	က	4	2	9	7	8	6	10	1	12	13	4	15	16	17	18	19
A. lifanensis (1)																			
A. mantzorum (2)	0.144																		
A. loloensis (3)	0.146	0.037																	
A. jinjiangensis (4)	0.163	0.051	0.033																
A. granulosus (5)	0.154	0.052	0.055	0.058															
A. tuberodepressus (6)	0.163	0.051	0.059	0.062	0.063														
A. wuyiensis (7)	0.238	0.230	0.226	0.237	0.211	0.219													
A. hainanensis (8)	0.208	0.212	0.222	0.217	0.230	0.214	0.189												
A. hongkongensis (9)	0.219	0.222	0.227	0.226	0.216	0.218	0.212	0.215											
A. daiyunensis (10)	0.194	0.197	0.202	0.208	0.189	0.209	0.189	0.196	0.088										
A. marmoratus (11)	0.263	0.219	0.215	0.216	0.208	0.226	0.219	0.234	0.253	0.244									
A. mengyangensis (12)	0.181	0.149	0.161	0.162	0.152	0.160	0.209	0.226	0.254	0.205	0.249								
A. panhai (13)	0.261	0.227	0.236	0.245	0.226	0.226	0.245	0.251	0.234	0.254	0.245	0.240							
A. ricketti (14)	0.239	0.231	0.225	0.228	0.244	0.228	0.122	0.189	0.192	0.181	0.223	0.218	0.231						
A. medongensis (15)	0.168	0.115	0.119	0.126	0.112	0.115	0.204	0.210	0.204	0.195	0.206	0.146	0.204	0.199					
A. aniqiaoensis (16)	0.162	0.134	0.142	0.137	0.136	0.155	0.223	0.230	0.250	0.203	0.228	0.110	0.241	0.243	0.135				
A. nyingchiensis (17)	0.150	0.116	0.119	0.124	0.122	0.126	0.202	0.225	0.232	0.205	0.227	0.110	0.216	0.213	0.117	0.111			
A. chayuensis (18)	0.160	0.107	0.111	0.118	0.131	0.118	0.220	0.212	0.228	0.203	0.212	0.168	0.223	0.197	0.113	0.135	0.145		
A. bellulus (19)	0.166	0.135	0.136	0.144	0.139	0.148	0.214	0.222	0.241	0.230	0.230	0.118	0.218	0.225	0.130	0.119	0.055	0.148	
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The numbers in the first line represent the species showed in first column.



Figure 2 Different views of the male holotype (KIZ 016432) of Amolops nyingchiensis sp. nov. in life (Photos by Ke JIANG)

A and B: dorsolateral and ventral view in preservative, respectively; C: dorsal view; D: ventral view.

black mottling. The upper margins of the dorsolateral fold are light gray. Lateral surfaces of the body are dusky brown. The throat, chest, abdominal region, the ventral surfaces of the fore limbs, and the ventral surfaces of the shank and feet are white, while the ventral surfaces of the thigh are yellowish. The iris becomes uniform gray in preservative.

**Variation:** Measurements of type series are summarized in Table 3 and Table 4. Sexually dimorphism is observed. Males have distinct nuptial pads (*v.s.* absence in females) and have thick, robust forearms (*v.s.* thin and slender in females). In addition to the morphometric variations, differences of coloration are also observed among the type specimens. In two adult male specimens (KIZ 012634 and KIZ 016433), both black and light gray mottling patterns are observed on the dorsal surfaces of the head and body, while other three adult males specimens (KIZ 012633, KIZ 012635, and KIZ 016415) do not possess any mottling pattern on the dorsal



Figure 3 Different views of the female paratopotypes in life, from Medog (KIZ016434) (Photos by Ke JIANG and Kai WANG)

A: dorsolateral view; B: ventral view; C and D: dorsolateral and ventral view from Mainling (KIZ012636), respectively.

surfaces of the head and body. Similar difference of coloration is also observed among the 12 adult females: KIZ 016435 and 016423 have dark gray spots only, KIZ 016418 and 016421 has light gray spots only, KIZ 016417, 016424, and 016422 have both light and black mottling patterns, and KIZ 016419, 016420, 016416, and 016434 have no mottling pattern at all.

**Comparison:** In the *Amolops monticola* group, *Amolops nyingchiensis* sp. nov. is most morphological similar to *A. aniqiaoensis*, *A. bellulus*, *A. chayuensis*, *A. chunganensis*, and *A. monticola*. But the new species could be distinguished from the four species except for *A. bellulus* by the absence of vocal sac in male (*v.s.* presence), and further differs from *A. aniqiaoensis* by the absence of white spine on dorsum (*v.s.*presence of both sexes) and the distinct transverse bands on dorsal limbs (*v.s.*absent or indistinct), differs from *A. chayuensis* by coloration of dorsum (light brown or yellowish brown *v.s.* green), differs from *A. chunganensis* by the smooth nuptial pad (*v.s.*the presence of tubercles on nuptial pad).

Amolops nyingchiensis sp. nov. differs from A. bellulus by the presence of circummarginal groove on all fingers (v.s. absence of first finger) and different color of posterior and lower flank (gray with yellow v.s. bluish green to olive green) and iris (upper one fourth golden yellow, lower part red brown v.s.upper half golden yellow, lower half dark brown) in life; Amolops nyingchiensis sp. nov. differs from A. gerbillus by the distinct tympanum (v.s. indistinct), and difference of dorsal coloration (light brown or yellowish brown v.s. dark gray with light spots).

**Etymology:** The species name "nyingchiensis" is the Latin form of name of Nyingchi Prefecture, which includes the two localities of the new species. According to the Latin name, we suggest the English common name as "Nyingchi cascade frog", and the Chinese common name as "Lin Zhi Tuan Wa" (林芝湍蛙).

#### **Ecological notes**

The new species, *A. nyinchiensis* is found in the small to medium sized, fast flowing streams near mixed forest at relative high elevation (Figure 4). At the type locality (Gedang), the stream is much faster, and has rocky shores, while at the other locality (Zhibai) the stream is slower, and the shores are covered by vegetation and not rocky. Such distinct microhabitats suggest a wide ecological niche of the new species. In Gedang, only the new species was observed, and in Zhibai, one amphibian species, *Nanorana parkeri* (Stejneger, 1927) was co-distributed with the new species, and one reptile species *Pseudoxenodon macrops* (Blyth, 1854) was also observed. Reproductive season of the new species is unknown.

#### **DISCUSSION**

The type species of the *A. monticola* group, *A. monticola*, was described based on a single female specimen from Darjeeling, northeast India (Anderson, 1871). Later, Boulenger (1920)

Table 3 Morphological measurements (mm) of the type series of Amolops nyingchiensis sp. nov. from Gelin, Medog

Number	Sex	Status		SVL	HL	HW	SL	INS	IOS	UEW
KIZ016432	Male	Holotype	• <u> </u>	58.3	19.7	18.8	7.5	7.0	4.9	4.9
KIZ016433	Male	Paratype	e	52.3	18.2	17.3	7.1	6.0	4.6	4.6
KIZ016415	Male	Paratype	e	56.9	20.1	19.4	8.2	7.3	4.5	4.9
		Range		52.3-58.3	18.2-20.1	17.3-19.4	7.1-8.2	6.0-7.0	4.5-4.9	4.6-4.9
		Average		55.8	19.3	18.5	7.6	6.8	4.7	4.8
		Ratio to S	SVL (%)		34.6	33.2	13.6	12.2	8.4	8.6
KIZ016418	Female	Allotype		70.7	24.2	24.3	9.1	8.1	6.2	5.7
KIZ016416	Female	Paratype	e	67.8	23.9	22.9	9.0	8.2	6.2	5.3
KIZ016417	Female	Paratype	e	68.4	22.9	23.0	9.0	7.9	6.0	5.3
KIZ016419	Female	Paratype	9	64.7	22.8	20.9	8.9	7.4	5.5	5.7
KIZ016420	Female	Paratype	9	57.6	21.2	19.3	8.4	7.0	5.6	4.8
KIZ016421	Female	• •		59.3	22.2	21.5	8.4	7.0	5.3	5.7
KIZ016422	Female	* *		69.6	23.9	24.0	9.1	8.2	5.9	6.1
KIZ016423	Female	Paratype	9	69.8	24.2	23.4	9.2	7.9	5.9	5.7
KIZ016424	Female	Paratype	9	68.7	24.9	24.7	9.5	8.2	5.8	5.7
KIZ016435	Female	Paratype	9	68.2	23.3	22.8	9.6	7.8	6.2	6.0
		Range		57.6-70.7	21.2-24.9	19.3-24.7	8.4-9.6	7.0-8.2	5.3-6.2	4.8-6.1
		Average		66.1	23.3	22.6	9.0	7.7	5.8	5.6
		Ratio to			35.2	34.2	13.6	11.6	8.8	8.5
Number		EHD	TD	FAHL	FAW	HAL	FML	TBL	TFL	FOL
KIZ016432		7.9	2.7	29.7	7.0	17.1	33.6	37.8	49.2	32.4
KIZ016433		7.9	2.4	26.6	6.9	15.7	30.0	34.6	45.7	30.5
KIZ016415		7.8	2.4	30.7	7.2	17.6	35.3	40.5	52.2	34.1
Range		7.8-7.9	2.4-2.7	26.6-30.7	6.9-7.2	15.7-17.6	30.0-35.3	34.6-40.5	45.7-52.2	30.5-34.
Average		7.9	2.5	29.0	7.0	16.8	33.0	37.6	49.0	32.3
Ratio to SVL	(%)	14.2	4.5	52.0	12.5	30.1	59.1	67.4	87.8	57.9
KIZ016416		9.0	3.3	33.9	6.3	19.7	40.0	44.8	58.7	39.9
KIZ016417		9.3	3.0	33.7	6.6	21.2	40.4	45.0	57.8	38.2
KIZ016418		10.2	3.2	36.4	7.2	21.3	40.6	44.8	59.5	37.8
KIZ016419		9.1	2.5	31.9	6.5	19.9	36.6	43.0	54.7	35.0
KIZ016420		8.3	2.7	30.4	5.2	18.1	33.5	40.3	51.1	32.6
KIZ016421		9.7	2.6	32.4	6.4	18.4	34.4	41.1	53.7	35.3
KIZ016422		10.3	3.0	35.1	6.0	21.4	36.5	43.6	54.9	36.7
KIZ016423		9.2	3.1	34.8	7.6	21.1	38.3	43.1	58.2	38.6
		9.7	3.0	34.0	6.9	21.1	39.1	42.3	58.2	38.1
KIZ016424		8.5	2.6	34.8	6.5	20.7	39.1	45.7	58.9	39.1
		0.5				-			· <del>-</del>	
KIZ016435				30.4-36.4	5.2-7.6	18.1-21.4	33.5-40.6	40.3-45.7	51.1-59.5	32.6-39.
KIZ016424 KIZ016435 Range Average		8.3-10.3 9.3	2.5-3.3 2.9	30.4-36.4 33.7	5.2-7.6 6.5	18.1-21.4 20.2	33.5-40.6 37.7	40.3-45.7 43.3	51.1-59.5 56.4	32.6-39. 37.0

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.

Table 4 Morphological measurements (mm) of the type series of Amolops nyingchiensis sp. nov. from Zhibai, Paizhen, Mainling

Number	Sex	Status	SVL	HL	HW	SL	INS	IOS	UEW
KIZ012634	Male	Paratype	50.0	18.6	17.2	8.0	6.0	4.3	4.7
KIZ012633	Male	Paratype	48.9	19.1	17.3	8.1	6.2	4.9	4.6
KIZ012635	Male	Paratype	48.5	18.0	16.4	7.5	6.4	4.2	4.3
		Range	48.5-50.0	18.0-19.1	16.4-17.3	7.5-8.1	6.0-6.4	4.2-4.9	4.3-4.7
		Average	49.1	18.6	17.0	7.9	6.2	4.5	4.5
		Ratio to SVL (%)		37.9	34.6	16.1	12.6	9.2	9.2
KIZ012632	Female	Paratype	60.7	21.9	20.3	9.1	7.0	5.0	5.9
KIZ012636	Female	Paratype	59.0	22.2	20.3	9.0	7.1	5.4	5.5
		Range	59.0-60.7	21.9-22.2	20.3	9.0-9.1	7.0-7.1	5.0-5.4	5.5-5.9
		Average	59.9	22.1	20.3	9.1	7.1	5.2	5.7
		Ratio to SVL (%)		36.9	33.9	15.2	11.9	8.7	9.5
Number	EHD	) TD	FAHL	FAW	HAL	FML	TBL	TFL	FOL
KIZ012634	6.3	2.3	27.6	4.7	16.0	28.1	33.2	44.1	28.9
KIZ012633	6.2	2.6	27.2	5.7	16.4	27.9	31.5	42.4	29.1
KIZ012635	6.3	2.8	26.2	4.8	15.2	26.7	30.3	41.7	27.7
Range	6.2-6	5.3 2.3-2.8	26.2-27.6	4.7-5.7	15.2-16.4	26.7-28.1	30.3-33.2	41.7-44.1	27.7-29.1
Average	6.3	2.6	27.0	5.1	15.9	27.6	31.7	42.7	28.6
Ratio to SVL (%	) 12.8	5.3	55.0	10.4	32.4	56.2	64.6	87.0	58.2
KIZ012632	7.4	3.1	30.7	5.3	18.4	31.2	34.9	48.5	34.6
KIZ012636	6.9	3.1	29.9	4.6	18.2	32.7	35.1	47.3	32.0
Range	6.9-7	7.4 3.1	29.9-30.7	4.6-5.3	18.2-18.4	31.2-32.7	34.9-35.1	47.3-48.5	32.0-34.6
Average	7.2	3.1	30.3	5.0	18.3	32.0	35.0	47.9	33.3
Ratio to SVL (%	) 12.0	5.2	50.6	8.3	30.6	53.4	58.4	80.0	55.6

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.

redescribed the species and added a single topotypic male specimen in the description, which possesses a pair of external vocal sacs. However, since no additional descriptions or phylogenetic studies of the topotypic *A. monticola* have been published ever since, the diagnosis of the species is limited, and the species boundary of *A. monticola* remained unclear. Yet based on available information from the description of topotypes by Boulenger (1920), our new species can still be diagnosed from the true *A. monticola* by the absence of vocal sacs in males.

Within Tibet, although *A. monticola* is recorded from several localities (Bom [=Bomi], Medog, Zayü [=Chayu], and Cona [=Cuona]) in Nyingchi Prefecture and Shannan Prefecture (Fei et al., 1977; Hu, 1987; Li et al., 2010), only one study provided information of vouchered specimens and their descriptions, and the vouchered specimens did not include any adult male (Hu, 1987). Given the lack of comparable information of the true *A.* 

monticola and the lack of male specimens of Amolops from Tibet, Hu (1987) temporarily identified those above specimens as A. monticola, and clearly stated that further studies are needed to clarify the taxonomic status of these Tibetan Amolops. Our examinations of Hu's (1987) specimens of A. monticola from Medog (one adult female, CIB 35332) shows that the specimens differ from the original description of A. monticola by the presence of white spine on dorsum (v.s. absence) and the absence of distinct transverse bands on dorsal limbs (v.s. presence), but resemble to A. aniqiaoensis by above two diagnoses. Therefore, the record of A. monticola from Medog in Hu (1987) is a misidentification of A. anigiaoensis and should be replaced by the latter. For the remaining specimens in Hu's (1987) description of A. monticola from Bom, Zayü, and Cona (tadpole only), we cannot assign their taxonomic statuses with confidence due to the conservative morphology of females, the lack of male





Figure 4 Habitat of *Amolops nyingchiensis* sp. nov. at Gelin, Medog (A) and Zhibai, Mainling (B), Tibet, China (Photos by Ke JIANG and Kai WANG)

specimens, and the lack of comparable materials of the true *A. monticola*. We recommend that future studies should collect and compare additional specimens from Bom, Zayü, and Cona of Tibet and topotypic *A. monticola* from India to clarify the taxonomic status of these Tibetan *Amolops* populations.

#### **ACKNOWLEDGEMENTS**

We thank to the volunteers, Mr. Tao LIANG, Mr. Duan YOU, Mr. Ya-Di HUANG and Mr. Ya-Qiang SUN, who helped with fieldwork in Tibet, Prof. Yue-Zhao WANG (CIB), Prof. Yue-Ying CHEN (CIB) and Prof. Sheng-Quan LI (CIB) for kindly letting us examine specimens under their care.

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#### **APPENDIX**

The following specimens were examined:

Amolops aniqiaoensis (n=6): KIZ 011138-39 (2 $\circlearrowleft$  $\circlearrowleft$ ), KIZ 011136-37, KIZ011158 (3 $\circlearrowleft$  $\circlearrowleft$ ), CIB35332 (1 $\circlearrowleft$ ), Medog, Tibet.

Amolops chayuensis (n=19): KIZ 014016, KIZ 014019-21, KIZ 014028-34 (123), KIZ 014017-18, KIZ 014022-26 (79), Baxoi, Tibet.

Amolops chunganensis (n=1): CIB 33536 (1 $\circlearrowleft$ ), Chong'an, Fujian.

Amolops medogensis (n=5): KIZ 06634 (1\$\delta\$), KIZ 06635-37 (3\$\Qeq\$), KIZ 016438 (1\$\Qeq\$), Medog, Tibet.

Amolops cf. monticola (by Hu, 1987) (n=2): CIB 35331 (1 $\updownarrow$ ), Zayü, Tibet, CIB 35333 (1 $\updownarrow$ ), Bom, Tibet.

# Two new species of *Japalura* (Squamata: Agamidae) from the Hengduan Mountain Range, China

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#### **ABSTRACT**

Until recently, the agamid species, *Japalura flaviceps*, was recognized to have the widest geographic distribution among members of the genus occurring in China, from eastern Tibet to Shaanxi Province. However, recent studies restricted the distribution of J. flaviceps to the Dadu River valley only in northwestern Sichuan Province, suggesting that records of J. flaviceps outside the Dadu River valley likely represent undescribed diversity. During two herpetofaunal surveys in 2013 and 2015, eight and 12 specimens of lizards of the genus Japalura were collected from the upper Nujiang (=Salween) Valley in eastern Tibet, China, and upper Lancang (=Mekong) Valley in northwestern Yunnan, China, respectively. These specimens display a unique suite of diagnostic morphological characters. Our robust comparisons of phenotype reveal that these populations can be distinguished readily from J. flaviceps and all other recognized congeners. Herein, we describe the two Japalura lineages as new species, Japalura laeviventris sp. nov. and Japalura iadina sp. nov.. In addition, we provide updated conservation assessments for the new species as well as imperiled congeners according to the IUCN criteria for classification, discuss the importance of color patterns in the diagnosis and description of species in the genus Japalura, and discuss directions for future taxonomic studies of the group.

**Keywords:** Coloration; Conservation; Hengduan Mountains; Hydropower development; *Japalura flaviceps* 

#### INTRODUCTION

The family Agamidae, a radiation of more than 300 species of iguanian lizards, is one of the most taxonomically diverse lizard groups in Eurasia, with members of the family pos-

sessing a wide range of morphologies and life history traits (Manthey, 2010; Moody, 1980; Townsend et al., 2011). Due to this large variation in phenotype, agamid lizards represent a great system for comparative studies in multiple fields of biology, including phylogeography (Macey et al., 1998; Melville et al., 2009; Moody, 1980), ethology (Kastle & Schleich, 1998; Murphy et al., 1978; Qi et al., 2011; Wei & Lin, 1981), and evolutionary biology (Grismer, 2010; Schulte et al., 2002; Stuart-Fox & Ord, 2004). Across mainland Asia, China harbors the greatest diversity of agamid lizards, currently possessing 52 currently recognized species in the country representing four subfamilies (Ananjeva et al., 2011). Of this diversity, nearly half (25 species) are believed to be endemic to China, including majority of the enigmatic Mountain Dragons of the genus Japalura Gray, 1853 (Ananjeva et al., 2011; Wang et al., 2015).

To date, the genus *Japalura* is composed of 30 species, distributed across much of Asia (Wang et al., 2015). The vast majority of this diversity occurs in the territory of Greater China (16 species found in Mainland China, five species on the islands of Taiwan; Ota et al., 1998; Wang et al., 2015). Until recently, *Japalura flaviceps* Barbour & Dunn, 1919 was recognized as having the widest geographic range in China, with populations distributed from eastern Tibet to Gansu Province in central China (Pope, 1935; Zhao et al., 1999). However, subsequent examinations of morphological variation among populations of *J. flaviceps* not only revealed the widespread species to be a complex of distinct evolutionary lineages, but also restricted the range of true *J. flaviceps* to the valleys of the Dadu River in northwestern Sichuan only (Gao & Hou, 2002; Li et al.,

Received: 12 November 2015; Accepted: 10 December 2015
Foundation items: This work was supported by the Ministry of Science
and Technology of China (2014FY210200, 2011FY120200) and the
Animal Branch of the Germplasm Bank of Wild Species of Chinese
Academy of Sciences (the Large Research Infrastructure Funding).

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DOI: 10.13918/j.issn.2095-8137.2016.1.41

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2001; Manthey et al., 2012; Wang et al., 2015). Furthermore, Manthey et al. (2012) and Wang et al. (2015) suggested that unexamined populations of *J.* cf. *flaviceps* outside of the Dadu River drainage may represent additional, unique evolutionary lineages within the species complex, worthy of additional systematic study.

During herpetofaunal surveys of eastern Tibet in 2013 and of northwestern Yunnan in 2015, we captured eight and 12 specimens of lizards in the upper Nujiang (=Salween) Valley, eastern Tibet, China, and the upper Lancang (=Mekong) Valley, northwestern Yunnan, China, respectively. All individuals were identified to the genus Japalura. Although these populations are considered to be part of the J. flaviceps species complex, they can be distinguished readily from true J. flaviceps sensu Manthey et al. (2012) and other members of the genus by suites of distinct morphological characters. In this study, we describe these populations as two distinct, new species within the genus. We review the taxonomic history of the J. flaviceps species complex, comment on the importance of coloration for species delimitation in the genus and discuss directions for future taxonomic studies of the group. Additionally, we assess the new species and other imperiled congeners against the IUCN criteria for classification (IUCN, 2013).

#### **MATERIALS AND METHODS**

Eight specimens of one new species were collected from the upper Nujiang Valley near the Nujiang Bridge at Baxoi, Qamdo Prefecture of eastern Tibet, China, including two adult males, four adult females, and two juveniles. Twelve specimens of a second new species were collected from the upper Lancang Valley at Ninong, Deqin, northwestern Yunnan, China, including 11 adult males and one adult female.

Following euthanasia, tissue samples were taken from livers and preserved in 95% ethanol, and voucher specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol for long-term preservation. With the exception of a single female specimen collected from Tibet that possesses an incomplete tail (KIZ 014040), all adult specimens were chosen as the type series. All specimens (including KIZ 014040) are deposited in the Museum of Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Measurements were made with digital calipers to the nearest 0.1 mm, except for snout-vent length (SVL) and tail length (TAL), which was made with a ruler to the nearest 1 mm. With the exception of several new traits measured in this study, focal characters and character definitions follow Wang et al. (2015): snout-vent length (SVL), tail length (TAL), head width (HW), snout-eye length (SEL), fore-limb length (FLL), hind limb length (HLL), supralabial count (SL), infralabial count (IL), middorsal scale (MD), Toe IV subdigital lamellae (T4S), Toe IV length (T4L), trunk length (TRL), interorbital distance (IOD), number of scales between nasal and first supralabials (NSL), supraciliary count (SCL), and number of scale rows between sixth supralabial and orbit circle (SOR). Additionally, in this study we examined the following morphometric characters (definitions provided after colon): enlarged, conical, post-tympanic scale

count (PTY): large conical scales posterior to tympanum; and enlarged, conical, post-rictal scale count (PRS): large conical scales posterior to the rictus. Values for paired characters (SL, IL, NSL, SOR) were recorded from both sides of the body, with counts provided in left/right order.

Summaries of specimens examined are listed in Appendix I. For comparisons, morphological data of the following phenotypically similar species were collected from vouchered specimens (from type or topotype specimens when available): J. batangensis, J. dymondi, J. micangshanensis, J. flaviceps, J. splendida, J. varcoae, J. vela, J. yunnanensis, and J. zhaoermii. Currently, with recognized populations of J. splendida distributed across multiple distinct zoogeographic regions of China (Xie et al., 2004; Zhao et al., 1999), and known to possess considerable variability in morphological characters (Manthey et al., 2012; Yang & Rao, 2008; Zhao et al., 1999), it is likely that the widespread species represents a complex of unique evolutionary lineages. Therefore, to avoid unnecessary confusion in drawing comparisons with potentially unique but undescribed diversity, we include morphological data from the type specimen of J. splendida as well as specimens from localities geographically proximate to the species' type locality along the Yangtze River (e.g. Chongqing). Morphological data of the following species were obtained from the literature: J. brevicauda (Manthey et al., 2012), J. kumaonensis (Schleich & Kästle, 2002), J. luei (Ota et al., 1998), J. makii (Ota, 1989), and J. yulongensis (Manthey et al., 2012).

Comparisons of coloration in life are based on type descriptions and available color photographs (Manthey, 2010; Yang & Rao, 2008; Zhao et al., 1999). Museum abbreviations for specimens examined follow Sabaj Perez (2015), and include: Chengdu Institute of Biology, Chinese Academy of Sciences (CIB); Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ); Museum of Comparative Zoology at Harvard University (MCZ), Boston, MA, USA; and National Museum of Natural History (USNM), Washington D.C., USA.

The topographic map shown in Figure 1 was created by N. A. Huron in ArcMap v.10.3.1 using the digital elevation model (DEM) layers based on NASA's Shuttle Radar Topographic Mission (SRTM). The SRTM data are available for free at approximately 90 meters resolution (3 arc-second projections; Reuter et al., 2007; CIAT-CSI SRTM, 2015).

#### **RESULTS**

Japalura laeviventris sp. nov. Wang, Jiang, Siler, and Che (Figures 1-4)

**Synonyms** 

Japalura flaviceps Pope, 1935: 467; Zhao & Jiang, 1977: 293-298; Hu et al., 1987: 112; Zhao et al., 1999: 111-115; Li et al., 2010: 115.

**Holotype**: KIZ 014038, adult male, collected near the Nujiang Bridge in the upper Nujiang Valley at Baxoi (=Basu), Qamdo (=Changdu), eastern Tibet (=Xizang), PR China (N30.10034°, E97.22787°, 2 739 m elevation); collected by Ke JIANG on 3 July 2013.

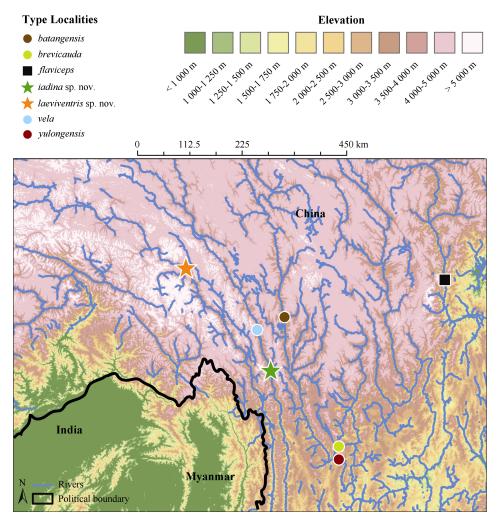


Figure 1 Distribution map of *Japalura* in the Hengduan Mountain Range, southwest China (Map created by Nicholas A. HURON and Cameron D. SILER)

Color-coded shapes show the distribution of type localities for the new species (stars), true *J. flaviceps* sensu Wang et al. (2015) (square), and other referenced members of the *J. flaviceps* Species Complex (circles).

**Paratopotypes**: One adult male (KIZ 014037) and three adult females (KIZ 014041-43); collected by Ke JIANG, Kai WANG, and Ya-Qiang SUN.

**Diagnosis:** Following Inger's definition of the genus (Inger, 1960), the new species is assigned to the genus *Japalura* based on a number of diagnostic characters, including: (1) dorsal scales unequal in size; (2) enlarged crest scales present; (3) gular pouch present; (4) lateral fold of skin in axilla-groin region present; (5) supraciliary scales greatly imbricate; (6) head relatively long, flat; (7) tail long, slender; (8) tail cylindrical in shape; and (9) precloacal and femoral pores absent.

Japalura laeviventris sp. nov. can be distinguished from all congeners by the combination of the following suite of morphological characteristics: (1) small adult body size (SVL 67-72 mm in males, 64-70 mm in females); (2) moderate TAL (TAL/SVL 168%-200%); (3) moderate HLL (HLL/SVL 64.3%-78.4%); (4)

NSL 1; (5) T4S 22-26; (6) SOR 3; (7) strongly-protuberant, conical, post-tympanic scale absent; (8) strongly-protuberant, conical, post-rictal scale absent; (9) tympanum concealed; (10) nuchal crests relatively raised on weak skin folds; (11) dorsal crests weakly developed without distinct skin folds in males; (12) transverse gular fold present; (13) gular pouch distinct, present; (14) scales of ventral surface of body smooth or weakly keeled; (15) MD 57-59, (16) ground dorsal coloration off-white in males, brownish-gray in females; (17) dorsal, lateral, and ventral surface of head, dorsal forelimbs, and lateral surface of body speckled with black; (18) distinct radial streaks around eyes; (19) dorsolateral stripes present, smooth-edged, pale-yellow in males; (20) dark-brown, "M"-shaped pigmentation patterns along dorsal midline in males; and (21) small, triangular, orange gular spots in adults of both sexes.

Description of holotype: Adult male, SVL 67 mm, TAL 133 mm,

FLL 30.6 mm, HLL 49.8 mm, HW 15.5 mm, HL 21.3 mm. Rostral rectangular, three times broader than high, in contact with six small scales excluding supralabial. Nasal sub-circular, bordered by 10/9 small scales. Single scale between rostral and first supralabial. Supralabials 7/8, smooth, posteriormost longest. Loreal scales irregularly arranged and weakly keeled. Ciliaries circular, much smaller than other dorsal scales, forming orbit circle; supraciliaries 9/11, prominent, elongated, above orbit; first 5/7 supraciliaries overlapping one-half to two-third of its length with subsequent ones, last four slightly overlapping; three rows of scales between orbit circle and sixth supralabials on both sides of head, all weakly keeled, with scales of middle row largest. Scales posterior to eyes strongly keeled; five enlarged scales between orbit and tympanum on both sides; tympanum covered with much smaller scales. Dorsal head scales heterogeneous in size, approximately circular shaped, mildly keeled, convex, somewhat granular in appearance; single row of five scales forming a weak ridge along snout midline from one scale posterior rostral to mid-point between anterior corner of eyes; parietal smooth, enlarged, with a distinct pineal eyespot; post-occipital and posterior lateral head scales strongly keeled; conical scale 1/0 on occipital region, weakly developed; no large conical scales posterior to tympanum or rictus.

Ventral head scales homogeneous in size, mostly smooth, weakly keeled posterolaterally; transverse gular fold distinct, well developed; gular pouch present; shoulder fold posterior to gular fold on each side, from ventral surface of neck to just about 5 mm above pectoral joint; axillary fold present on each side of body. Middorsal crest scales 59, roughly equal in size to neighboring scales, imbricate along body midline; nuchal crest relatively raised on skin fold, while dorsal crest weakly developed without skin folds. A row of enlarged and distinctively keeled scales running parallel to dorsal crest from pectoral region of body to pelvis on each side of crest above dorsolateral stripes. Ground dorsal scales heterogeneous in size; scales of axilla much smaller than remaining dorsals; large, distinct, flat scales distributed irregularly across dorsal surface of body, circular in shape, roughly four times larger than ground scales, at times arranged in proximally transverse rows. Dorsal limb scales distinctively keeled, roughly equal in size to ventral scales, homogeneous in size on forelimbs, heterogeneous on hind limbs; Toe IV subdigital lamellae 25/24. Tail scales keeled in lateral rows; cloaca scales small. Ventral scales of body and limbs mostly homogeneous in size, smooth or weakly keeled close to lateral sides.

Coloration of holotype in life: In life, the dorsal surface of the head is light yellowish, speckled with small black scales, of which some connect to form dark X-shaped patterns. Two of these X-shaped patterns are observed on the snout, and another can be observed on the dorsal surface of the head between the orbits. The lateral surface of the head is off-white and is also speckled with black scales. Some black scales form radial stripes around the eyes, with the stripes directed posteriorly being the broadest. The ground coloration of the dorsal and lateral surfaces of the body is off-white. Smoothedged, pale-yellow dorsolateral stripes run along the entire length of the body on each side of the vertebral crest. Black vermiculate pigmentation patterns are present on the lateral

surfaces of the body, beneath and proximate to the dorsolateral stripes. A series of three V-shaped brownish-black patterns of dark pigmentations are observed on the dorsal surface of the body, distributed from the occipital region of the head to the pectoral joint of the body along the dorsal midline. These shapes are followed posteriorly by a series of four brownish-black, M-shaped pigmentations, of which the last one is gradually faded and somewhat unclear. Numerous brownish-gray, transverse bands are present on the posteriormost two-thirds of the tail. The bands do not form complete rings around the tail ventrally. The dorsal surfaces of the forelimbs are off-white and speckled with black scales. The black scales in this region are connected in places by gray-blotched scales, and together, these colors create irregular transverse bands of dark coloration. The dorsal surfaces of the hind limbs are light gray, with dark-gray transverse patterns similar to those observed on the fore-limbs. The ground coloration of the ventral surface of the head is uniform white, but heavily speckled with black scales. Sporadically, small series of these black scales from short, longitudinal, vermiculate stripes. A triangular, bright orange gular spot is present in the center of the gular pouch. The ventral surfaces of the body, limbs, and tail are uniform white, except for the ventral surfaces of the hands and feet, which are more yellowish (Figure 2).

Coloration of holotype in preservative: The coloration of the holotype in preservative closely matches its coloration in life; however, the following differences are observed: (1) the ground coloration of the dorsal surface of the head as well as the coloration of the dorsolateral stripes is light gray, and (2) the triangular orange gular spot turns white (Figure 3).

Variation and sexual dimorphism: Variations in pholidosis and measurements are summarized in Table 1. The male paratopotype (KIZ 014037) is slightly darker in dorsal coloration than the holotype in preservative. Females are sexually dimorphic from males by having shorter snouts (SEL/HL), less speckled ventral surfaces of the heads (v.s. heavily speckled), more consistently arranged transverse rows of enlarged scales on the dorsal surfaces of the body (v.s. irregularly distributed), smaller orange gular spots (v.s. larger), much more distinct transverse patterns of pigmentations at bases of the tails (v.s. less distinct), as well as by the absences of M-shaped dark pigmentation patterns along the dorsal midline of the body (v.s. presence), and the presence of dark transverse bands on the dorsal surface of the body that extend to the lateral surfaces of the body (v.s. absence). Yellow dorsolateral stripes are absent in most females, except for a single individual (KIZ 014043), which has wavy dorsolateral stripes. Juveniles (KIZ 014039, 014044) closely resemble adult females, except for the absence of orange gular spots. A single juvenile (KIZ 014039) was observed to possess a light-gray, dorsal, vertebral stripe from the pectoral region of the body to pelvis. This trait was not observed in any adult females.

**Comparisons:** Populations of the new species were identified previously as *J. flaviceps*. However, the new species can be



Figure 2 Lateral and ventral views of adult male holotype KIZ 014038 (A and B) and adult female paratopotype KIZ 014043 (C and D) of *Japalura laeviventris* sp. nov. in life (Photos by Kai WANG)

Note the dark reddish orange color posterior to the shoulder fold in the lateral view is the color of ectoparasites and not a coloration pattern of the new species.

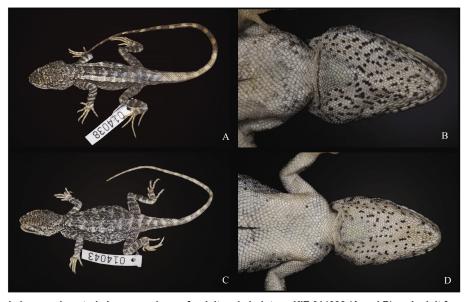


Figure 3 Dorsolateral views and ventral close-ups views of adult male holotype KIZ 014038 (A and B) and adult female paratopotype KIZ 014043 (C and D) of *Japalura laeviventris* sp. nov. in preservative (Photos by Kai WANG)

distinguished readily from the latter by having smooth or weakly keeled scales on the ventral surface of the head and the body (v.s. distinctively keeled), a greater number of MD (57-59 v.s. 43-48), heavily speckled ventral surfaces of the head, with speckles rarely forming short lines (v.s. speckles absent, but broad, dark stripes present and interconnected into a mosaic pattern), as well as by the absence of strongly-protuberant, conical, post-rictal scale (v.s. presence), presence of X-shaped

patterns of dark pigmentation on the dorsal surface of the head (v.s. absence, or presence of few transverse streaks), presence of distinct radial patterns around the eyes (v.s. absence), presence of M-shaped patterns of dark pigmentation along the dorsal midline between the two dorsolateral stripes in males (v.s. rhombus-shaped patterns with distinct yellow centers), and presence of distinct orange gular spots in both sexes (v.s. absence in both sexes).

Table 1 Meristic and mensural data of the type series of Japalura laeviventris sp. nov.

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No.	Status	Sex	SVL	TAL	TAL/SVL	HL	HL/SVL	۸L	HW	HW/SVL	SEL	SEL/HL	)	IOD
KIZ 014038	Holotype	Male	29	133	1.99	21.3	0.32		15.5	0.23	7.1	0.33	6	9.8
KIZ 014037	Paratopotype	Male	72	142	1.97	22.7	0.32		16.3	0.23	8.0	0.35	_	10.9
		Range	67-72	133-142	1.97-1.99	21.3-22.7	.7 0.32		15.5-16.3	0.23	7.1-8.0	0.33-0.35		9.8–10.9
		Average	20	138	1.98	22.0	0.32		15.9	0.23	7.6	0.34	_	10.4
KIZ 014041	Paratopotype	Female	64	118	1.84	18.2	0.28		12.4	0.19	6.5	0.36	6	0.0
KIZ 014042	Paratopotype	Female	70	128	1.83	20.6	0.29		13.7	0.20	7.0	0.34	6	8.6
KIZ 014043	Paratopotype	Female	70	118	1.69	20.2	0.29		13.5	0.19	9.7	0.38	_	10.2
		Range	64-70	118-128	1.69-1.84	18.2-20.6	.6 0.28-0.29	0.29	12.4-13.7	0.19-0.20	6.5-7.6	0.34-0.38		9.0-10.2
		Average	89	121	1.79	19.7	0.29		13.2	0.19	7.0	0.36	6	9.7
No.	FLL	FLL/SVL	HLL	HLL/SVL		T4L	TRL	SL	-	NSL	MD	T4S	SCL	SOR
KIZ 014038	30.6	0.46	49.8	0.74		12.3	30.7	8/2	6/6	1/1	59	25/24	10/10	3/3
KIZ 014037	32.0	0.44	50.7	0.70		12.4	35.4	8/8	11/11	1/1	57	25/25	9/10	3/3
	30.6-32.0	0.44-0.46	49.8-50.7	.7 0.70-0.74		12.3-12.4	30.7-35.4	7 or 8	9-11	_	57-59	24 or 25	9 or 10	ဇ
	31.3	0.45	50.3	0.72		12.4	33.1	<b>∞</b>	10	_	58	25	9.8	က
KIZ 014041	30.3	0.47	47.4	0.74	_	10.3	33.3	6/6	6/6	1/1	59	22/24	10/10	3/3
KIZ 014042	30.3	0.43	50.1	0.72	_	12.5	35.7	8//	8/7	1/1	58	23/23	10/10	3/3
KIZ 014043	31.0	0.44	44.4	0.63	_	11.0	33.3	8/8	2//8	1/1	58	23/24	8 or 9	3/3
	30.3-31.0	0.43-0.47	44.4-50.1	.1 0.63-0.74		10.3-12.5	33.3-35.7	7-9	7-9	_	58 or 59	22-24	8-10	ဇ
	30.5	0.45	47.3	0.70		11.3	34.1	8.3	8	-	58	23	10	3

Summary statistics are provided for male and female specimens separately. For average calculation of paired measurements, each one of the paired measurements is treated independently. Measurements of SVL, TAL, HL, HW, T4L, TRL, FLL, and HLL are in millimeters (mm), all meristic data are given in decimals, and paired meristic characters are given in left/right order. Abbreviations: SVL: snout-vent length; TAL: tail length; HL: head length; HV' head width; SEL: snout-eye length; IOD: interorbital distance; FLL: fore-limb length; HLL: hind limb length; T4L: Toe IV length; TRL: trunk length; SL: supralabial count; IL: infralabial count; NSL: number of scales between nasal and first supralabials; MD: middorsal scale; T4S: Toe IV subdigital lamellae; SCL: supraciliary count; SOR: number of scale rows between sixth supralabial and orbit circle.

Table 2 Meristic and mensural data of the type series of Japalura iadina sp. nov.

2	0,1010	300	770	-<-	1/\0/ IV ±	5	1707	747		IIVO//O/I	100	17/12/0	2
.02	Status	067	GVL	<u> </u>	IAE/SVE	=	IIII/OVE			IVWOVL	3EL	SELITE	20
KIZ 019321	Holotype	Male	09	116	1.95	18.5	0.31	12.4	J	0.21	6.9	0.38	9.1
KIZ 019328	Paratopotype	Male	63	118	1.89	20.0	0.32	13.4	J	0.21	7.0	0.35	10.1
KIZ 09402	Paratopotype	Male	58	102	1.78	17.0	0.30	11.9	J	0.21	6.3	0.37	8.2
KIZ 09403	Paratopotype	Male	62	122	1.98	20.0	0.32	13.9	J	0.22	7.8	0.39	10.0
KIZ 019322	Paratopotype	Male	62	1	1	20.3	0.33	13.7	J	0.22	7.8	0.38	6.6
KIZ 019327	Paratopotype	Male	54	26	1.80	16.9	0.31	10.9	J	0.20	6.2	0.37	7.9
KIZ 019320	Paratopotype	Male	63	109	1.73	19.3	0.31	13.0	J	0.21	7.5	0.39	0.6
KIZ 019326	Paratopotype	Male	64	I	I	19.7	0.31	13.6	J	0.21	7.0	0.36	0.6
KIZ 019325	Paratopotype	Male	64	I	I	19.7	0.31	13.1	J	0.21	7.0	0.36	10.0
KIZ 09401	Paratopotype	Male	99	110	1.98	17.1	0.31	11.7	J	0.21	6.9	0.40	8.7
KIZ 019323	Paratopotype	Male	65	127	1.96	20.6	0.32	13.7	J	0.21	7.2	0.35	9.4
		Range	54-65	97–127	1.73–1.98	16.9–20.6	3 0.30-0.33	33 10.9–1	3.9	0.20-0.22	6.2-7.8	0.35-0.40	7.9–10.1
		Average	61	113	1.88	19.0	0.31	12.8	J	.21	7.1	0.37	9.2
KIZ09398	Allotype	Female	62	107	1.73	19.0	0.31	12.8	J	0.21	6.7	0.36	8.8
No.	FLL	FLL/SVL	HLL	HLL/SVL	T4L	TRL	SL	-	NSL	MD	T4S	SCL	SOR
KIZ 019321	28.8	0.48	44.3	0.74	10.3	28.3	9/10	9/10	1/1	40	20/21	9/9	3/3
KIZ 019328	28.6	0.46	43.6	0.70	10.5	28.8	2/8	10/10	1/1	35	20/23	2/9	3/3
KIZ 09402	26.3	0.46	41.4	0.72	9.3	24.1	9/10	10/11	0/1	40	23/22	9/9	3/3
KIZ 09403	30.5	0.49	49.6	0.80	11.7	29.4	8/6	9/10	1/1	42	22/22	2/9	3/4
KIZ 019322	29.9	0.48	45.4	0.73	11.7	28.0	8/8	6/6	2/1	44	24/23	1/1	3/3
KIZ 019327	25.1	0.46	41.5	0.77	9.3	24.5	9/10	10/11	1,1	44	22/22	9/9	3/3
KIZ 019320	29.5	0.47	48.7	0.77	14.4	28.5	8/8	10/8	1/1	46	22/22	9//	3/3
KIZ 019326	30.2	0.47	47.5	0.74	10.6	29.2	6/8	9/10	0/0	40	20/21	9/9	3/3
KIZ 019325	28.9	0.45	48.3	92.0	10.9	30.4	8/8	9/10	1/1	46	21/21	9/9	3/3
KIZ 09401	27.0	0.49	41.2	0.74	10.4	25.3	6/8	9/10	0/1	39	21/21	9/9	4/4
KIZ 019323	30.5	0.47	6.03	0.78	11.3	30.2	8/8	9/10	1,1	45	21/21	9/9	3/3
	25.1–30.5	0.45-0.49	41.2–50.9	0.70-0.80	9.3-14.4	24.1–30.4	7–10	8–11	0 or 1	35-46	20–23	6 or 7	3 or 4
	28.7	0.47	45.7	0.75	10.9	27.9	8	10	_	42	22	9	3
KIZ 09398	28.5	0.46	45.6	0.74	9.7	30.3	2/8	6/6	1/1	41	19/19	1/1	3/3

For measurements and calculation details see Table 1. Summary statistics are provided for male and female specimens separately. Abbreviations: SVL: snout—vent length; TAL: tail length; HL: head length; HW: head width; SEL: snout—eye length; IOD: interorbital distance; FLL: fore-limb length; HLL: hind limb length; T4L: Toe IV length; TRL: trunk length; SL: supralabial count; IL: infralabial count; NSL: number of scales between nasal and first supralabials; MD: middorsal scale; T4S: Toe IV subdigital lamellae; SCL: supraciliary count; SOR: number of scale rows between sixth supralabial and orbit circle.

Table 3 Morphological comparisons of Japalura laeviventris sp. nov., J. iadina sp. nov., and phenotypically similar congeners.

200	noiogicai c	11164113	morphiological companisons of oaparata factivents sp. nov., o. factira sp. nov., and pricincy preaty similar congeners.		1 1 1		- fd frad p					
Sex	iaeviventris sp. nov.	sp. nov.			ladina sp. nov	  -			parangensis			
	Male <i>n</i> =2		Female <i>n</i> =3		Male <i>n</i> =11		Female <i>n</i> =1		Male <i>n</i> =6		Female <i>n</i> =5	
SL	7 or 8	8	7–9	8	7–10	6	7 or 8	80	9–11	10	9–11	10
_	9–11	10	7–9	80	8–11	10	6	6	9–12	7	9–12	10
SCL	9 or 10	10	8–12	10	6 or 7	9	7	7	7–9	œ	7 or 8	8
NSL	_	_	_	-	02	_	_	_	1 or 2	2	1 or 2	2
SOR	က	က	က	3	3 or 4	က	က	က	3 or 4	3	3 or 4	4
MD	57–59	58	58 or 59	28	35–46	42	41	41	39–54	44	40-44	42
PTY	0	0	0	0	1–3	_	2	2	1–3	2	0-2	2
PRS	0	0	0	0	1 or 2	2	1 or 2	2	0	0	0	0
T4S	24 or 25	25	22–24	23	20–24	22	19	19	20–22	21	21–24	22
SVL	67–72	69.5	64-70	0.89	54-65	61.0	61.8	61.8	50–62	58.5	53-64	58.6
TAL	133–142	137.5	118–128	121.3	97–127	112.6	107.1	107.1	85–115	105.6	95–111	104.7
TRL	30.7-35.4	33.1	33.3–35.7	34.1	24.1–30.4	27.8	30.3	30.3	20.5–30.3	26.7	25.3–31.7	28.2
로	21.3–22.7	22.0	18.2–20.6	19.7	16.9–20.6	19.0	19.0	19.0	16.0–19.3	17.8	17.2–19.4	18.3
MΗ	15.5–16.3	15.9	12.4–13.7	13.2	10.9–13.9	12.8	12.8	12.8	11.4–13.9	12.8	12.5–14.8	13.4
SEL	7.1–8.0	9.7	6.5-7.6	7.0	6.2–7.8	7.0	6.7	6.7	6.1–7.6	8.9	6.4–7.6	6.9
ОО	9.8-10.9	10.4	9.0-10.2	9.7	7.9–10.1	9.2	8.8	8.8	8.0-9.7	8.8	7.9–9.4	8.6
FLL	30.6–32.0	31.3	30.3-31.0	30.5	25.1–30.5	28.5	28.5	28.5	21.7–29.7	56.6	25.9–30.8	27.8
HLL	49.8–50.7	50.3	44.4-50.1	47.3	41.2–50.9	45.7	45.6	45.6	38.5-44.8	42.3	42.0-48.8	44.4
T4L	12.3–12.4	12.4	10.3–12.5	11.3	9.3–14.4	11.1	9.7	9.7	8.6–11.4	10.2	9.6–11.4	10.4
TRL/SVL	0.46-0.49	0.48	0.48-0.52	0.50	0.42-0.48	0.46	0.49	0.49	0.41-0.51	0.46	0.46-0.49	0.47
TAL/SVL	1.97-1.99	1.98	1.69–1.84	1.79	1.73-1.97	1.87	1.73	1.73	1.65-1.95	1.81	1.64–2.04	1.84
HL/SVL	0.32	0.32	0.28-0.29	0.29	0.29-0.33	0.31	0.31	0.31	0.29-0.32	0.3	0.29-0.32	0.31
HW//HL	0.72-0.73	0.73	0.67-0.68	0.67	0.64-0.70	0.67	0.68	0.68	0.68-0.77	0.72	0.71-0.77	0.73
SEL/HL	0.33 - 0.35	0.34	0.34-0.38	0.36	0.35-0.40	0.37	0.36	0.36	0.35-0.41	0.38	0.35-0.40	0.37
IOD/HL	0.46-0.48	0.47	0.48-0.50	0.49	0.46-0.51	0.49	0.46	0.46	0.45-0.53	0.5	0.45-0.50	0.47
FLL/SVL	0.44-0.46	0.45	0.43-0.47	0.45	0.45-0.49	0.47	0.46	0.46	0.43-0.50	0.45	0.42-0.51	0.47
HLL/SVL	0.70-0.74	0.72	0.63-0.74	0.70	0.69-0.80	0.75	0.74	0.74	0.69–0.77	0.72	0.68-0.80	0.75
T4L/HLL	0.24-0.25	0.25	0.22-0.25	0.24	0.22-0.30	0.24	0.21	0.21	0.22-0.26	0.24	0.23-0.25	0.24
VSK	٧		⋖		۵		۵		۵		۵	
TGF	۵		۵		۵		۵		۵		۵	
GS	۵		۵		۵		<u>а</u>		۵		S	
CGS	Orange		Orange		Blue		Yellowish green		Bluish green		Bluish green	
TSDH	۵		۵		۵		۵		۵		۵	
RPAE	۵		۵		۵		۵		۵		۵	
DSVH	۵		۵		⋖		4		۷		⋖	
NSVH	Ь		Ь		Ь		Ь		S		S	

												Continued
X-DX	flaviceps				micangshanensis	ısis			splendida			
Yan	Male <i>n</i> =5		Female <i>n</i> =3		Male <i>n</i> =6		Female <i>n</i> =3		Male <i>n</i> =4		Female <i>n</i> =2	
SL	9–11	10	9 or 10	10	6–10	8	7 or 8	7	6-2	8	8 or 9	8
_	10 or 11	7	10 or 11	7	6-2	8	8 or 9	6	7–9	8	9 or 10	10
SCL	7 or 8	8	7 or 8	7	7 or 8	7	7	7	7 or 8	7	7	7
NSL	1 or 2	_	1 or 2	2	_	_	_	_	0 or 1	0	_	_
SOR	4	4	4	4	က	3	က	က	ဗ	3	က	3
MD	43-48	45	43-45	44	38-43	40	37–42	40	44–48	45	46–48	47
ΡΤΥ	2–5	3	2–5	4	3–8	4	1–3	က	1 or 2	2	4	4
PRS	4-7	5	2–6	4	3–7	5	4	က	0-2	2	2–5	4
T4S	23–24	24	22–24	23	23–27	25	23–26	25	24–28	27	25–27	26
SVL	72–75	74.0	64-73	68.9	55–67	60.1	57–69	63.6	59–92	76.1	64–81	72.8
TAL	122–144	135.2	108–131	121.4	112–143	124.8	114–133	123.7	152–225	187.8	140–181	160.4
TRL	31.9–36.1	33.9	30.6–35.1	32.6	24.0–29.6	26.2	30.1–32.9	31.3	26.1-40.3	31.9	32.3-42.0	37.2
로	22.6–23.7	23.1	18.0–20.9	19.9	16.6–20.0	18.1	15.1–20.2	17.9	18.0–28.8	23.5	19.3–23.8	21.6
MH	16.7-17.4	17.1	13.2–15.3	14.5	11.2–14.1	12.7	11.8–14.8	13.1	12.8–22.0	17.0	13.1–15.4	14.2
SEL	8.8–10.1	9.6	7.8-8.7	8.4	5.9-8.0	7.2	6.9–7.7	7.4	7.3–11.1	0.6	8.2–10.1	9.2
ОО	10.4–10.9	10.7	9.0–10.4	6.6	8.3-10.4	9.5	8.6-10.4	9.5	9.7–13.3	11.2	9.6–11.9	10.8
FLL	29.3–31.5	30.4	26.5–30.2	27.9	26.0–29.7	27.6	25.0–29.0	26.7	25.3-44.2	34.6	29.0–36.9	32.9
HLL	46.5–52.6	9.09	45.1–47.7	46.4	43.9–52.1	46.8	43.7–46.6	44.8	47.4–73.8	61.0	44.9–62.3	53.6
T4L	12.3–13.5	12.8	10.7–12.2	11.6	11.7–12.9	12.4	10.8–12.8	11.6	13.8–17.8	15.4	11.4–15.4	13.4
TRL/SVL	0.44-0.49	0.46	0.44-0.50	0.47	0.41-0.48	0.44	0.47-0.53	0.49	0.33-0.45	0.41	0.50 - 0.52	0.51
TAL/SVL	1.70–1.91	1.82	1.67–1.82	1.76	1.92–2.22	2.08	1.79–2.04	1.95	2.39–2.58	2.47	2.17–2.23	2.20
HL/SVL	0.30-0.32	0.31	0.28-0.30	0.29	0.30-0.31	0.30	0.27-0.29	0.28	0.29-0.32	0.31	0.29-0.30	0.30
HW/HL	0.72-0.76	0.74	0.72-0.73	0.73	0.68-0.72	0.70	0.70-0.78	0.74	0.66-0.76	0.71	0.65-0.68	99.0
SEL/HL	0.39-0.44	0.41	0.41-0.43	0.42	0.36-0.42	0.40	0.38-0.45	0.41	0.31-0.44	0.38	0.42	0.42
IOD/HL	0.45-0.48	0.47	0.49-0.50	0.50	0.50 - 0.55	0.53	0.51-0.57	0.53	0.39-0.54	0.47	0.5	0.50
FLL/SVL	0.39-0.44	0.41	0.39-0.41	0.40	0.44-0.51	0.47	0.40-0.44	0.42	0.42-0.48	0.45	0.45	0.45
HLL/SVL	0.65-0.71	0.68	0.62-0.74	0.68	0.74-0.85	0.78	0.68-0.77	0.71	0.79-0.81	0.80	0.70-0.77	0.73
T4L/HLL	0.23-0.27	0.25	0.22-0.27	0.25	0.24-0.28	0.27	0.24-0.27	0.26	0.25-0.29	0.27	0.25	0.25
VSK	۵		۵		Ъ		۵		۵		۵	
TGF	۵		۵		۷		4		۵		۵	
GS	∢		⋖		۷		⋖		∢		⋖	
CGS	∢		⋖		⋖		⋖		∢		⋖	
TSDH	S		S		۵		Ъ		۵		۵	
RPAE	⋖		∢		Д		Ъ		۵		۵	
DSVH	⋖		⋖		⋖		⋖		S		⋖	
NSVH	Ь		Ь		Α		Α		Ь		Р	

												500
<b>X</b>	vela				yunnanensis	"			zhaoermii			
200	Male <i>n</i> =9		Female <i>n</i> =2		Male <i>n</i> =5		female <i>n</i> =3		Male <i>n</i> =5		Female <i>n</i> =2	
SL	7-10	6	6	6	8-9	8	7 or 8	80	9 or 10	10	8-10	6
_	9-11	10	10 or 11	1	6 or 9	7	8-10	6	9-11	10	9 or 10	6
SCL	7	7	7 or 8	8	6-2	8	6-2	80	6 or 7	7	7	7
NSL	1 or 2	_	1 or 2	2	0	0	0	0	_	_	_	_
SOR	3 or 4	က	ဗ	က	2 or 3	2	2-4	3	3 or 4	က	3-5	4
MD	41-47	44	47-51	49	43-45	44	39-46	14	41-45	43	40-42	41
ΡΤΥ	2 or 3	3	0-3	2	0	0	0-2	_	2-5	3	3 or 4	4
PRS	0	0	0	0	_	_	1 or 2	_	1-3	_	_	_
T4S	24 or 25	24	24 or 25	25	27-29	27	29-31	30	24 or 25	25	24 or 25	25
SVL	56–69	62.1	59-66	62.5	26-70	63.6	69-71	71.2	22-24	72.5	60-64	61.9
TAL	110–136	123.1	109-123	116.0	145-202	177.2	175-181	178.3	128-151	138.3	94-97	95.6
TRL	25.3-33.0	28.8	30.2-32.7	31.5	19.0-20.9	19.8	20.2-22.9	21.5	29.8-36.3	33.1	26.3-32.4	29.4
로	17.3–20.8	19.1	17.3-19.5	18.4	12.9-14.4	13.7	14.4-16.2	15.6	20.8-26.1	23.4	18.1-19.0	18.6
MH	12.2–14.5	13.5	12.4-14.0	13.2	7.9-9.3	9.8	8.2-9.6	8.8	14.7-20.3	17.1	11.7-12.9	12.3
SEL	6.5-7.7	7.1	6.4-7.1	8.9	10.1-12.4	11.7	10.8-12.0	11.3	8.5-8.9	8.7	7.0-7.4	7.2
OOI	8.4–9.6	9.1	8.8-9.1	8.9	29.9-37.1	32.4	33.1-34.8	34.0	9.9-11.3	10.6	8.6	8.6
FLL	25.5-31.5	29.2	28.0-32.0	30.0	44.5-54.7	50.1	52.9-57.2	54.9	29.1-37.1	32.1	25.4-28.8	27.1
HL	43.9-50.8	46.9	43.1-47.9	45.5	11.1-17.1	14.5	13.7-16.1	15.0	47.6-55.0	51.5	39.4-43.8	41.6
T4L	11.3–13.2	12.2	12.1-13.9	13.0	25.8-29.4	28.1	33.4-35.8	34.6	12.8-14.5	13.8	11.1-11.8	11.4
TRL/SVL	0.42-0.48	0.46	0.50-0.51	0.50	0.42-0.46	0.44	0.47-0.50	0.49	0.43-0.49	0.46	0.44-0.50	0.47
TAL/SVL	1.92–2.06	1.99	1.85-1.86	1.86	2.59-2.89	2.77	2.37-2.60	2.51	1.81-2.20	1.94	1.51-1.57	1.54
HL/SVL	0.30-0.34	0.31	0.29-0.30	0.29	0.29-0.35	0.31	0.29-0.31	0.30	0.30-0.34	0.32	0.29-0.30	0.30
HW/HL	0.68-0.72	0.71	0.72	0.72	0.66-0.72	69.0	0.71-0.76	0.73	0.62-0.80	0.72	0.62-0.71	99'0
SEL/HL	0.36-0.39	0.37	0.36-0.37	0.37	0.41-0.45	0.43	0.36-0.45	0.41	0.34-0.41	0.37	0.39	0.39
IOD/HL	0.42-0.53	0.48	0.47-0.51	0.49	0.52-0.59	0.56	0.47-0.56	0.53	0.44-0.50	0.47	0.45-0.47	0.46
FLL/SVL	0.45 - 0.50	0.47	0.47-0.48	0.48	0.46-0.54	0.51	0.46-0.49	0.48	0.41-0.48	0.44	0.43-0.45	0.44
HLL/SVL	0.72-0.81	92.0	0.73	0.73	0.78-0.80	0.79	0.74-0.81	0.77	0.67-0.79	0.72	0.66-0.68	0.67
T4L/HLL	0.24-0.29	0.26	0.28-0.29	0.29	0.25-0.31	0.29	0.25-0.29	0.27	0.25-0.29	0.27	0.27-0.28	0.28
VSK	۵		₾		۵		۵		۵		۵	
TGF	۵		۵		⋖		۷		۵		۵	
GS	⋖		⋖		۵		S		Д.		⋖	
ces	∢		⋖		Yellow		Yellow		Yellowish green		⋖	
TSDH	۵		۵		۵		۵		<u>а</u>		۵	
RPAE	۵		۵		۵		۵		<u>а</u>		۵	
DSVH	∢		⋖		∢		۷		⋖		⋖	
NSVH	۵		۵		۵		Ь		S		<u>а</u>	

scale count; T4S, Toe IV subdigital lamellae; SVL, snout-vent length; TAL, tail length; TRL, trunk length; HL, head length; HW, head width; SEL, snout-eye length; IOD, interorbital distance; FLL, fore-limb length; HLL, hind limb length; T4L, Toe IV length; VSK, ventral scales distinctively keeled; TGF, transverse gular fold; GS, gular spot; CGS, coloration of gular spot; TSDH, distinct range and of sead; RPAE, distinct radial patterns around eyes; DSVH, distinct dark speckles on ventral head; VSVH, distinct vermiculate stripes on ventral head; P. supralabials; SOR, number of scale rows between sixth supralabial and orbit circle; MD, middorsal scale; PTY, enlarged, conical, post-tympanic scale count; PRS, enlarged, conical, post-rictal For measurements and calculation details see Table 1. Abbreviations: SL, supralabial count; IL, infralabial count; SCL, supraciliary count; NSL, number of scales between nasal and first present; A: absent; S: sometimes present.

Continued

Japalura laeviventris sp. nov. is most similar to J. kumaonensis (Annandale, 1907) and J. yunnanensis Anderson, 1879 in coloration patterns. All three species possess radial patterns of dark pigmentation around the eyes and light dorsolateral stripes in males. However, the new species can be distinguished readily from the latter two by having smooth or weakly keeled scales on the ventral surfaces of the head and the body (v.s. distinctively keeled), a greater number of MD (≥57 v.s. ≤52), as we as by the absence of strongly protuberant, conical, post-tympanic scales (presence and in high numbers) and absence of strongly-protuberant, conical, post-rictal scale (v.s. presence). Additionally, Japalura laeviventris sp. nov. differs from from J. kumaonensis by having a greater number of SL (7-9 v.s. 5 or 6), a concealed tympanum (v.s. exposed), a relatively well developed gular pouch (v.s. weakly developed), M-shaped patterns along the dorsal-midline in males (v.s. chevron-shaped), the posteriorly directed radial-stripes of the eyes less prominent and short, ending before reaching the tympanums (v.s. distinct, broad, enclosing the tympanums), as well as by the presence of a transverse gular fold (v.s. absence) and presence of orange gular spots in both sexes (v.s. absence in both sexes); and from J. yunnanensis by having a shorter tail (TAL/SVL ≤200% v.s. ≥235%), fewer T4S (22-26 v.s. 27-31), greater number of NSL (1 v.s. 0), broad dorsolateral stripes with smooth edges in males (v.s. narrow and jagged), an off-white ground coloration on the dorsal surface of the body (v.s. green or brown), the terrestrial lifestyle (v.s. arboreal), as well as by the presence of a transverse gular fold (v.s. absence) and presence of orange gular spots in both sexes (v.s. light yellow gular spots in males, sometimes absence in females).

In addition to the four species compared above, the new species can be diagnosed from all remaining congeners by having smooth or weakly keeled scales on the ventral surfaces of the head and the body (v.s. distinctively keeled), an off-white ground coloration of the body (brown, black, or green), heavily speckled surfaces of the head and lateral surfaces of the body (v.s. absence or weakly speckled lateral body only), M-shaped dark patterns of pigmentations along the dorsal midline of the body between the two dorsolateral stripes in males (v.s. rectangular blotches of dark pigmentations), and the orange gular spots in both sexes in life (v.s. other colorations, in males only), as well as by the absence of large, conical post-rictal and post-tympanic scales (v.s. presence and in high numbers).

Distribution and Natural History: Japalura laeviventris sp. nov. is known only from its type locality in the upper Nujiang Valley (Figure 1); however, the species may also occur along geographically proximate stretches of Nujiang. Little vegetation exists in the Hot-and-Dry Valley (Figure 4). The new species is terrestrial, with individuals have been observed on the sandy hills alongside the river, utilizing rock crevices and rodent burrows as shelters. No larger reptiles have been recorded from the area. Males of the new species are more conspicuous in behavior than females, and can be observed basking for greater lengths of time in open habitat. In contrast, females appear to stay in closer proximity to shelters.



Figure 4 Preferred microhabitat of *Japalura laeviventris* sp. nov. near the Nujiang Bridge, Baxoi County, Qamdo Prefecture, eastern Tibet, China (Photo by Ya-Qiang SUN)

Etymology: We derive the new species name from the Latin word "laeviventris," meaning "smooth venter," in reference to one of the major diagnostic characteristics of the new species: smooth or weakly keeled ventral body scales. Suggested common name: Smooth-venter Mountain Dragon (English); Hua Fu Pan Xi (Chinese; 滑腹攀蜥).

### Japalura iadina sp. nov. Wang, Jiang, Siler, and Che (Figures 5-7) Synonyms

J. flaviceps Zhao et al., 1999: 293-298; Yang and Rao, 200: 200-201; 8; Xu and Zhang 2011: 202-203; J. splendida Xu and Zhang 2011: 202-203; J. cf. flaviceps Manthey et al., 2012

**Holotype**: KIZ 019321, adult male, collected by Kai WANG on 27 May 2015, from the Lancang Valley at Ninong, Deqin, northwest Yunnan, China (N28.370255°, E98.865287°, 2 062 m elevation).

**Allotopotype**: KIZ 09398, adult female. Collected by Da-Hu ZOU. Specimen shares the same locality and collection information as the holotype.

Paratopotypes: KIZ 09401-03, 019322, 019323, 019325-28, all adult males. Collected by Kai WANG, Ke JIANG, and Da-Hu ZOU.

**Diagnosis:** Following Inger's (1960) definition of the genus, the new species is assigned to *Japalura* based on a number of diagnostic characters, including: (1) dorsal scales unequal in size; (2) enlarged crest scales present; (3) gular pouch present; (4) lateral fold of skin in axilla–groin region present; (5) supraciliary scales greatly imbricate; (6) head relatively long, flat; (7) tail long, slender; (8) tail cylindrical in shape; and (9) precloacal and femoral pores absent.

The new species differs from all congeners by a combination of the following morphological characters: (1) gular fold present; (2) distinct gular pouch present; (3) relative hind-limb length moderate HLL/SVL 69.6%-80.1%; (4) relative tail length



Figure 5 Dorsolateral (A), ventral (B), and ventral head close-up views (C) of the adult male holotype (KIZ 019321) of *Japalura iadina* sp. nov. in life (Photos by Kai WANG)

moderate TAL/SVL 173%-198%; (5) F4S 15-17; (6) T4S 19-25; (7) MD 35-46; (8) three lateral rows of enlarged scales present on the dorsal surface of the body parallel to the dorsal crest; (9) nuchal and dorsal crests moderately raised on skin folds; (10) nuchal and dorsal crests relatively low and discontinuous in males; (11) dorsal and lateral surfaces of body emerald green in males, yellowish brown speckled with large, light yellow scales in females; (12) tail yellowish green in males, brownish in females; (13) ventral surface of body bluish or whitish gray, sometimes with black speckles, in males, uniform yellow in females; (14) two smooth-edged, light-green, dorsolateral stripes present in males; (15) vermiculate stripes on ventral surface of head present, distinct, blackish blue in males, black in females; (16) gular region dark blue in males, yellow in females.

**Description of holotype:** Adult male, SVL 60 mm, TAL 116 mm, TrL 28.3 mm, HL 18.5 mm, HW 12.4 mm, IOD 9.1 mm, SEL 6.9 mm, FLL 28.8 mm, HLL 44.3 mm, T4L 10.3 mm. Rostral rectangular, three times broader than high, in contact with six small scales excluding supralabial; nasal sub-circular; single scale between

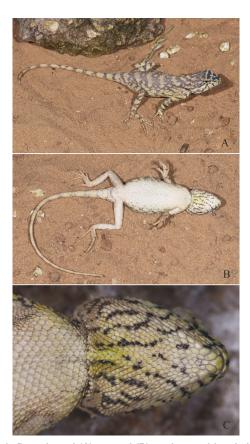


Figure 6 Dorsolateral (A), ventral (B), and ventral head close-up views (C) of the adult female allotype of *Japalura iadina* sp. nov. (KIZ 09398) in life (Photos by Kai WANG)



Figure 7 Habitat of *Japalura iadina* sp. nov. in the Dry-Hot Valley of Lancang River, Deqin, Northwest Yunnan, China (Photo by Kai WANG)

nasal and first supralabial; supralabials eight on both sides of head, weakly keeled; loreal scales irregularly arranged and moderatly keeled; ciliaries circular, much smaller than other scales of lateral head, forming orbit circle; supraciliaries six on both sides of head, prominent, elongated; three rows of scales between orbit circle and sixth supralabial on both sides of head, all weakly keeled; scales posterior to eyes strongly keeled; orbit

and tympanum separated by five enlarged scales on both sides of head; tympanum covered with small scales; dorsal head scales heterogeneous in size, distinctively keeled, convex; single row of five scales forming weak ridge along snout midline from just posterior to rostral to point in line with anterior corner of eyes; parietal keeled, enlarged; pineal eyespot present; post-occipital and posterior lateral head scales strongly keeled; conical scales on occipital region numerous, strongly protuberant; conical scales posterior to tympanum two, large; conical scale posterior to tympanum or rictus single, large, on each side of head.

Ventral head scales homogeneous in size, distinctively keeled; transverse gular fold present, distinct; gular pouch present; shoulder fold posterior to gular fold on each side of body present, distributed from ventral surface of throat to dorsolateral stripes; axillary fold present on each side of body. Middorsal scales 40, larger than neighboring scales, imbricate along dorsal midline; nuchal and dorsal crests slightly raised on skin folds, with distinct break separating regional folds; three dorsolateral rows of enlarged, distinctively keeled scales running parallel to dorsal crest from pectoral region of body to pelvis on each side of body, with one dorsal to dorsolateral stripe and two along upper and lower edges of dorsolateral stripe; ground dorsal scales heterogeneous in size; scales of axilla smaller than dorsal scales; large conical scales present, on dorsal and lateral surface of body, distinctly keeled, randomly scattered; dorsal limb scales distinctively keeled, slightly larger than ventral scales, homogeneous in size on fore-limbs, heterogeneous on hind limbs; F4S 16/16; T4S 20/21. Tail scales keeled, in lateral rows; cloaca scales small. Ventral scales of body and limbs near homogeneous in size, distinctively keeled.

Coloration of holotype in life: The ground coloration of the dorsal, lateral, and ventral surfaces of the head is emerald green. Four broad, black transverse bands are observed on the dorsal surface of the head, and are equally spaced across the region between the nares and a point in line with the posterior edge of the orbits. Black reticulated patterns are observed posterior to the last transverse bands on the dorsal surface of the head. Nine black streaks are observed radiating around the eye on each side of the head. The streaks extend ventrally to the supralabial scales, with the posteriormost streaks broadest. The infralabial scales and the margin of the lower jaw are emerald green with black bars present on several of the infralabial scales. These infralabial bars match up with streaks present on the supralabial scales, and extend extend further posteroventrally, gradually transitioning into blackish blue, vermiculate stripes on the ventral surface of the head. The vermiculate stripes connect posteriorly with the large, triangular shaped, blue gular spot located in the center of the gular pouch.

A black vertebral stripe runs from the occipital region of the head to the pelvis along the dorsal midline of the body. Several crest scales and a few small, ground scales along the vertebral stripe are green. The lateral and dorsolateral surfaces of the body are emerald green, with reticulated patterns of black pigmentation on the lateral surfaces of the body. These reticulated patterns of pigmentation form a thin, black, dorsolateral line

along the ventral edge of the dorsolateral stripe on each side of the body. The dorsolateral stripes are green and smooth-edged, running from the posterior occipital region of the head to the pelvis. A series of enlarged, distinctively keeled, green scales are observed in a dorsolateral series on each side of the dorsal crest, running from the neck to the pelvis and distributed along the black vertebral stripe. The ventral surface of the body is uniform whitish blue and is slightly lighter anteriorly.

The ground coloration of the dorsal surfaces of the limbs is emerald green, with numerous transverse bands running from the proximal to distal regions of the limbs. The ventral surfaces of the fore-limbs and hind limbs are whitish blue and greenish yellow, respectively. The ventral surfaces of the hands and feet are gray in coloration.

The dorsal surface of the tail is greenish yellow, with numerous dark gray, transverse bands running along its length. The transverse bands do not form complete rings around the tail ventrally. The ventral side of the tail is significantly duller gray in coloration (Figure 5).

Coloration of holotype in preservative: The coloration of the holotype in preservative closely resembles its coloration in life; however, the following differences are observed: 1) the emerald green coloration on some parts of the dorsal surfaces of the head, body, and limbs changed to light blue, and 2) the blue coloration of the gular spot on the ventral surface of the head and body faded significantly.

**Variation and sexual dimorphism:** Variation in morphometric characters and pholidosis patterns is summarized in Table 2. The new species is sexually dimorphic, with females possessing distinct coloration and pigmentation patterns from males. The female allotype of the new species differs from males by having light, yellowish brown ground coloration on the dorsal surfaces of the head, body, and limbs ( $\nu$ .s. emerald green), distinct, light brown transverse bands on the dorsal surface of the body ( $\nu$ .s. single, black vertebral stripe), a greenish yellow gular spot ( $\nu$ .s. blue), and a white ground coloration on the ventral surfaces of the body and limbs ( $\nu$ .s. blue; Figure 6).

Comparisons: The new species Japalura iadina sp. nov. has been confused previously with J. flaviceps; however, it can be distinguished readily from the latter by having a smaller adult body size (SVL 54-65 mm v.s. 64-75 mm), a tendency towards a shorter relative snout length (SEL/HL 34.9%-40.2% v.s. 40.0%-44.2%), smaller, less protuberant nuchal and dorsal skin folds in males (v.s. strongly protuberant), distinct ground coloration on the dorsal surfaces of the head, limbs, and body in males (emerald green v.s. brown), and distinct patterns of pigmentation along the dorsal midline of the body in males (vertebral black stripes v.s. a series of dark rhomboid-shaped patterns of pigmentation), as well as by the presence of distinct gular spots in both sexes (v.s. absence), and by the presence of distinct black radial stripes around the eyes (v.s. absence).

The new species is morphologically most similar to *J. splendida*, *J. makii*, and *J. luei*, of which all four species have the green

background coloration. However, Japalura iadina sp. nov. can be diagnosed from J. splendida by having a terrestrial life style (v.s. arboreal), a smaller adult body size (SVL 54-65 mm v.s. 59-92 mm), fewer T4S (19-24 v.s. 24-28), a shorter relative tail length (TAL/SVL≤198% v.s. ≥217%), distinct tail coloration in males (greenish yellow v.s. brownish gray), as well as by the absence of white lip stripes (v.s. presence), presence of gular spots in both sexes (v.s. absence), and absence of green coloration on the dorsal surfaces of the body in females (v.s. presence); from J. makii by having a smaller adult body size (SVL 54-65 mm v.s. 61-79 mm), a relatively shorter tail (TAL/SVL 1.73-1.98 v.s. 2.09-2.61), fewer T4S (19-24 v.s. 24-30), a pink coloration of tongue and oral cavity in life (v.s. yellow), a terrestrial lifestyle (vs. arboreal), as well as by the absence of a gular fold (v.s. presence), absence of green, transverse bands across the dorsal surface of body (v.s. presence), and absence of lip strips below eyes (v.s. presence); and from J. luei by having a smaller adult body size (SVL 54-65 mm v.s. 65-75 mm), a relatively shorter tail (TAL/SVL 1.73-1.98 v.s. 2.31-2.48), fewer T4S (19-24 v.s. 24-28), a terrestrial lifestyle (v.s. arboreal), continuous dorsolateral stripes in males (v.s. a dorsolateral series of disconnected, irregular, large markings), as well as by the absence of a gular fold (v.s. presence), and absence of lip stripes below eyes (v.s. presence).

Japalura iadina sp. nov. differs from J. laeviventris by having a smaller adult body size (SVL 54-65 mm v.s. 64-72 mm), distinctively keeled ventral scales of head and body (v.s. smooth or weakly keeled), fewer MD (35-46 v.s. 57-59), distinct ground coloration of the dorsal surfaces of head and body in males (emerald green v.s. off-white), distinct coloration of gular spots (blue in males, greenish yellow in females v.s. orange in both sexes), and distinct patterns of pigmentations on the dorsal surfaces of the body along the dorsal midline (black vertebral stripes speckled with green v.s. M-shaped patterns of dark brown pigmentation).

Additionally, when compared with species distributed in close geographic proximity along the Lancang River, J. iadina sp. nov. can be distinguished from J. vela by having less protuberant, discontinuous vertebral crests in males (v.s. sail-like, strongly protuberant, continuous), distinct ground coloration on the dorsal surfaces of the body (emerald green v.s. black) and tail (yellow v.s. gray) in males, uniquely shaped dorsolateral stripes in males (smooth edged v.s. strongly jagged), as well as by the presence of gular spots in both sexes (v.s. absence in both sexes), and by the absence of weakly defined, reddish dorsolateral lines in females (v.s. presence); and from J. yunnanensis by having a terrestrial lifestyle (v.s. arboreal), shorter relative tail length (TAL/SVL ≤198% v.s.≥237%), fewer T4S (19-24 v.s. 27-31), distinct coloration of gular spots (blue in males, greenish yellow in females vs. yellow in both sexes when present or absence in females), as well as by the presence of a transverse gular fold (v.s. absence).

**Distribution and natural history:** Although locally abundent, *Japalura iadina* sp. nov. is known only from the type locality (Figure 1). The new species is terrestrial, inhabiting dry, rocky habitats along the Lancang River (Figure 7). The emerald green

coloration of the males makes them stand out from the rocky, environmental background. Tail autotomy has been observed for this population.

**Etymology:** The Latin name "iadina" means "emerald like," which describes the diagnostic emerald green body coloration of males of the new species. Suggested common name: Emerald Mountain Dragon (English), Fei Cui Pan Xi (Chinese; 翡翠攀蜥).

#### **DISCUSSION**

Body coloration, particularly the coloration of the gular region, has been suggested to play an important role in the recognition of conspecifics, sexual selection, and the general diversification of agamid lizards in general (Bastiaans et al., 2014; LeBas & Marshall, 2000; Stuart-Fox & Ord, 2004), particularly members of the genus Japalura (Kästle & Schleich, 1998; Wei & Lin, 1981). Within the genus Japalura, color patterns vary among species from mainland Asia, but appear to be conservative within species, and hence serve as good diagnostic characters for systematic studies of species diversity (Wang et al., 2015). However, to date, detailed descriptions of coloration in life, have not been published for many species in the genus, including J. brevicauda, J. chapaensis, J. grahami, J. hamptoni, J. otai, J. yulongensis; (Mahony, 2010; Manthey, 2010; Manthey et al., 2012; Ota & Weidenhöfer, 1992; Zhao et al., 1999). Since specimen coloration is recognized to fade significantly after preservation for many specimens in natural history collections, color descriptions of preserved specimens may not be as applicable to diagnostic comparisons. Therefore, we recommend future research focusing on describing coloration patterns based on new observations of species of Japalura in life.

Despite the importance of the coloration patterns in the taxonomic studies of members of the genus Japalura, the diagnostic coloration of *J. flaviceps* has been historically confused. which has continued to the species' recognized wide spread distribution. Originally described by Barbour & Dunn (1919) from the Tung River Valley (today Dadu River Valley) in northwest Sichuan Province, J. flaviceps was described as possessing a uniform, "dusky brown" dorsal head coloration and lacked "strongly marked stripe from the eye to the angle of the mouth." Together with photographs of the holotype of J. flaviceps (MCZ R-12469) and topotypic males from the Dadu River Valley, Manthey et al. (2012) argued that J. flaviceps did not possess dorsal and lateral pigmentation patterns on the head, and proposed that the absence of such patterns should be used as a diagnostic feature of the species. Although most topotypic specimens of J. flaviceps that we examined possess no distinct, radial pigmentation patterns around the eyes, we did observe several individuals, particularly subadults, that possess several distinct transverse bands of darker pigmentation across the dorsal region of the head (CIB 2333 and 2549). Therefore, we suggest that the absence of radial patterns of darker pigmentation around the eyes may be a more consistent feature of J. flaviceps for use in diagnostic comparison. Furthermore, we propose that the diagnostic feature of the true J. flaviceps should be restricted to the following combination of morphological characters: SVL 70-83 mm in adult males, 58-78 mm in adult females; TAL/SVL 165%-192%; HLL/SVL 62%-78%; SEL/HL 39%-43%; SOR 4; SL 9-11; IL 9-11; MD 43-54; T4S 21-24; tympanum concealed; distinct gular fold present; distinct gular pouch present; enlarged, conical, post-rictal, post-occipital, and post-tympanic scales present, numerous, prominent; ventral scales distinctively keeled; dorsal ground coloration of head and body brownish gray; distinct radial stripes around eyes absent; dark, broad, interconnected, vermiculated stripes on the ventral surface of head present; gular spot absent; smooth-edged, yellow dorsolateral stripes in males present; a series of rhomb-shaped patterns of dark brown pigmentation with distinct yellow centers present along the dorsal midline of the body.

Due to the continued taxonomic confusion over comparisons of populations of the *J. flaviceps* species complex, *J. flaviceps* was thought to be a widespread species, and its conservation status was assessed as Least Concern (IUCN, 2013). However, because of the absence of data allowing for confirmation of species level diversity within the *J. flaviceps* Species Complex, and the paucity of available information about intraspecific ecological and genetic diversity, we recommend that *J. flaviceps* and all conspecifics in the species complex be considered Data Deficient, and recommended immediate research be focused on better understanding this unique complex of agamid lizards.

Unfortunately, habitats in the Hengduan Mountain Range are experiencing currently considerable human-mediated modifications as a result of the rapid development of hydropower plants (Pan et al., 2002; Chen & Rao, 2010; personal communication with Mr. Li CHENG). Although populations of Japalura can be abundant, continued alteration of these valley habitats could result in the extirpation of micro-endemic populations, or worse, unique evolutionary lineages not yet recognized formally as species. Additionally, many species of Japalura are experiencing over-exploitation through the illegal pet-trade, both domestically and internationally (personal communication with Mr. Jia-Wei WU and Mr. Mian HOU). Therefore, it is critical that researchers and conservation and government agencies work together to assess the conservation statuses and ecological requirements of species in the genus, particularly species and populations endemic to the river valleys in the range of the Hengduan Mountain Range.

#### **ACKNOWLEDGEMENTS**

We would like to thank Dr. Gernot Vogel (Society for Southeast Herpetology, Germany), Mr. Xin-Lei ZHAO (Chinese Academy of Medical Sciences), Mr. Scott Farnsworth (Washington State University), and Dr. Su LIU (Shanghai Chenshan Botanical Garden) for providing suggestions on the scientific names of the new species, Mr. Duan YOU and Mr. Ya-Qiang SUN for assisting our fieldwork in Tibet, Mr. Wynn Addison (NMNH), Professor Yue-Zhao WANG (CIB), Professor Yue-Ying CHEN (CIB), Mr. Ke LU (CIB), and Mr. Gui-Wu HE (KIZ) for kindly letting us examine specimens under their care, Mr. Nicholas A. Huron (University of Oklahoma) for assistance with ArcGIS and in the creation of the Figure 1 map, Ms. Jing-Ting LIU (Washington State University) and Ms. Dan-Lin LI (University of Idaho) for editing the photos, Mr. Mian HOU for providing valuable information, and members of the Siler Lab for their generous comments on the manuscript.

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#### **APPENDIX**

The following specimens were examined:

- J. batangensis (n=16): CIB 2227, 2233, 2243, 1902-1908, KIZ 84011, 801081, Batang, Sichuan, P.R. China; KIZ 019314, KIZ 09404, 019311, 019312 Markam Tibet China
- J. dymondi (n=7): CIB 87234, 1869, Panzhihua, Sichuan, P.R. China; KIZ 95I1001, 1002, 1016, 1018, 1022, Dayao, Yunnan, China.
- $\textit{\textbf{J. grahami}} \ (\textit{n}\text{=}1)\text{: USMN 65500 (holotype)}, \ \textit{Yibin, Sichuan, China}.$
- J. micangshanensis (n=9): CIB 86351, 86348, Xianyang, Shaanxi, P.R. China; CIB 86360, 86361, 86356, 86357, Luonan, Shaanxi, China; CIB 2572, 2578, 2582, Wenxian, Gansu, China.
- *J. flaviceps* (*n*=13): CIB 2234, 2332, 2333, 2341, 2354, 2355, 2549, 2554,

2556, 2561, 2567; KIZ 05181, 05182; Luding, Sichuan, China.

- J. splendida (n=6): USNM 35522 (holotype), Yichang, Hubei, PR China; CIB 2588, 2591, 2596, 72468, 72469, Chongqing, China.
- J. varcoae (n=3): CIB 2651, 2650, KIZ 85II0006, Kunming, Yunnan, China.
- J. vela (n=11): KIZ 013801 (holotype), KIZ 013802, 013813, 013800, 013805–013811 (paratopotypes), Jerkalo, Tibet, China.
- J. yunnanensis (n=8): CIB 2684, 2686, 2687, 2689, KIZ 82081, Longling, Yunnan, PR China; KIZ 74II0240, 0248, 79I469, Tengchong, Yunnan, China.
- J. zhaoermii (n=12): CIB 86432, 86435, 85721, 85722, 86433, 86434, 86436, Wenchuan, Sichuan, PR China; CIB 2232, 2244, 2240, KIZ 84032, 85030, Lixian, Sichuan, China.

# AmphibiaChina: an online database of Chinese Amphibians

Jing CHE<sup>1,\*</sup>, Kai WANG<sup>2</sup>

#### **ABSTRACT**

AmphibiaChina, open-access. web-based an database, is designed to provide comprehensive and up-to-date information on Chinese amphibians. It offers an integrated module with six major sections. Compared to other known including databases AmphibiaWeb Amphibian Species of the World, AmphibiaChina has the following new functions: (1) online species identification based on DNA barcode sequences; (2) comparisons and discussions of different major taxonomic systems; and (3) phylogenetic progress on Chinese amphibians. This database offers a window for the world to access available information of Chinese amphibians. AmphibiaChina with its Chinese version can be accessed at http:// www.amphibiachina.org.

**Keywords:** Amphibia; Taxonomy; Species identification: DNA barcoding: Tree of life

Being the third largest country in the world, China possesses multiple biodiversity hotspots, which harbor a rich diversity of amphibians. Because of the rapid accumulation of many kinds of data for Chinese amphibians, it is desirable to have a versatile platform to integrate all new advances and promote their accessibility to scientists, governmental agencies, and the inquisitive public.

Currently, two online databases deal exclusively with amphibians: "AmphibiaWeb" (2015) and "Amphibian Species of the world" (Frost, 2015). The latter provides detailed taxonomic history and the former provides information on natural history, conservation, as well as its own taxonomic system for amphibians. Both databases host information on Chinese amphibians, yet they sometimes conflict with regard to the taxonomic validity of some species. This owes to multiple causes, such as being unaware of Chinese literature or not having access to it, the language barrier, the lack of

communications between scholars, and philosophical differences regarding assignments of species to genera and the recognition of other higher taxonomic categories. Such controversies create significant confusion, even for the professional herpetologists. For the public and most governmental agencies, it may be much worse. Various textbooks and popular presses use outdated nomenclature, invalid species names, and sometimes even convey the wrong information about species and the incorrect identification of species. Among a cornucopia of concerns, this situation causes serious problems and creates obstacles in further research, of biology, public education, effective conservation activity, wildlife management and law enforcement in China.

Here, we announce that the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS), has established the open-access, web-based platform "AmphibiaChina" (www. amphibiachina.org). It seeks to integrate available information on all known species of amphibians in China. Scientists, non-experts, and even the public can freely search the database and obtain relevant information, according to their interests.

AmphibiaChina aims to: (1) promote multidisciplinary studies on Chinese amphibians, (2) correct some errors, spread accurate knowledge and promote scientific outreach, (3) involve citizen scientists and herpetoculturists in documenting amphibian diversity and contributing to research in China, (4) encourage communication among herpetological researchers, law enforcement agencies, governmental departments, and the curious public, and advocate effective biodiversity conservation in

Received: 20 December 2015; Accepted: 31 December 2015

Foundation items: The development of the AmphibiaChina has benefitted from the data accumulated through the programs of the National Natural Science Foundation of China (31090252), the Chinese Academy of Sciences (KSCX2-YW-Z-0807, KSCX2-EW-Z-2), Ministry of Science and Technology of China (MOST2011FY120200), and the Animal Branch of the Germplasm Bank of Wild Species of CAS (the Large Research Infrastructure Funding).

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China, and (5) provide a window for researchers outside of China to understand and access information on Chinese species.

Presently, AmphibiaChina is available in Chinese only, although

an English version is planned. It contains six major sections: Systematics; Species Identifications; Information retrieval; Chinese Amphibian Tree of Life; News; and a Photo Gallery (Figure 1).

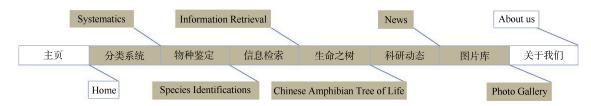


Figure 1 Six major sections of AmphibiaChina

#### **Systematics**

Users can navigate this section using the hierarchical system of formal taxonomy, or by geographic region (province).

This section provides the Chinese species list by three orders. Clicking on the Order name displays the families in that Order. Clicking on the Family name displays its genera. Clicking on the Genus will display its species. At the species-level, the most comprehensive and up-to-date information is available, including the valid scientific name, subjective synonym(s), local common name, level of protection, diagnosis, brief morphological description, available biological information, photos, distribution range and the distribution maps, type specimen(s) and type locality, and a discussion. In groups with taxonomic controversy, even chaos, this section gives alternative taxonomies on the right column of the screen based on the preference of AmphibiaChina (AmphibiaChina, 2015), AmphibiaWeb (AmphibiaWeb, 2015), Amphibian Species of the World (Frost, 2015), and the Colored Atlas of Chinese Amphibians and their Distributions (Fei et al, 2012). AmphibiaChina offers comments on taxonomic controversies.

Users can explore the amphibian diversity of a province by clicking on its name on the map of China, which generates a provincial amphibian species list. These will be extremely useful for conservation agencies and the public.

#### **Species Identifications**

This section designed multiple kinds of online identification tools including diagnostic keys, photos uploaded, and DNA barcoding. Presently, online identification from uploaded gene sequences (copy/paste sequences to the text box, or choose files) of samples of interest is available. At the moment of writing, the DNA sequence database contains 2048 sequences of the COI gene of 302 species of Chinese amphibians, covering more than 70% of the known diversity of this country. This system will increase its taxonomic coverage up to most extant amphibian species by 2018 (two years), and other genes (16S rRNA, and *CYTB*) will become available in the very near future.

The online species identification section will not only be useful for researchers, but also in fields such as biodiversity surveys, invasion species monitoring, forestry conservation management, pet trading, food safety, biological medicine, pest

control, and customs, etc..

#### Information Retrieval

This section will allow anyone to search this site by species, photos, and sequences.

#### **Chinese Amphibian Tree of Life**

This section gives the up-to-date phylogeny of Chinese amphibian according to the published data. Chinese amphibians are not a monophyletic group, of course. Users can move from higher to lower taxonomic ranks for each group. A detailed discussion of its phylogenetic position appears along with figures of the tree.

#### News

This section presents news on current amphibian research progress, meetings, etc. It will emphasize Chinese species. Researchers can use this platform to communicate their initiatives.

#### **Photo Gallery**

This section contains photographs of living Chinese amphibians and their habitat. Anyone can navigate the gallery by taxonomic groups, geographic area (province), or individual photographer. People are encouraged to register as photographers of this system and upload photos under their accounts. While allowing for the maximum involvement of photographers, the gallery is reviewed by experts on a weekly basis, correcting the taxonomic information associated with the photos. Users are the builders of this section.

**NOTE**: Currently, only the Chinese version of AmphibiaChina is available. We are considering an English version of the site. We encourage professional herpetologists, students, and amphibian enthusiasts to contribute to the site by uploading photos to the database and provide comments and suggestions through email: amphibiachina@mail.kiz.ac.cn.

#### **ACKNOWLEDGEMENTS**

This database has been developed and is being maintained by the Laboratory of Herpetological Diversity and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences. Thanks for the durable encouragements from Prof. David Wake and Prof. Ya-ping Zhang.

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我们致力于报道中国两栖类每一个物种的数据信息,因此鼓励爱好两栖类的专业之士及志愿者积极参与本网站数据提供和更新。网站实行实名登录,"中国两栖类"信息系统的发展将受益于每一个参与人员。

本网站力求实现信息实时更新, 资源共享及信息交流,以期在大家的共 同努力下,促进中国两栖类学科更多研 究方向的发展,关爱和保护中国两栖 类,维护生态环境平衡,促进自然与人 类的和谐共处!



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Edited by Editorial Office of Zoological Research

(Kunming Institute of Zoology, Chinese Academy of Sciences, 32 Jiaochang Donglu, Kunming,

Yunnan, Post Code: 650223 Tel: +86 871 65199026 E-mail: zoores@mail.kiz.ac.cn)

Sponsored by Kunming Institute of Zoology, Chinese Academy of Sciences; China Zoological Society©

Supervised by Chinese Academy of Sciences

Published by Science Press (16 Donghuangchenggen Beijie, Beijing 100717, China)

Printed by Kunming Xiaosong Plate Making & Printing Co, Ltd

Domestic distribution by Yunnan Post and all local post offices in China

International distribution by China International Book Trading Corporation (Guoji Shudian) P.O.BOX 399,

Beijing 100044, China

Advertising Business License 广告经营许可证: 滇工商广字66号

Domestic Postal Issue No.: 64-20

Price: 10.00 USD/60.00 CNY Post No.: BM358



