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Genetic variation and tadpoles of the westernmost Himalayan lazy toad *Scutiger occidentalis*

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ABSTRACT

While the diversity of *Scutiger* is high in the central and eastern parts of the Himalaya-Tibet orogen, only a single species, *Scutiger occidentalis* Dubois, 1978, can be found in the westernmost regions, in Northwest India and Pakistan. Little is known about its genetic variation, ecology, and distribution and far less about its larval morphology. Here, we provide new records, detailed morphological data of tadpoles, and genetic data (mtDNA and nDNA) of the species from two, so far unknown localities in northern Pakistan. Molecular analysis confirmed the taxonomic identity of the tadpoles and indicated shallow intraspecific genetic differentiation. Tadpole characterizations were illustrated by detailed imagery of live and preserved specimens. Measurements and qualitative traits on oral apparatus provide relevant taxonomic characteristics to distinguish the tadpoles of this species from other lazy toads.

The genus *Scutiger* (Theobald 1868; family Megophryidae) comprises 24 taxa that are found in high altitudes across the Himalayan mountain arc from northern Pakistan, and northern India, through Nepal, Sikkim, and Bhutan, and in the valleys of southern and eastern Tibet, eastwards to mountains of Chinese provinces Gansu, Sichuan, and Yunnan, and southward to the mountains of Myanmar (Frost 2021). Morphologically, this group is characterized by one or two pairs of keratinized spine patches on the chests of males, the absence of a tympanum, and a reduced columella (Chen et al., 2009).

While the diversity of *Scutiger* is high in the central and eastern part of the Himalayan arc, only a single species, *Scutiger occidentalis* (Dubois 1978), can be found in the westernmost regions, in NW India and Pakistan (Hofmann et al., 2017 and references therein), where they inhabit torrents, small natural channels, or clear ponds (pers. observ.). This species was first described from Jammu and Kashmir (Dubois 1978) and further reported from Ladakh, and Himachal Pradesh (Frost 2021; Litvinchuk et al., 2019, and references therein). Previously, it was considered a synonym of *Scutiger nyingchiensis* (Dubois 1987) but variably treated as distinct or as a synonym of that species without critical discussion until recently (Hofmann et al., 2017). Apart from the molecular evidence for the distinctiveness of *S. occidentalis*, this recent study of Hofmann and colleagues addressed the phylogenetic placement of the taxon for the first time, indicating a probably basal position of it relative to all other *Scutiger* species.

Little is known about the life history, ecology, and distribution of *S. occidentalis* and far less recent data is available about the larvae of this taxon. In Pakistan, the Asian lazy toad can be found in the wetlands of Deosai Plains (~4200 m a.s.l.), in pools of clear water connected by small channels running through the swamps (pers. observ.). Breeding starts probably between June and July, with the first summer rain, like in other *Scutiger* species (Jiang et al., 2016). No acoustic data of the mating call exists, and it is still unknown,

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whether tadpoles may develop from eggs deposited the preceding year or if larvae can overwinter in water. So far, tadpoles of *S. occidentalis* have not been morphologically described. However, Annandale (1917) provided a description of tadpoles of *Rana* (*Nanorana*) *pleskei* from Kashmir, which may have been erroneously assigned, and were actually larvae of *S. occidentalis*. A photo of the oral disc and mouth of the larva of *S. occidentalis* was provided by Hofmann et al. (2017) and Litvinchuk et al. (2019), without further description.

In the present study, we provide detailed photographs, and a description of *S. occidentalis* tadpoles from two, so far unknown distribution sites of northern Pakistan. We used mitochondrial and nuclear DNA sequence data to gain information on the intraspecific genetic variation of the species, and to validate the identity of our specimens by assigning them to existing *S. occidentalis* sequences. These rare data may support future research on this taxon in the Northwest Himalaya.

A total of nine larvae (ZFMK 103372–77, ZFMK 2009138–40) were collected in September 2020 during daytime in Lulusar, Khyber Pakhtunkhwa, Pakistan (35.08°N, 73.93°E, 3399 m a.s.l.; locality no. 17, see Fig. 1a, Supplementary Table S1). A further tadpole (ZFMK 103262) was collected at lower elevation near Tarishing, Astore District, Gilgit-Baltistan, Pakistan (35.24°N, 74.73°E, 2941 m a.s.l.; locality no. 19, see Fig. 1a, Supplementary Table S1). The larvae in Lulusar were observed in a small, clear pool of water that run off on a slope next to a road (Supplementary Figs. S1a and b), while the tadpole in Tarishing was captured in a small puddle next to a creek running through a meadow (Supplementary Fig. S1c).

A small piece of the tail was taken from two of the tadpoles (ZFMK 103262, ZFMK 2009138), transferred into absolute ethanol, and stored at −20 °C. Specimens are deposited in the Zoological Research Museum Alexander Koenig (ZFMK), Bonn, Germany.

Tadpoles were processed as previously described (Hofmann et al., 2021a): They were staged according to Gosner (1960), preserved in 70% ethanol, and morphologically described under a stereomicroscope (Zeiss, Stemi 508). Photos of entire tadpoles were taken at the lab with a Nikon D750 digital camera, a 105 mm macro lens, and a teleconverter 2.0x for detail shots. Morphometric measurements were taken with a digital caliper (accuracy ± 0.1 mm). Tadpole terminology follows Altig and McDiarmid (1999); the following measurements were taken: TL (total length), BL (body length), TAL (tail length), TMH (tail muscle heigh at tail base), TMW (tail muscle width at tail base), IOD (interorbital distance), IND (internarial distance), EN (eye-nostril distance), ODW (oral disc width). Characteristics of the oral disc were described according to the system suggested by Altig (1970). The formula of keratodonts (= labial tooth rows) is abbreviated LKRF and is presented according to Altig and McDiarmid (1999), with the anterior (A-) and posterior (P-) rows indicating gaps in brackets and a backslash separating the upper and lower jaw sheaths (Schulze et al., 2015).

Genomic DNA was extracted from tail tissues of the two larvae ZFMK 103262 and ZFMK 2009138 using the DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands) following the manufacturer's protocol. Approximately 668 bp and 985 bp of the mitochondrial cytochrome oxidase subunit 1 (co1) and cytochrome *b* (cytb) genes, and a fragment of 957 bp of the nuclear recombination activating protein 1 (rag1) gene were amplified via the polymerase chain reaction (PCR) using primers and PCR conditions as previously described (Hofmann et al., 2017). PCR products were purified using the mi-PCR Purification Kit (Metabion, Planegg, Germany) and

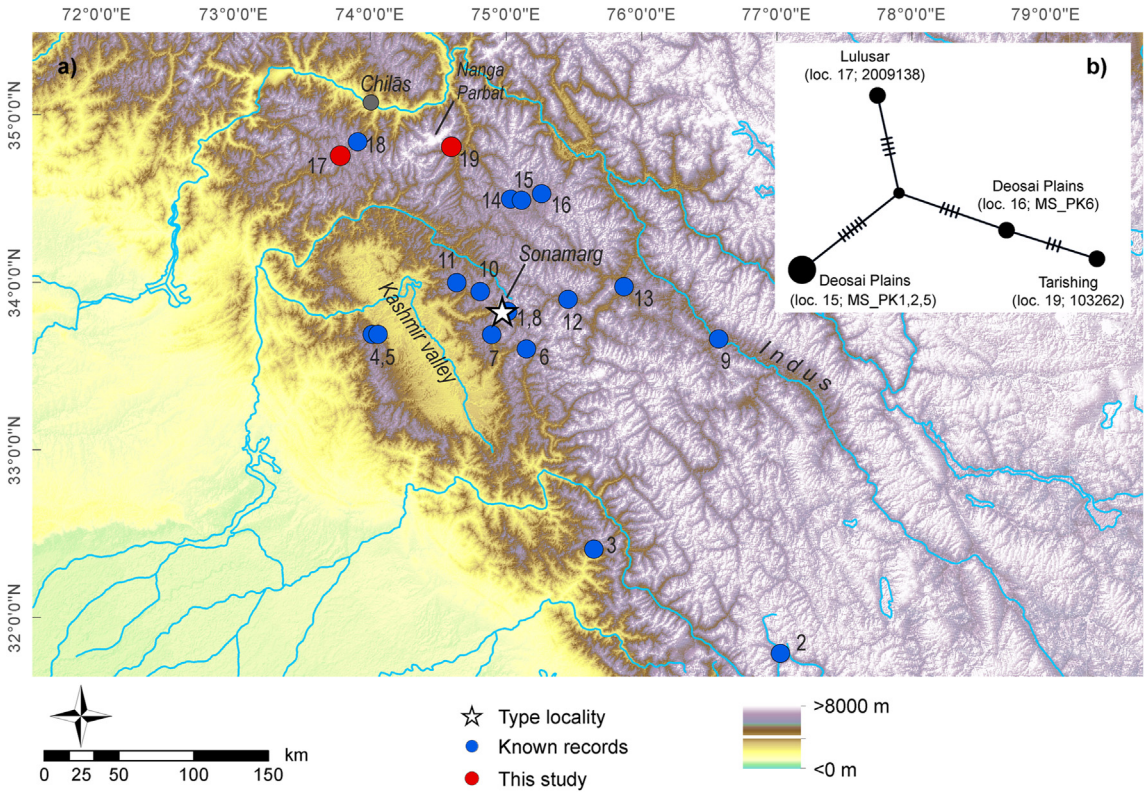

Fig. 1. Distribution map and haplotype network of *Scutiger occidentalis*: **a)** Map showing the localities of the *Scutiger occidentalis* specimens reported herein and further known records; for details of locality numbers see Supplementary Table S1. The type locality of *S. occidentalis* is indicated by a white star. **b)** Median-joining haplotype network inferred from the concatenated mitochondrial and nuclear DNA sequences showing the intraspecific relationships of *S. occidentalis* from three different localities in northern Pakistan.

sequenced in both directions by Macrogen Europe (Amsterdam, Netherlands; http://www.macrogen.com).

We aligned the new sequences (N total = 6; accession numbers co1: MZ073378, MZ073379; cytb: MZ126690, MZ126691; rag1: MZ126688, MZ126689) to a subset of data compiled in our previous study (Hofmann et al., 2017) by eye, using *Leptolalax boringii* as outgroup. No ambiguities, such as deletions, insertions, or stop codons, were found in the alignment. The final concatenated mtDNA + nDNA sequence dataset consisted of 52 taxa and contained 2610 alignment positions of which 436 were phylogenetically informative. We inferred a Bayesian inference (BI) tree using MrBayes v. 3.2.6 (Ronquist et al., 2012). The dataset was partitioned a priori by gene and codon fragments, and PartitionFinder 1.1.1 (Lanfear et al., 2012) was applied to optimize partitions using linked branch lengths, the corrected Akaike Information Criterion (AICc), the greedy search algorithm, and the substitution models implemented in MrBayes. We ran MrBayes for ten million generations (4 runs, 4 chains), sampling trees every 1000th generation. Inspection of the standard deviation of split frequencies after the final run as well as the effective sample size value of the traces was done using Tracer v. 1.7.1 (Rambaut et al., 2018). Uncorrected p-distances were calculated between the *S. occidentalis* sequence data using Mega-X v.11.0 (Kumar et al., 2018) with the pairwise deletion option, 1000 bootstrap replications, and by considering both transitions and transversions. Finally, a median-joining haplotype network was created with PopART (Leigh & Bryant 2015; Bandelt et al., 1999).

Our tadpole sequences (N total = 6) from Tarishing and Lulusar, Pakistan, nested in the clade of *S. occidentalis* that contained sequences from the Deosai Plains, Pakistan (Hofmann et al., 2017); the placement within this clade was highly supported (posterior probability 0.99; Supplementary Fig. S2).

The haplotype network showed little variation among *S. occidentalis* (Fig. 1b), where sequences from Tarishing (locality 19, Fig. 1a) were most distantly related to sequences from one of the localities at Deosai Plains (locality 15, Fig. 1a; p-distance ~1%); airline distance between these two sites is *ca.* 60 km. Noteworthy, the genetic distance between sequences from Tarishing and the second locality at Deosai Plains (locality 16) was considerably lower (~0.2%), although the geographic distance was about the same as to locality 15 (~70 km). Between the two localities at Deosai Plains (locality no. 15 and 16), the p-distance was about 0.6%, while the geographic distance between them was less than 15 km. Pairs with the Lulusar locality (locality no. 17) showed p-distance values from 0.6 to 0.9%. Overall, the uncorrected p-distance ranged between 0.16 and 0.96% among sequences from the different localities (Table 1).

The new record of *S. occidentalis* (locality no. 17; Fig. 1a) is located near the known record site at Babusar (vouchers deposited at Florida Museum of Natural History FLMNH 82455, 82456, 82394 to 82407, 82409, leg. Auffenberg and Rehmann), while the record no. 19 extents the known distribution range of the species to the foothills of the Nanga Parbat (Fig. 1a). All known localities of *S. occidentalis*, including the new records we report herein, are located south and east of the Indus River.

The following tadpole description is based on ten specimens at Gosner stages 26–28 (n = 4) and 31–38 (n = 6) from Northern Pakistan (for details see Table 2): total length 28.2–42.6 mm, 55.3–75.1 mm; body length 9.3–14.8 mm, 17.7–25.5 mm, respectively. Large globular body, ovoid in dorsal and ventral view (Supplementary Figs. S3a–b, S4a–b, S5a–b, and S6a-b), compressed in lateral view (Supplementary Figs. S4c, S5c, and S6c); snout oval in dorsal and ventral view, and slightly tapering in lateral view (Supplementary Figs. 4a–c, S5a-c, and S6a-c). Eyes and nostrils small, positioned and directed dorsolaterally (Supplementary Figs. S3a, S4a and c, S5a and c, and S6a and c). Oral disc large, situated and directed ventrally, laterally emarginated (Supplementary Figs. S4d, S5d, S6d). Marginal papillae uniseriate with a small (tadpole from Tarishing, see Supplementary Fig. S4d) to medium wide (tadpoles from Lulusar; see Supplementary Figs. S5d and S6d) gap on anterior labium, uniseriate also anterolaterally to laterally and on posterior labium. Submarginal papillae present laterally, in the wrinkle between labia (Supplementary Fig. S7). Jaw sheaths serrated, upper one arc-shaped (Supplementary Fig. S7), lower one V-shaped; LKRF 4(2–4)/4(1–3), 4(2–4)/5(1–4), 5(2–5)/4(1–3), or 5(2–5)/5(1–4) (see Table 2). Spiracle sinistral, opening directed posterodorsally, opening less wide than tube (Supplementary Figs. S4e, S5e, S6e).

The typical habitat of *S. occidentalis* on observed localities in northern Pakistan is characterized by swampy meadows and clear ponds, often supplied by flowing water (Deosai Plains; Supplementary Fig. S8a), and on a rocky ground or close to rocky areas as seen in Lulusar and Tarishing (Supplementary Figs. S1a–c). Wet stones and rocks are important for the species to take refuge in crevices during the daytime (Supplementary Fig. S8b). At Lulusar and Deosai both habitats are around lakes.

Adult specimens were active at night when weather conditions and food availability were favorable; at Lulusar, first adult individual came out around 8:00 p.m., at air temperature of ~10 °C. Tadpoles were active during the whole day in September and differed substantially regarding Gosner stage (26–38, see above); the species was syntopic with *Bufotes latastii* that was more common including larvae. Photos of adult specimens from Deosai (Supplementary Fig. S9a), Lulusar (Supplementary Fig. S9b), and Tarishing (Supplementary Figs. 9c–d) indicate a certain morphological variation, at least regarding coloration; further morphological studies are currently ongoing.

So far, *S. occidentalis* is known only from a few places in the Western Himalaya, namely Gilgit-Baltistan, Pakistan, Kashmir and Ladakh regions of the Jammu and Kashmir State, and Himachal Pradesh, India (Dubois 1978; Frost 2021; Litvinchuk et al., 2019), see Fig. 1a. According to a recent species distribution model, the most suitable areas for the species are alpine habitats in Deosai Plains in Pakistan, mountains around the Kashmir Valley, and along the Dras and Suru river valleys in western Ladakh, India; the vertical distribution ranges between 2700 and 4300 m a.s.l (Litvinchuk et al., 2019). All known records, including our own, are located south and east of the Indus River, which suggests that *S. occidentalis*, as the westernmost taxon of the whole genus, reaches its western and northern distribution limit near this broad river valley. The Indus valley probably played a key role for dynamic range shifts of ancestral taxa along the southern slopes of the Himalaya during the uplift of the orogen (Hofmann et al., 2017; Schmidt et al., 2012).

Genetic distances within *S. occidentalis* were small, although the sampled localities in northern Pakistan seem to be geographically remote and well isolated from each other by deep valleys. Moreover, we observed striking phenotypic differences between the adult individuals from different localities (Supplementary Figs. 9a–d). Similar findings have been reported for other high-altitude amphibian species and explained by recent isolation events (Kok et al., 2012). We assume that in *S. occidentalis* gene flow is still maintained or has been maintained until recently and occurs primarily along riverine corridors.

The tadpole of *S. occidentalis* has so far not been described or illustrated in detail, although photographs of the mouthpart have been published previously (Hofmann et al., 2017; Litvinchuk et al., 2019). Annandale (1917) addressed the oral disc of *Rana* (*Nanorana*) *pleskei* tadpoles from Lake Kreshen Sar, Kashmir (locality no. 10 in Fig. 1a), which probably were misidentified and represented in fact *S. occidentalis* (see also Boulenger 1920, who characterized the tadpoles as *Pelobates*-like and assigned them to *Aelurophryne*

Table 1
Uncorrected genetic distances between *Scutiger occidentalis* sequences from different localities (loc.) in northern Pakistan as shown in Fig. 1a. Given are p-distance values (%) for the concatenated sequences (lower-left) and standard error estimates (upper-right). MS_PK1–PK6 = sample numbers as given in Hofmann et al., 2017.

	MS_PK1	MS_PK2	MS_PK5	MS_PK6	Tarishing	Lulusar
Deosai Plains loc. 15 (MS_PK1)	–	0.00	0.00	0.00	0.00	0.00
Deosai Plains loc. 15 (MS_PK2)	0.00	–	0.00	0.00	0.00	0.00
Deosai Plains loc. 15 (MS_PK5)	0.00	0.00	–	0.00	0.00	0.00
Deosai Plains loc. 16 (MS_PK6)	0.52	0.73	0.74	–	0.00	0.00
Tarishing loc. no. 19	0.79	0.95	0.96	0.16	–	0.00
Lulusar loc. no. 17	0.62	0.70	0.70	0.77	0.87	–

Table 2
Measurements (in mm) and counts of voucher specimens (V-ID) of tadpole series of *Scutiger occidentalis* at different Gosner stages. TL: total length; BL: body length; TAL: tail length; TMW: tail muscle width at tail base; IOD: interorbital distance; TMH: tail muscle high at tail base; IND: internarial distance; EN: eye-nostril distance; ODW: oral disc width. Specimens with sequence data are marked bold.

V-ID (ZFMK)	locality	stage	oral formular	TL	BL	TAL	TMW	IOD	TMH	IND	EN	ODW
103262	Tarishing	35	5(2–5)/5(1–4)	60.00	21.56	39.97	4.34	6.00	5.46	3.97	4.03	5.28
103372	Lulusar	37	5(2–5)/5(1–4)	69.99	24.17	46.59	5.15	6.91	5.64	4.33	4.14	5.46
103373	Lulusar	28	4(2–4)/5(1–4)	42.63	14.80	28.41	2.23	4.21	3.30	3.01	2.49	3.42
103374	Lulusar	37	5(2–5)/4(1–3)	70.35	23.42	43.66	5.31	7.92	5.80	4.34	3.62	5.84
103375	Lulusar	38	4(2–4)/5(1–4)	75.08	25.49	48.57	5.71	7.27	6.75	4.33	4.27	6.38
103376	Lulusar	33	5(2–5)/5(1–4)	60.22	20.02	41.05	3.82	5.44	4.47	3.79	3.29	4.62
103377	Lulusar	31	5(2–5)/5(1–4)	55.31	17.72	37.39	3.20	5.55	3.73	3.83	3.18	4.22
2009138	Lulusar	26	4(2–4)/4(1–3)	28.17	9.26	17.67	1.24	2.62	1.66	1.77	1.66	2.00
2009139	Lulusar	27	4(2–4)/4(1–3)	32.65	10.56	20.59	1.38	3.35	2.07	2.35	2.26	2.41
2009140	Lulusar	26	4(2–4)/4(1–3)	31.05	10.88	19.84	1.69	2.83	1.84	2.48	1.92	2.53

[*Scutiger*]). The keratodont row formula given by Annandale (1917) for *Rana pleskei* tadpoles (LKRF 4[2–4]/5[1–4]) differs from the formula provided by Fei et al. (2012) for *Nanorana pleskei* (which does not occur in the Kashmir region; LKRF 3[2–3]/3[1]). It is also substantially distinct from the oral apparatus specified for *Nanorana vicina* (Gill et al., 2020), but similar to that we present for *S. occidentalis*.

Based on our data, larvae of *S. occidentalis* have a large body (total length 31.1–42.6 mm, stages 26–28; 55.3–75.1 mm, stages 31–38) with a strong muscular tail; body length 10.9–14.8 mm, and 17.7–25.5 mm, respectively. The mouth is located almost ventrally but close to the snout tip. The oral disc is prominent, bordered by marginal papillae with a small gap on the upper lip, with some submarginal papillae at the libs' commissure, and the serrated jaws are robust. The number of keratodonts varies between four and five in the anterior and posterior part of the mouth, with only the outermost (A_1, $P_{4(5)}$) uninterrupted, and with A_2–$A_{4(5)}$ and P_1–$P_{3(4)}$ being discontinous rows, which is consistent with the arrangement of keradonts seen in Hofmann et al. (2017) and Litvinchuk et al. (2019).

Morphologically, specifically with respect to the keradont row formula, the larvae of *S. occidentalis* seem to be most closely related to *Scutiger glandulatus, S. muliensis*, and *S. nyingchiensis* (5[2–5]/5[1–4]), as well to *S. mammatus* (5[2–5]/6[1–5] or 5[2–5]/5[1–4]), and *S. ningshanensis* (6[2–6]/6[1–5]) (Fei et al., 2012). Interestingly, all of these species occur in the East Himalaya (*S. nyingchiensis*) and in regions of the southeastern margin of the Tibetan Plateau (*S. glandulatus, S. mammatus, S. muliensis*, *S.ningshanensis*). The morphological relatedness of the westernmost lazy toad *S. occidentalis* to these taxa, which occur at the diametrically opposite end of the Himalaya-Tibet orogen, is in line with the apparent paradoxical phylogenetic pattern within the genus (Hofmann et al., 2017). Similar findings have been reported in spiny frogs (tribe Paini, Dicroglossidae) and explained by trans-Tibet dispersal from east to west during the Tertiary (Hofmann et al., 2021b).

All specimens investigated in the present study were observed in a small pool (diameter less than one meter) of small flowing water under (warm-)temperate conditions in the high-montane to sub-alpine zone. Thus, given the habitats in which we found *S. occidentalis*, we assume that this taxon is not a torrent species but occupies clear, stagnant water pools of slow-running creeks. We also suspect, that larvae of *S. occidentalis* can overwinter in shallow standing or flowing permanent waters, since we found tadpoles at early Gosner stages in September (tadpoles of the species were even found in October, see Litvinchuk et al., 2019), making it unlikely that they will metamorphose before the winter. Overwintering of tadpoles have been also reported in *Scutiger spinosus* (Jiang et al., 2016), which occurs in the Eastern Himalaya.

Apart from information on genetic variation and new records of *S. occidentalis* in Pakistan, we provide the first detailed illustration and description of a tadpole series of the species that should facilitate the identification of this species, e.g., during tadpole surveys in Pakistan and India. Our study highlights the still limited knowledge about the amphibian biodiversity in Pakistan, and particularly of their larval stages. We encourage further research across Pakistan that focus on amphibians, for a better understanding of their distribution, taxonomy, and diversity, especially also for monitoring and conservation efforts.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2021.08.008.

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Supplementary information

Genetic variation and tadpoles of the westernmost Himalayan lazy toad *Scutiger occidentalis*

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Table S1. List of localities where *Scutiger occidentalis* is known to occur. Coordinates are given in decimal degrees. The record by Annandale (1917) is marked with an asterisk; the reported specimens might have been misidentified as *Rana* (*Nanorana*) *pleskei* but were in fact *S. occidentalis*.

ID/No	*Locality*	*Long (N)*	*Lat (E)*	*Altitude* (m a.s.l.)	*Reference*
1	India, Sonamarg	34.3000	75.2900	2920–2940	Dubois (1978)
2	India, Chandra Sar Lake	32.4752	77.6171	3900	Ahmad (1946)
3	India, Trakar Rani Kot	32.9517	76.1904	2896	Litvinchuk et al. (2019)
4	India, Khilanmarg	34.0400	74.3700	2680–2850	Dubois (1978)
5	India, Gulmarg	34.0484	74.3804	2690	Dubois (1978)
6	India, Shesh Nag lake	34.0943	75.4971	3658	Ahmad (1946)
7*	India, Lidarwat	34.1500	75.2300	2740	Annandale (1917)
8	India, Shukdhari	34.3019	75.2894	2920–3200	Dubois (1978)
9	India, Khalsi	34.3188	76.8776	2750	Sahi & Duda (1985)
10	India, Kreshen Sar [Krishansar] Lake	34.3971	75.1004	3677	Annandale (1917)
11	India, Gangabal Lake	34.4300	74.9200	3567	Annandale (1917)
12	India, Dras	34.4300	75.7440	~3082	Chabanaud (1922)
13	India, Kargil	34.5543	76.1366	3660	Duda & Sahi (1977)
14	Pakistan, Deosai, Sheosar Lake	34.9800	75.2200	~4100	Hofmann et al. 2017
15	Pakistan, Deosai	34.9833	75.3000	4300	Ficetola et al. 2010
16	Pakistan, Deosai	35.0400	75.4400	4300	Hofmann et al. 2017
17	Pakistan, Lulusar	35.0800	73.9300	3399	this study
18	Pakistan, 4.2 km N Babusar	35.1800	74.0400	~3300	Hofmann et al. 2017
19	Pakistan, Tarishing	35.2400	74.7300	2941	this study

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Figure S1. Tadpole of *Scutiger occidentalis* and collection sites in Pakistan: **a)** tadpole at Lulusar, Khyber Pakhtunkhwa, Pakistan (3399 m a.s.l.); **b)** habitat at Lulusar; **c)** collection site at Tarishing, Astore District, Gilgit-Baltistan, Pakistan (2941 m a.s.l.); photo credits: Daniel Jablonski (a, b), Sylvia Hofmann (c).

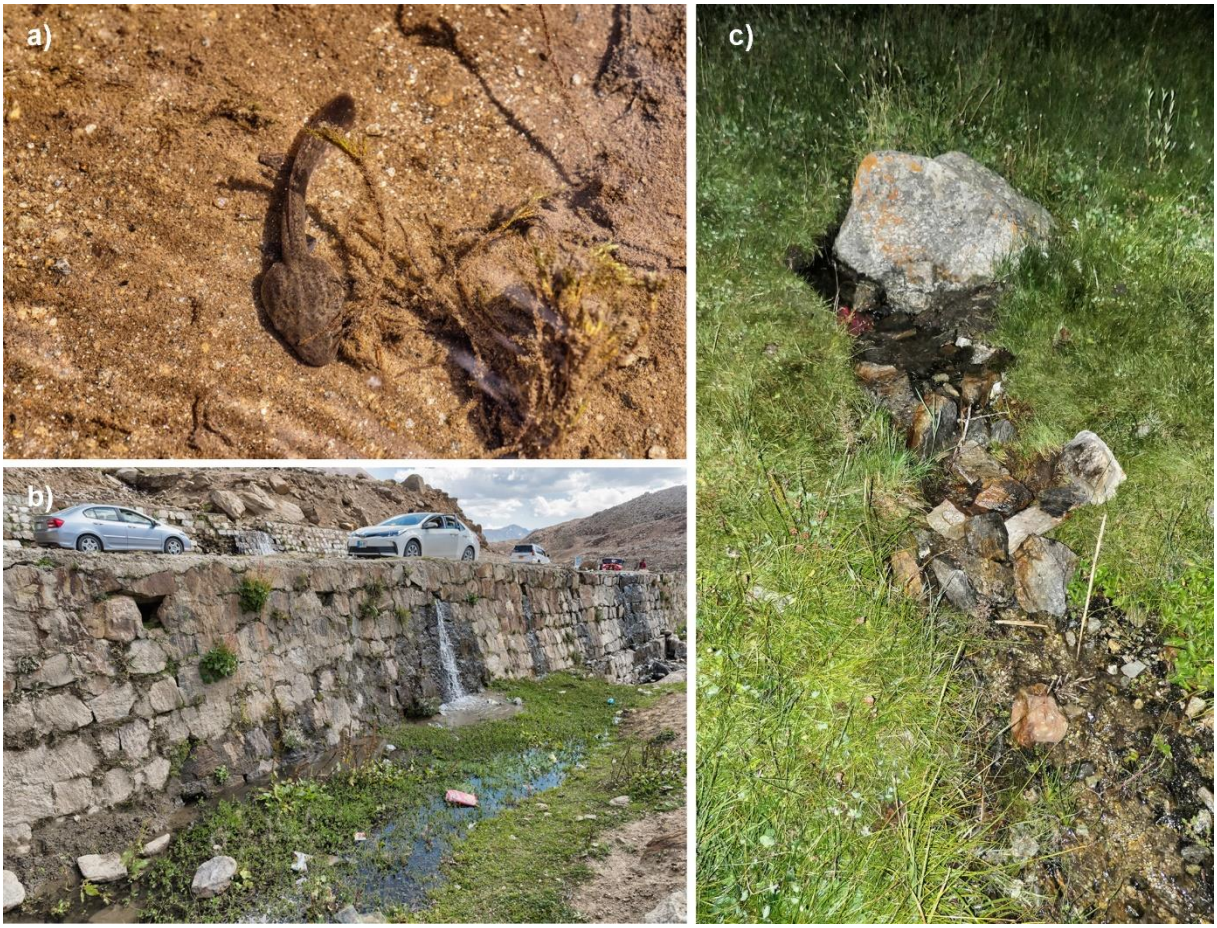

Figure S2. Bayesian inference tree based on concatenated mtDNA and nDNA sequence data inferred with MrBayes v. 3.2.6 (Ronquist et al. 2012). *Scutiger* (*S.*) is followed by the species name and by sample ID (Hofmann et al. 2017). Branch nodes supported by a posterior probability (pp) value ≥ 0.95 are marked with a small rectangle. ZFMK-IDs of the two tadpoles sequenced herein are in red.

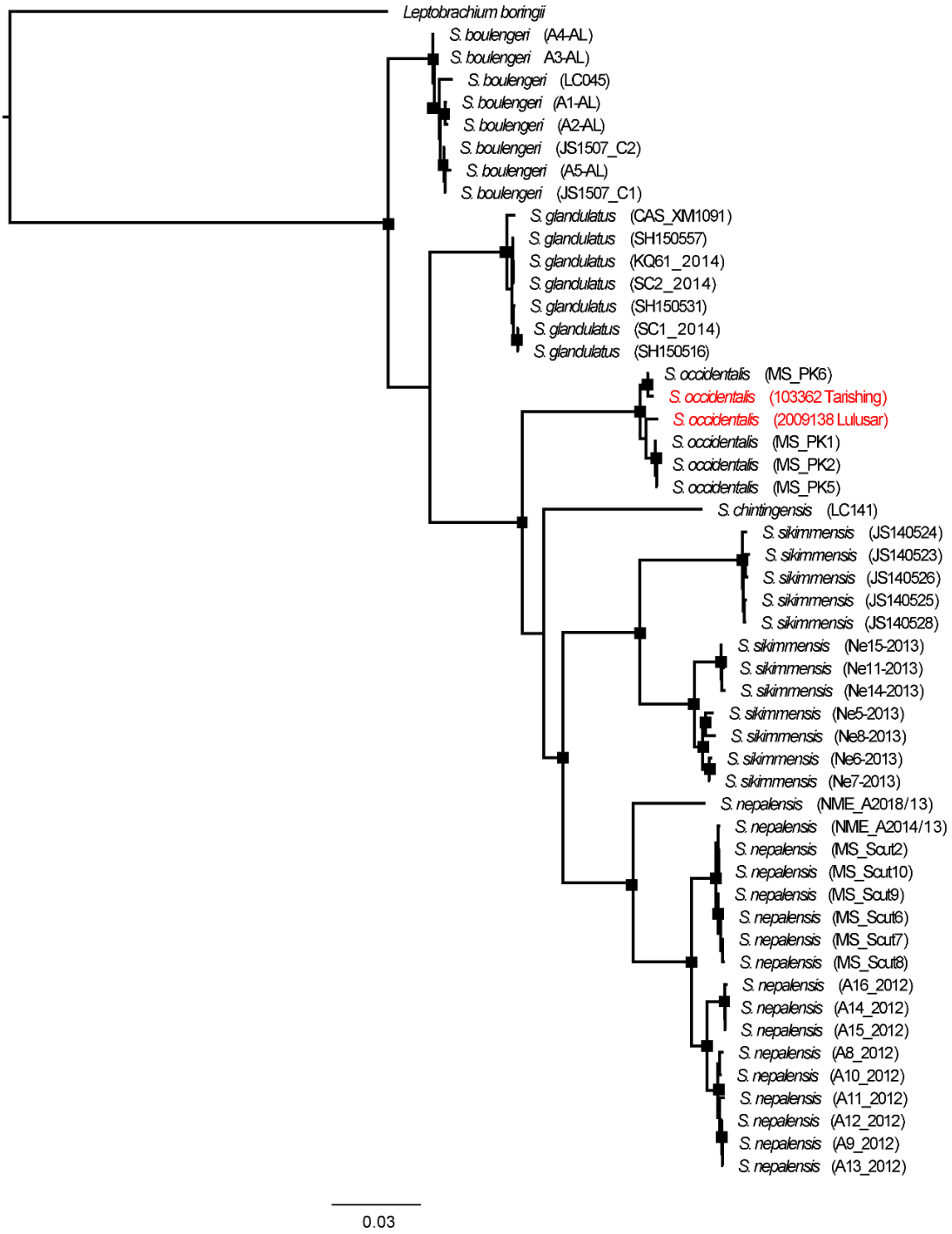

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Figure S3. Live tadpole of *Scutiger occidentalis* from Lulusar, Khyber Pakhtunkhwa, Pakistan (3399 m a.s.l.): **a)** dorsolateral view, and **b)** ventral view. Photo credit: Daniel Jablonski.

Figure S4. Preserved tadpole specimen (ZFMK 103262; Gosner stage 35) of *Scutiger occidentalis* from Tarishing (Trashing), Astore District, Gilgit−Baltistan, Pakistan (2941 m a.s.l.): **a-c)** dorsal, ventral, lateral views, **d)** mouthpart, and **e)** closeup of the spiracle.

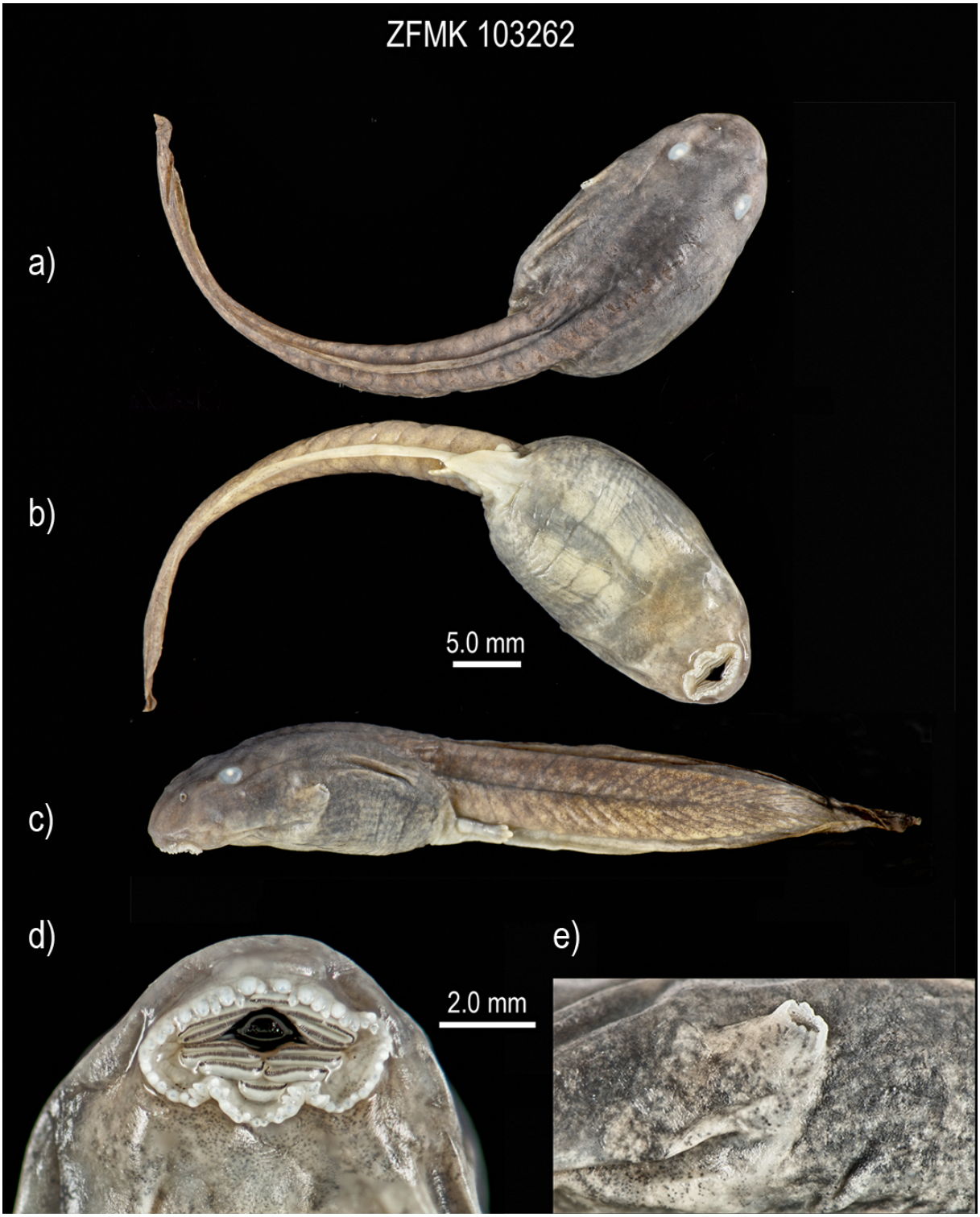

Figure S5. Preserved tadpole specimen (ZFMK 103372; Gosner stage 37) of *Scutiger occidentalis* from Lulusar, Khyber Pakhtunkhwa, Pakistan, 3399 m a.s.l.: **a-c)** dorsal, lateral, and ventral view, **d)** mouthpart, and **e)** spiracle marked with a red arrow.

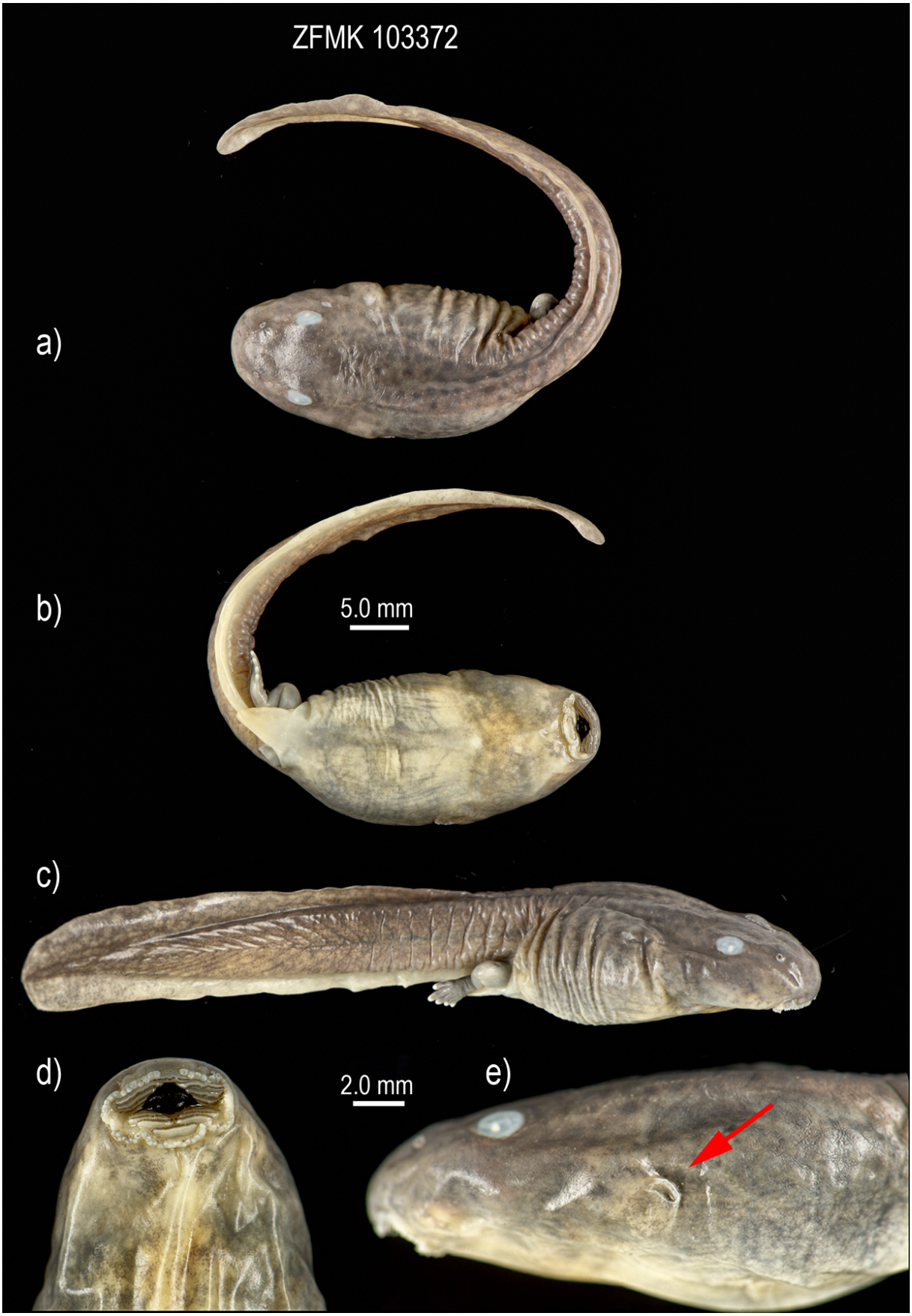

Figure S6. Preserved tadpole specimen (ZFMK 103375; Gosner stage 38) of *Scutiger occidentalis* from Lulusar, Khyber Pakhtunkhwa, Pakistan, 3399 m a.s.l.: **a-c)** dorsal, lateral, and ventral view, **d)** mouthpart, and **e)** spiracle marked with a red arrow.

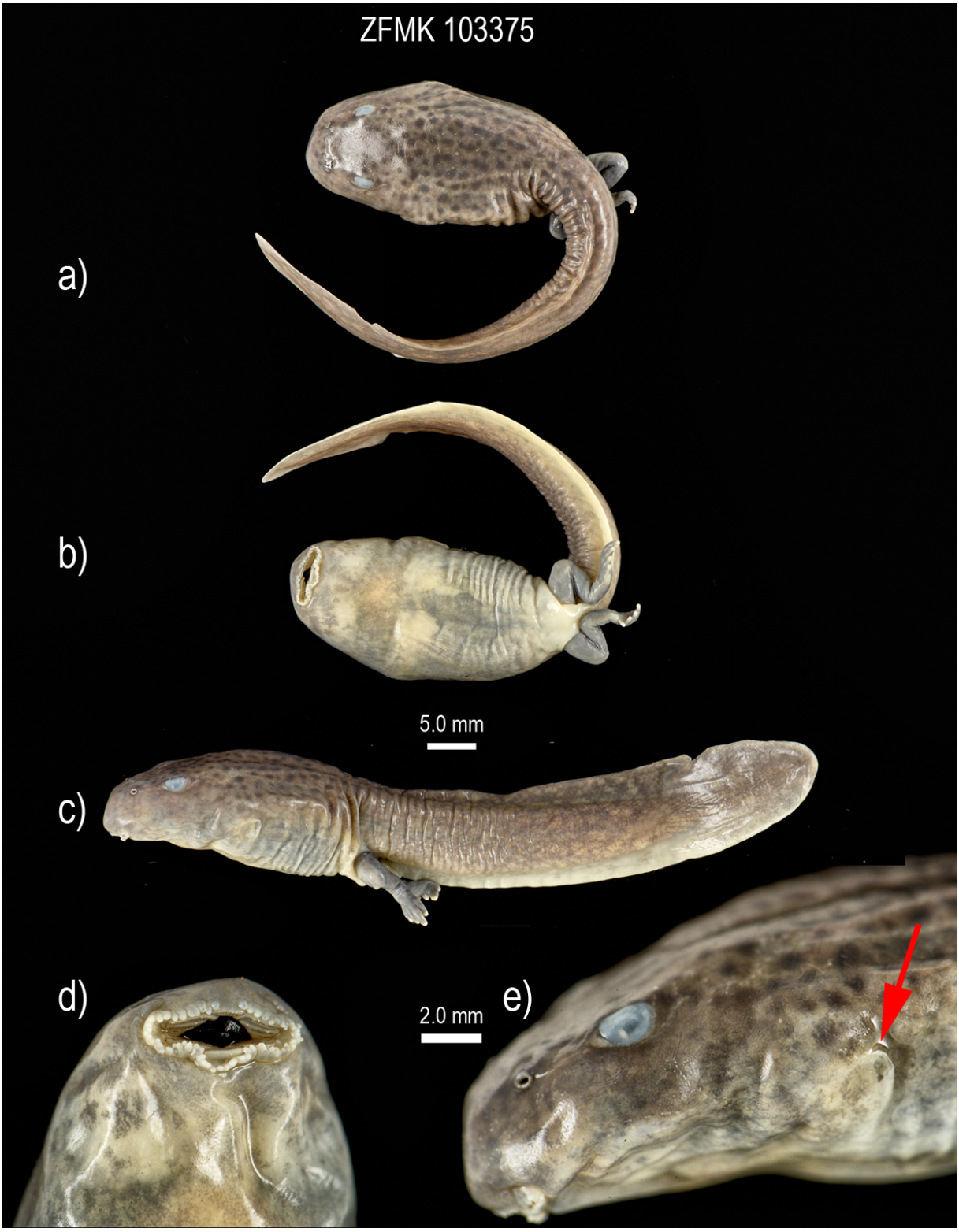

Figure S7. Drawing of oral disc of *Scutiger occidentalis* tadpole (credit: Sylvia Hofmann).

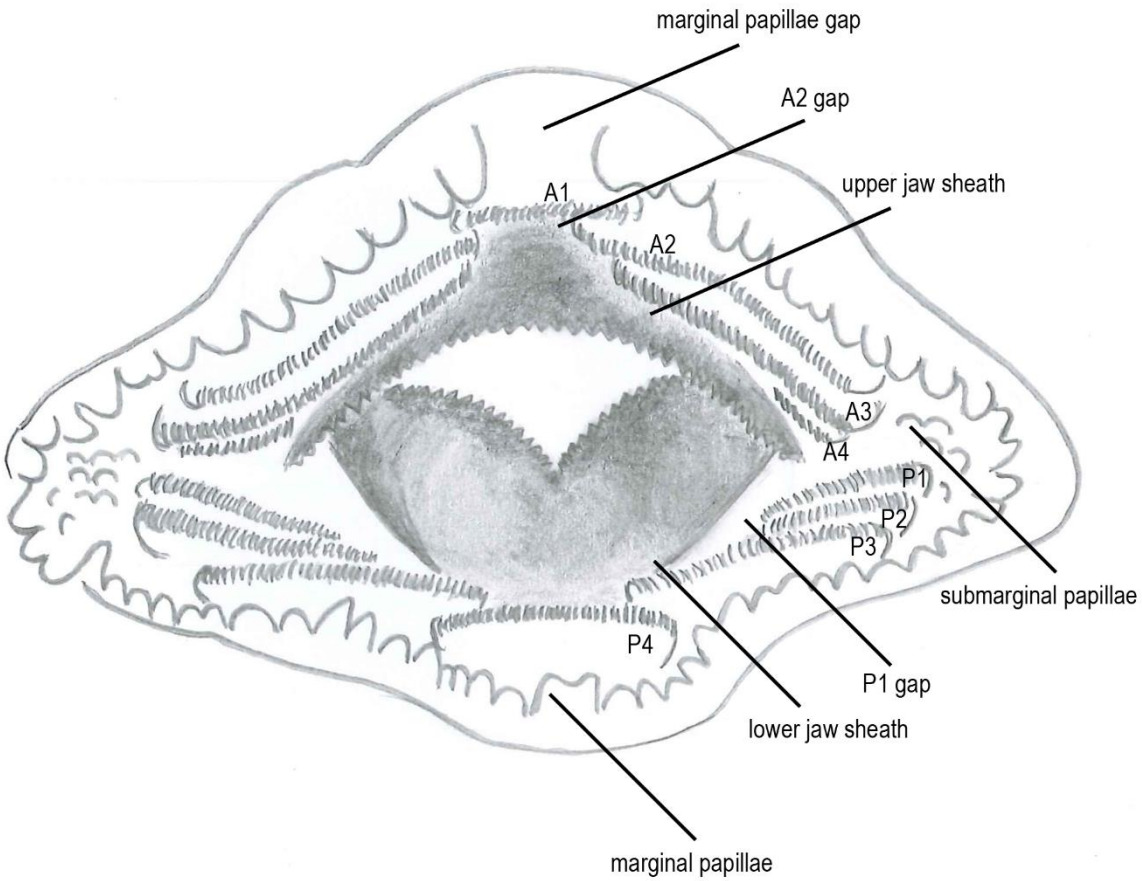

Figure S8. Habitat types of *Scutiger occidentalis*. Photo credits: Sylvia Hofmann (a), Daniel Jablonski (b).

Figure S9. Adult specimens of *Scutiger occidentalis*: **a)** from Deosai Plains, Astore District, Gilgit−Baltistan, Pakistan (4200 m a.s.l.), photo credit: Sylvia Hofmann; **b)** from Lulusar, Khyber Pakhtunkhwa, Pakistan (3399 m a.s.l.), photo credit: Daniel Jablonski; **c, d)** from Tarishing, Astore District, Gilgit-Baltistan, Pakistan (2941 m a.s.l.), photo credit: Sylvia Hofmann.