

The genus *Pelophylax* (Amphibia, Ranidae) in Pakistan: museum collections and possible distribution

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Abstract

We provide the first comprehensive data on the questionable distribution of the genus *Pelophylax* and the family Ranidae from Pakistan. Based on a literature review and two specimens of the genus from Tasp, Panjgur District in Pakistani Balochistan (USNM 26194–95), stored in the Smithsonian National Museum of Natural History, Washington, DC, USA, we discuss the possible occurrence and affiliation of these frogs in the context of Central Asia. Our comparison shows that the nearest records of *Pelophylax* in relation to the Tasp specimens are reported from more than 280 km (air-line) away in Iran and Afghanistan, which are currently separated by hot and mostly desert environments. We suggest that possible surviving populations of this genus may still be present in Balochistan (Rakhshan River) or Khyber Pakhtunkhwa (Kabul River) Provinces of Pakistan. This would, however, need further field investigations.

Key Words

Afghanistan, biogeography, Central Asia, Iran, museum data, *Bufo*, *Chrysopaa*, *Nanorana*, *Rana*

Introduction

The genus *Pelophylax* Fitzinger, 1843 currently represents 20–21 species and 2–3 hemiclones distributed from northern Africa to the Far East in Asia. They are morphologically very similar and often mentioned as particular species complexes (groups) with unresolved phylogenetic relationships (Akin et al. 2010). Populations from the Middle East and Central Asia are ranked under the *P. ridibundus* group and are geographically associated with the

following species: *P. ridibundus* (Pallas, 1771), *P. bedriagae* (Camerano, 1882), *P. caralitanus* (Arikan, 1988) and *P. terentievi* (Mezhzhherin, 1992). All these taxa have phenotypic, ecological and etiological similarities, yet the ongoing research shows that they will need comprehensive evolutionary-taxonomic re-evaluation (Mazepa et al., in prep.). The members of this group are paraphyletic, containing cryptic taxa (Plötner 2005). Populations of the *ridibundus* group have a continuous distribution and are most abundant in the temperate and subtropical zones of

the Western Palearctic, with a scattered distribution in the southern dry regions in the Middle East where water bodies are available. Overall, they are distributed from eastern Spain to western China, through Central Europe, the Balkans, the Anatolian Peninsula, the Caspian Region and Central Asia (Akin et al. 2010). In the division between Central (Afghanistan, Iran, Turkmenistan, Uzbekistan) and Southern Asia, where the territory of Pakistan falls, three species are currently recognised: *P. bedriagae*, *P. ridibundus* and *P. terentievi* (Wagner et al. 2016; Pesarakloo et al. 2017; Showler 2018; Frost 2021).

Pakistan is a country in the transition zone between the Palearctic and Oriental faunas, which is based on the fact that representatives of (not only) amphibians from both these two zoogeographic regions may be found in the country (Khan 2006; Jablonski et al. 2020, 2021). Currently, the territory of Pakistan contains four families of amphibians: Bufonidae Gray, 1825; Dicroglossidae Anderson, 1871; Megophryidae Bonaparte, 1850; and Microhylidae Günther, 1858 (Masroor 2012; Sarwar et al. 2016; Frost 2021). Whereas Bufonidae contains both Palearctic (*Bufo* Rafinesque, 1815) and Oriental (*Duttaphrynus* Frost et al., 2006; *Firouzophrynus* Safaei-Mahroo & Ghaffari, 2020) genera, other families are represented only by genera with mostly Oriental affiliation (e.g. *Euphlyctis* Fitzinger, 1843; *Minervarya* Dubois, Ohler, Biju, 2001; *Microhyla* Tschudi, 1838; *Scutiger* Theobald, 1868).

Previous reports of the family Ranidae from Pakistan (Khan 2006) are for species currently recognized in the family Dicroglossidae. However, historical data mention the genus *Pelophylax* (formerly *Rana* Linnaeus, 1758), a true member of the family Ranidae, for Pakistan. The first *Pelophylax* record ("*Rana esculenta*, L.") was provided by Blanford in Hubrecht (1880: 621) from the locality Nál (Naal) of central Balochistan [coordinates given by Blanford as 29°30'N, 66°E, however, the correct position should be ~27.682°N, 66.183°E sensu St. John (1889)]. Another specimen was collected in Baghunna (~27.907°N, 66.298°E), "*so badly preserved that doubts about its identity with this or any other species may be entertained*" (Hubrecht 1880). These specimens were part of a small collection of amphibians and reptiles made by "*Dr. C. Duke in April and May 1877*" and deposited in the Leiden Museum (currently known as Naturalis Biodiversity Center, Leiden, The Netherlands; Hubrecht 1880). However, in the digital catalogue, we were able to find only a specimen RMNH. RENA.4024 of *Trapelus agilis* (Olivier, 1807) from that collection. We, moreover, think that both localities belong to *Chrysopaa sternosignata* (Murray, 1885), aquatic species that was described later than Blanford in Hubrecht published their observations. Authors thus could affiliate them erroneously with "*Rana esculenta*".

Later, "*Rana esculenta*, L., var. *ridibunda*, Pallas" was mentioned by Boulenger (1898: 353) for Pakistan from the collections of the BMNH as two collection items (i.e. each in a separate jar): i) a tadpole originating from the area "Quetta to Nushki" (~29.71°N, 66.40°E; loc. 2), yet we were able to recover no traces of this specimen (first mentioned by Boulenger in Aitchison 1889: 105) and

ii) a male and a female, NHMUK 1955838 (= BMNH 1884.11.20.6–7) (Boulenger 1882, 1891, 1920: 89–90).

The *Pelophylax* larva was collected by James E. T. Aitchison, naturalist of the Afghan Boundary Commission, on 22–29 September 1884 (Alam 2009). The following settlements were indicated along the road between Quetta and Nushki: Spangaoli, Gobraak, Khanak, Panj-paj, Chaman and Kaisar (Aitchison 1889). Notably, along this route, Aitchison collected an adult specimen of "*Bufo viridis*" NHMUK 1880497 (= 1886.9.21.128) that was later defined as a paratype of *Bufo* *zugmayeri* (Eiselt and Schmidtler 1973). Possible concerns on larval affiliation to *Pelophylax* (identification made by Boulenger) may be ruled out by the fact that Aitchison's collection also contained larvae of a green toad "*Bufo viridis*, Laur.": "*Numerous Tadpoles from streams at base of Mt. Do-Shakh*" (Boulenger in Aitchison 1889: 105).

The adult frog vouchers came from the locality "*near Sarawan, Baluchistan*" (~28.67°N, 65.53°E; loc. 3) and were deposited by George E. Mason (Boulenger 1891: 380). The origin of some other amphibian specimens obtained from Mason was doubted by Boulenger (1920: 89), while no such doubt was raised for the Sarawan specimens.

All abovementioned records come from north-eastern Balochistan, near the border with Afghanistan (Fig. 1).

Materials and methods

During extensive research on the biogeography and taxonomy of the genus *Pelophylax*, we came across a forgotten collection comprising two specimens of *Pelophylax* originating from south-western Pakistan (Balochistan Province), deposited in the Smithsonian Institution National Museum of Natural History, Washington, DC, USA (USNM). We, therefore, provide important data and confirmation of (at least) the historical presence of these frogs for Pakistan (western Balochistan), supported by well available museum material. We took 14 morphometric characters (Table 1) and detailed photos of both specimens (Figs 2, 3). We also compiled, updated, or even corrected (see localities of vouchers CAS [California Academy of Sciences] 115915, FMNH [Field Museum of Natural History] 161114–16 in Suppl. material 1: Table S1 and literature therein) the available field and museum records of the genus *Pelophylax* from eastern Iran and Afghanistan to present the possible geographical affinity to the closest populations and obtain a better view on the origin of Pakistani populations (see Suppl. material 1: Table S1). Moreover, we attempted to genotype the mitochondrial *ND3* locus of specimens (USNM 26194–95) from Pakistan, as well as vouchers from one of the closest known localities of the genus from Iran (Zabol, MVZ [Museum of Vertebrate Zoology] 243552–63 and MVZ 243612–13). However, due to the age and preserved medium of specimens from Pakistan, we were able to extract DNA and sequences from only four of the Zabol vouchers, namely MVZ 243553–54 and 243612–13 (GenBank Accession Numbers: MW590982–MW590985).

Table 1. Morphological data of two specimens of *Pelophylax* from Pakistan, stored in the Smithsonian National Museum of Natural History. Morphometric data are presented in millimetres and bilateral characters are presented with the left/right sides.

Measurements	USNM 26194 (♂)	USNM 26195 (♀)
Total length	78	80
Head length	23	26
Distance between nostrils	4	4
Distance between front end of eye to tip of snout	10	11
Distance between the eyes	11	11
Distance between eye lids	2	2
Width of eye lids	9/9	8/8
Diameter of ear membrane	6/6	5/5
Length of femur	39/39	32/34
Length of tibia	40/41	42/43
Length of small toe	12/12	11/10
Length of metatarsal tubercle	4/4	4/4
Width of the metatarsal tubercle	2/1	2/2
Length of the second shank	20/20	19/19

Results

Two *Pelophylax* specimens (“*Rana ridibunda*”; Figs 2, 3) were collected from Tasp (26.99°N, 64.06°E; Fig. 1, loc. 1), south-western Balochistan, Pakistan, by George E. Mason (London, England) and deposited at the National Museum of Natural History, Smithsonian Institution,

Washington, DC, USA, as an exchange for invertebrates (Fig. 4). The time of their collection is unknown, but before they were catalogued in July 1899 (Fig. 4). Both specimens were adult and have voucher numbers USNM 26194 (male; Fig. 2) and USNM 26195 (female, Fig. 3). Together with the material from Sarawan (BMNH 1884.11.20.6–7; see Introduction), these two specimens represent the only vouchered specimens of the genus from Pakistan. The specimens from Tasp are also the western- and southernmost known records of the genus for Pakistan. Morphological characters, together with the visible colour pattern, correspond to the *P. ridibundus* group. We compared the locality of Tasp with available locality records from Pakistan, Afghanistan and eastern Iran (overall 57 identified localities representing 147 vouchered specimens; Fig. 1 and Suppl. material 1: Table S1) to evaluate their geographic distances and closest locality. The nearest locality is “*Hodar, Sarbaz River*” (MMTT [Muzeye Melli-e Tarikh-e Tabiei, Tehran] 877; Fig. 1, loc. 57; Safaei-Mahroo and Ghaffari 2020) in Iran, approximately 280 km (air-line) from Tasp in Pakistan. Other Iranian and Afghan localities are more than 500 km from Tasp (Fig. 1). We also compared the distances to previously known historical records of the genus in Pakistan mentioned by Blanford in Humbrecht (1880) and

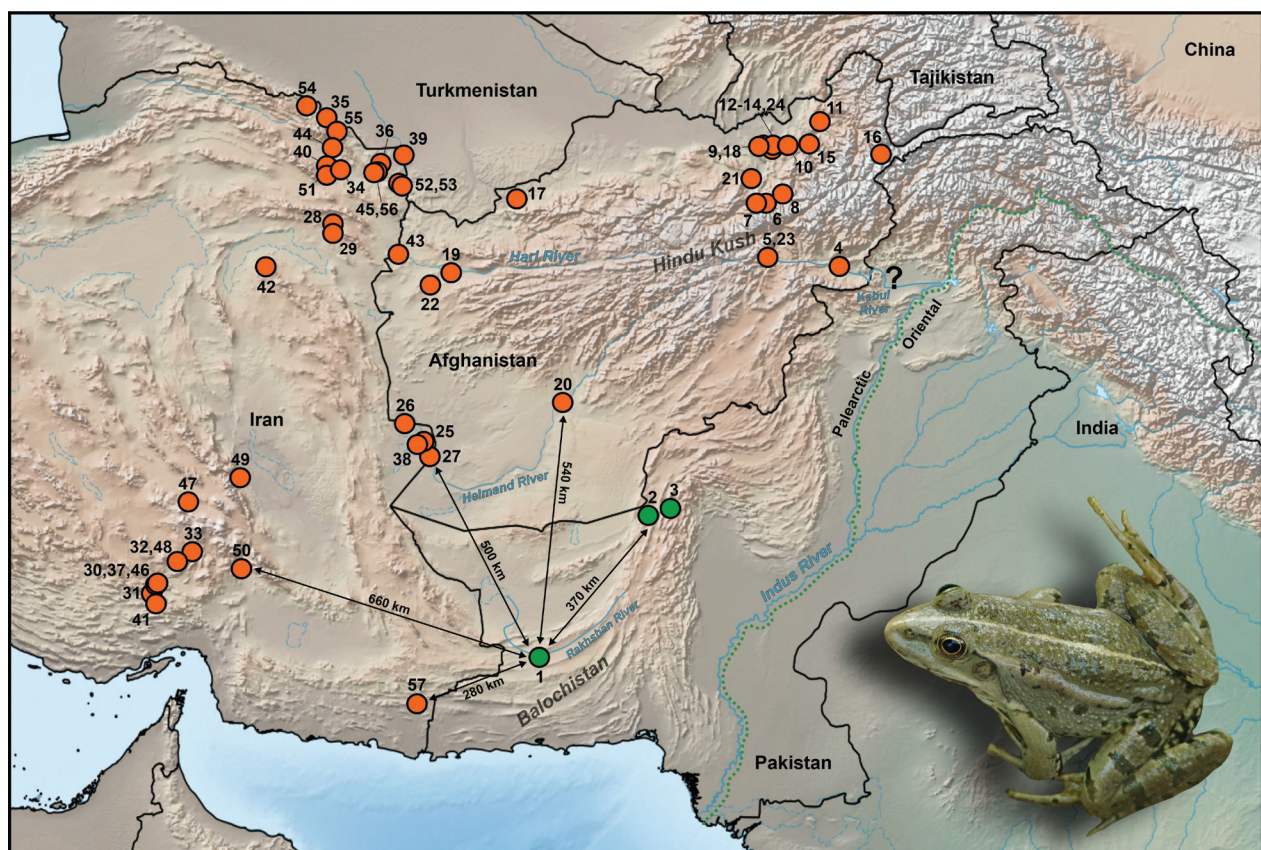


Figure 1. Locality data of the genus *Pelophylax* in eastern Iran and Afghanistan, based on museum voucher specimens and published field records (orange circles) in the context of data from Pakistan (green circles). The nearest localities to Tasp (USNM 26194–95; locality no. 1) are marked with arrows. The question mark represents possible presence of the genus *Pelophylax* in Pakistan on the Kabul River. The dashed green line represent the border between the Palearctic and Oriental zoogeographic realms. For details and locality numbers, see Suppl. material 1: Table S1. The individual in the figure originated from Kunduz Province, Afghanistan, photographed by Sjoerd van Bemmelen, BemmelenPhotography.



Figure 2. The voucher specimen of *Pelophylax* sp. USNM 26194, adult male. Scale bars correspond to 1 cm. Photo credit Esther M. Langan, modified by Glib Mazepa.

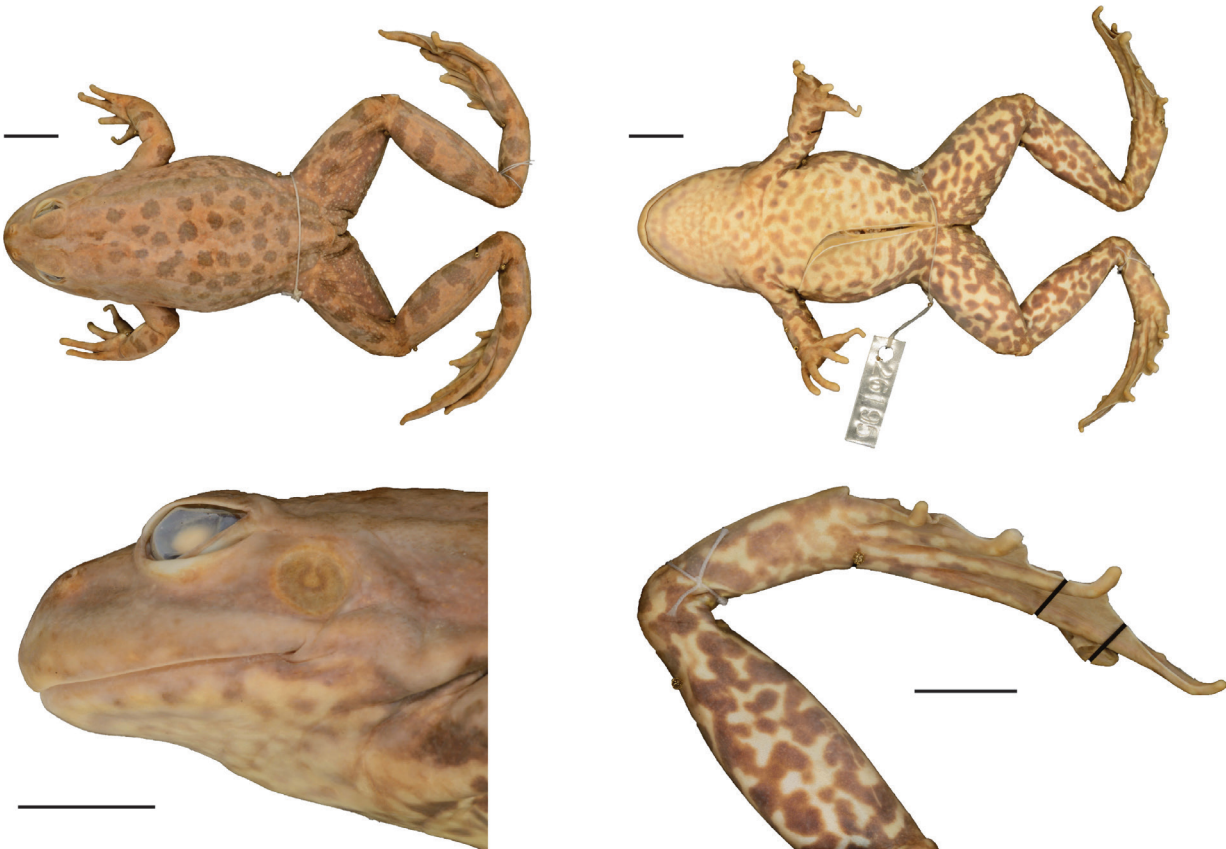


Figure 3. The voucher specimen of *Pelophylax* sp. USNM 26195, adult female. Scale bars correspond to 1 cm. Photo credit Esther M. Langan, modified by Glib Mazepa.

CURRENT NO.	ORIGINAL NO.	NAME	SEX	LOCALITY	DATE COLLECTED	NATURE OF SPECIMEN	CATALOGUE NUMBER	MEASUREMENT	RECEIVED FROM	COLLECTED BY	DATE	WHEN SPECIMEN WAS TAKEN	NO. OF SPECIMENS	REMARKS
2615	1	<i>Pelophylax</i> <i>terentievi</i>		Mediating Co. Concordville, Pa.			35278		Miss. Coover		July 19, 1899	1	1	PARATYPE
	2	<i>Pelophylax</i> <i>terentievi</i>		Holdy, Pa.			35286		V. R. Bodley			1	1	
	3	<i>Pelophylax</i> <i>terentievi</i>		Blair, N.Y.			35284		Thos. C. Seal			15	1	
	4	<i>Terrapene</i> <i>carolina</i>											2	eggs with young
	5	<i>Rana</i> <i>esculenta</i> <i>typica</i>		as Moulton, Pa. Prof. J. H. H. Aug 15 1899			35238		Geo. E. Mearns	Geo. E. Mearns	July 18, 1899	1	1	
	6	"	juv		14							1	1	
	7	"										1	1	
	8	"										1	1	
	9	"										1	1	
2619	01	"	juv									1	1	
	14	"										1	1	
	24	"		Ridgeway, Susana, Baluchistan, Persia								1	1	
	30	"		Ridgeway, Susana								1	1	
	44	"		Ridgeway, Tasp, Baluchistan								1	1	
	50	"										1	1	
	6	<i>Natrix</i> <i>sepioides</i>		juv Franklin, N. Va.	July 1899		35328		J. H. Riley		July 19, 1899	1	1	
	7	<i>Diemictophis</i> <i>viridescens</i>										1	1	
	8	"										1	1	
	9	"										1	1	
26210	1	"										1	1	
	2	"										1	1	
	3	"										1	1	
	4	<i>Amphispelops</i> <i>ruber</i>										1	1	
	5	<i>Varanus</i> <i>monticola</i>		pat Tomy, Siam Malayam Jan 3 1899			35322	head body 172 tail 23 total 195	Dr. W. L. Abbott			1	1	Thos. C. Seal

Figure 4. The Smithsonian Institution, National Museum of Natural History (USNM) catalogue ledger with USNM 26194 and 26195 voucher entries for *Pelophylax* from Pakistan. Photo credit Division of Amphibians and Reptiles, National Museum of Natural History, Smithsonian Institution.

Boulenger (1889, 1891, 1920), namely the area of Quetta. These localities are ca. 370 km (air-line) from Tasp. Based on the distribution of the Tasp specimens, we expect that they should be identified as *P. terentievi*.

The ND3 haplotypes of the sequenced specimens from the Zabol area, Iran (MVZ 243553–54 and 243612–13) are most similar to *P. terentievi* (GenBank Accession Number NC029199) from Ashgabat, Turkmenistan. Unfortunately, specimens from the Pakistani locality of Tasp (USNM 26194–95) were not successfully sequenced. However, we expect the same genetic affinity for the Pakistani specimens as is recorded for the Zabol specimens, but further confirmation is needed.

Discussion

As there exist no recent observations on *Pelophylax* from Pakistan and only four exact localities are historically known with only two corroborated by available voucher specimens, it is difficult to evaluate if *Pelophylax* is currently a member of the herpetofauna of the country. According to Masroor (2012), Sarwar et al. (2016), Frost (2021) and Jablonski et al. (2021), 20 to 24 species of amphibians are currently reported from the country, without any mention of the family Ranidae or the genus *Pelophylax*. Pakistani Balochistan is herpetologically the most diverse region of the country (Khan 2006), yet the predominantly arid conditions do not generally provide

suitable and stable habitats for amphibians. *Pelophylax* frogs need permanent water sources around rivers or lakes, but they are also able to live in oases, for example, in northern Africa or the Middle East (e.g. Nicolas et al. 2015). Although such habitats are available in Balochistan, they are often ephemeral. This is also the case of the Rakhshan River flowing in the vicinity of Tasp. This river currently possesses little or no water; however, in Panjgur (near Tasp), it expands into a series of bright clear water pools (*kor joh*) connected by small water channels. The banks are bordered by numerous date palms and most of the water is used for irrigation. Speculatively, in the time of the collection (150 years ago), the situation with suitable habitats might have been different, providing conditions for a stable, broadly distributed population. However, we presume that some small populations of the genus *Pelophylax* might still occur there. This needs further investigations to determine not only the presence of *Pelophylax*, but also to investigate its origin with respect to the nearest localities in Iran and Afghanistan, which are situated more than 280 km from Tasp (Fig. 1). Due to the physiography of today's locality, the possible populations of *Pelophylax* may represent relicts, surviving in Balochistan after a more suitable past climate. Further field investigation is also needed from areas of historical records mentioned by Blanford in Hubrecht (1880) and Boulenger (1889, 1891, 1920) in the area of Quetta.

Moreover, potential localities of the genus in Pakistan may be found around the Kabul River in the Khyber Pakh-

tunkhwa Province in the north-western part of the country (Fig. 1). The Kabul River is a biogeographic corridor for the Oriental fauna into the Palearctic area that probably allowed colonisation of valleys of Hindu Kush mountains around the river from Pakistan to eastern Afghanistan [e.g. *Euphylyctis cyanophlyctis* (Schneider, 1799); *Calotes versicolor* (Daudin, 1802); *Ophisops jerdonii* Blyth, 1853, *Varanus bengalensis* (Daudin, 1802); *Fowlea piscator* (Schneider, 1799); Wagner et al. 2016; Jablonski and Lesko 2018; Jablonski et al. 2019]. Given the records of *Pelophylax* in Afghanistan, for example, from Paghman near Kabul (FMNH: 161055; Anderson and Leviton 1969; Fig. 1 loc. 5) and especially from the locality “ca. 25 km SE (by air) Jalalabad” (MVZ: 236876; Fig. 1 loc. 4), only 60 km from the Pakistani border, such vice versa colonisation and presence of the genus *Pelophylax* in north-western Pakistan is highly possible. In this context, we checked the specimens stored in the Florida Museum of Natural History at the University of Florida (UF) and vouchered as UF 82322–24, collected in Yakh Tangi, Khyber Pakhtunkhwa Province, Pakistan (approximately 34.30°N, 71.65°E). These specimens were stored in the collection under the name “*Rana* “*farooqi*” nov. sp. Auffenberg”, raising serious questions on the identification of these specimens as *Pelophylax*. However, available photos reveal that these specimens might be members of the genus *Nanorana* Günther, 1896, most likely *N. vicina* (Stoliczka, 1872).

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Supplementary material 1

Table S1. An overview of localities and voucher specimens from Afghanistan, eastern Iran, and Pakistan used in this study

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Data type: species data

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