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Larval description of *Theloderma albopunctatum* (Liu & Hu, 1962) (Anura: Rhacophoridae) from Northern Vietnam, with comparison between the North-Vietnamese and North-eastern Thai clades

PHILIPP GINAL[1*], LISA M. SCHMITZ[1] & DENNIS RÖDDER[1]
[1]*LIB, Museum Koenig Bonn, Leibniz Institute for the Analysis of Biodiversity Change, Adenauerallee 127, 53113 Bonn, Germany*
L.Schmitz@leibniz-lib.de
D.Roedder@leibniz-lib.de; https://orcid.org/0000-0002-6108-1639
[*]*Corresponding author. Philipp.ginal@gmx.de; https://orcid.org/0000-0001-6491-554X*

The mossy frogs of the genus *Theloderma* Tschudi comprise 28 described taxa (Sivongxay *et al.* 2016; Frost 2022), which are distributed from north-eastern India and Myanmar to southern China, across the peninsula of Indochina and Malaysia, to Indonesia (Poyarkov *et al*. 2015; Frost 2022). *Theloderma albopunctatum* is a small-sized taxon that is assigned to the *T.-asperum* species complex (Poyarkov *et al*. 2015, 2018; Sivongxay *et al*. 2016; Dever 2018). For long time, it has been believed to be a synonym of *T. asperum*. However, genetic analyses revealed that both taxa show significant differences. Currently, populations south of the Isthmus of Kra (southern Thailand, Malayan peninsular) are assigned to *T. asperum*, while populations north of it (southern China, northern and central Vietnam, adjacent Laos, south-eastern Cambodia) are assigned to *T. albopunctatum*. In addition, this species complex might contain further cryptic species (cf. Nguyen *et al*. 2015; Poyarkov *et al*. 2015) and according to Chunskul *et al*. (2021) four genetic groups do exist: group A comprises *T. albopunctatum* from southern and central Vietnam, Laos, central and north-eastern Thailand; group B is composed of populations from northern Vietnam and China; group C ranges from north-western Vietnam to northern Thailand and Myanmar; and group D is distributed in northern Vietnam (Thanh Hoa).

Several publications concerning members of *Theloderma* focus on the larval description. At the moment, 15 tadpole descriptions out of 28 recognized species are available, including those of *T. albopunctatum* (Table 1). The tadpole description of *T. albopunctatum* is based on specimens collected in north-eastern Thailand (group A). Considering the genetic divergence and the potential occurrence of cryptic species in the *T.-albopunctatum* complex, herein we describe the tadpoles of *T. albopunctatum* from the northern Vietnamese clade (group B) and compare them to tadpoles of the Thai clade and all other described tadpoles of the genus.

Our captive breeding group consisted of six adult *T. albopunctatum*, obtained from a private breeder. Species identification was already confirmed in Ginal *et al*. (2021) by DNA barcoding using a fragment of the mitochondrial 16S rRNA. Sequences were generated following Koch *et al*. (2013) and subsequently compared with newly generated sequences of specimens from the ZFMK collection as well as 167 sequences of *Theloderma* spp. obtained from GenBank. We used the web version of Automatic Barcode Gap Discovery (ABGD; Puillandre *et al*. 2012; https://bioinfo.mnhn.fr/abi/public/abgd/) to assign sequences to species. Furthermore, uncorrected p-distances were calculated using the ape package (Paradis & Schliep 2019) for R (R Core Team 2019) to investigate sequence divergences. All sequences produced by this study were uploaded to GenBank (accession numbers MZ376706 – MZ376725). The results of the DNA barcoding by ABGD assigned the genetic samples to *T. albopunctatum*. Observed p-distances of our samples of *T. albopunctatum* to *T. albopunctatum*. Observed p-distances MZ376706 – MZ376725 of our samples of T. *albopunctatum* to other *T. albopunctatum* range from 0.00% to 5.46%, with the total intraspecific variance of p-distances ranging from 0.00% to 6.81% (n = 34). The samples most similar to ours were from Sa Pa, northern Vietnam (p-distance 0.00% to 0.33%) and belong to group B. All other species of *Theloderma* were divergent by 6.77% to 20.06%.

Specimens were kept in a terrarium measuring 40 x 40 x 50 cm. The enclosure was equipped with several cork bark pieces and plants (i.e., *Epipremnum* sp., *Scindapsus* sp.). The bottom was filled with a dilution of black tea at a height of c. 5–7 cm. Black tea was used to reconstruct the microhabitat of a water-filled tree hole as it contains a high content of tannins. According to our experiences, this is necessary to keep the frogs at healthy conditions. The ambient temperature ranged between 18 and 28°C, depending on daytime and season. The frogs were misted manually once or twice a week.

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Twice a week, the adult frogs were fed with domestic crickets (*Acheta domesticus*) and fruit flies (*Drosophila* sp.). Before the insects were fed to the frogs, they were gut loaded with a variety of vegetables and fruits to supply additional nutrients. *Theloderma albopunctatum* have been reproducing regularly in an interval of approx. two weeks and the tadpoles as well as juveniles were raised in the same enclosure. Twice a week, tadpoles were fed with Repashy Superfood (Savory Stew Omnivore Gel Premix). Additionally, tadpoles were feeding on drowned insects, the faeces of the adult frogs and other micro-food items (i.e., algae) that permanently persist in the water of the enclosure.

TABLE 1. Descriptions of tadpoles of *Theloderma*. Colour, labial tooth row formula (LTRF), and mean values of total length (TL), body length (BL), and tail length (TAL) in millimetres are consigned.

Species	Main features	Reference
T. albopunctatum (Liu & Hu)		
Northern Vietnam	Body dark grey to black; LTRF 3(2-3)/3(1); TL 32.3, 14.7, TAL 18.7 (Stages 29–38)	this study
North-eastern Thailand	Body dark grey to black; LTRF 3(2-3)/3(1), 2(2)/2(1): TL 17.0, BL 6.7, TAL 11.3 (Stage 25)	Chunskul *et al*. 2021
T. asperum (Boulenger)	Body ash grey; LTRF 3(2-3)/3(1); TL 40.0, BL 15.6, TAL 24.4 (Stage 31)	Leong & Lim 2003
T. auratum Poyarkov *et al.*	Body dark grey; LTRF 5(3–5)/3; TL 29.9, BL 11.4, TAL 18.5 (Stage 29)	Poyarkov *et al*. 2018
T. bicolor (Bourret)	Body black to dark blue with red, brown and grey spots; LTRF 3(2-3)/3; TL 29.9, BL 12.0, TAL 19.0 (Stage 25)	Gawor *et al*. 2012; Kropachev *et al.* 2013
T. corticale (Boulenger)	Body dark grey to black; LTRF 4(2–4)/3(1); TL 50.9, BL 21.9, TAL 29.0 (Stage 32)	Gawor *et al*. 2012; Kropachev *et al.* 2013
T. gordoni Taylor	Body black; LTRF 4(2–4)/3; TL 52.5, BL 19.1, TAL 33.4 (Stage 31)	Kropachev *et al*. 2018
T. laeve (Smith)	Body unpigmented, semi-transparent, fleshy, pinkish appearance; LTRF 4(2-4)/3; TL 25.0, BL 10.2, TAL 14.8 (Stage 31)	Orlov *et al*. 2012
T. lateriticum Bain *et al.*	Body dark brown; LTRF 3(2-3)/3; TL 26.0–28.7, BL 10.1–10.7, TAL 15.9–18.0 (Stages 32–38)	Chen *et al*. 2019; Le 2020
T. moloch (Annandale)	Body dark grey to black; LTRF 4(2-4)/3; TL 55.0, 58.0, BL 17.0, 20.0, TAL 38.0 (Stage not detailed)	Annandale 1912
T. nebulosum Rowley *et al.*	Body dark brown; LTRF 4(2-4)/3; TL 24.6, BL 10.3, TAL 14.3 (Stage 28)	Rowley *et al*. 2011
T. palliatum Rowley *et al.*	Body dark brown; LTRF 4(2-4)/3; TL 21.2–23.0, BL 9.2–10.1, TAL 11.9–12.9 (Stages 27–28)	Orlov *et al*. 2012
T. ryabovi Orlov *et al.*	Body black; LTRF 4(2-4)/3, 4(2-4)/3(1); TL 65.4, BL 22.9, TAL 42.5 (Stage 31)	Kropachev *et al*. 2019
T. horridum (Boulenger)	Body dark brown to blackish; LTRF 4(2-4)/3; TL 15–50 (Stage not detailed)	Boulenger 1903
T. stellatum Taylor	Body dark brown; LTRF 4(2-4)/3(1); TL 30.6, BL 13.3, TAL 17.3 (Stage 28)	Wassersug *et al*. 1981
T. vietnamense Poyarkov *et al.*	Body dark brown to yellowish brown; LTRF 3(2-3)/3, 4(2-4)/3, 5(2-5)/3; TL 26.1, 30.8, BL 11.6, 13.5, TAL 14.5, 17.3 (Stage 31)	Inger *et al*. 1999, Poyarkov *et al*. 2015

Nine tadpoles at Gosner Stages 29 (n = 3), 31 (n = 1), 34 (n = 1), 36 (n = 1), 37 (n = 2) and 38 (n = 1; Gosner 1960) were euthanized using a solution of saturated chlorbuthanol until no movement was visible anymore for several minutes. The tadpoles were fixated in a 4% formalin solution for 24 hours and subsequently transferred into ethanol 45% for 24 h, ethanol 55% for 24 h and ethanol 75% as final storage solution at the herpetological collection of ZFMK (ZFMK

103953). The nine freshly preserved tadpoles were measured with a digital calliper (Type: INOX Hardened 150 mm) under a binocular (Type: Zeiss Stemi 508) to the nearest of 0.1 mm. The following 20 metrics were taken according to prior studies describing tadpoles (Poyarkov *et al*. 2015; Kropachev *et al*. 2018; Klein *et al*. 2020): MBH, maximum body height; BL, body length; MBW, maximum body width; ED, eye diameter; IND, internarial distance (between centres of the nares); IOD, interorbital distance (between pupils of the eyes); UF, maximum upper fin height; LF, maximum lower fin height; MTH, maximum tail height; END, naro-pupilar distance; ODW, oral disc width; RND, rostro-narial distance (tip of snout to centre of nares); RSD, rostro-spiracle distance (tip of snout to spiracle); TAL, tail length; TL, total length; TMH, tail musculature height at base; TMW, tail musculature width at base; BWE, body width at eye level; BWN, body width at nostril level; RED, rostro-eye distance (tip of snout to centre of eye).

Tadpole description. The description of the tadpoles is based on one specimen at Stage 31. Body shape (Fig. 1A) oval and slightly elongated (MBW/BL 0.69); snout short and slightly pointed (RED/BL 0.30, BWN/BWE 0.65); nares small, rounded, located on dorsal side of body with lateral orientation, situated closer to snout than to eyes (RND/RED 0.44); eyes large (ED/BL 0.10), located on dorsal side of body with lateral orientation; internarial distance slightly smaller than interorbital distance (IND/IOD 0.83); single, sinistral spiracle not visible from dorsal view. Body moderately depressed in lateral view (MBH/MBW 0.75) with rounded snout; spiracle located below the longitudinal axis, on second half of body (RSD/BL 0.65), inner wall free from body, opening is round, spiracle tube short; maximum body height from front of base of tail on dorsal side to second half of body of ventral side; tail slightly longer than body, rounded at tip (TAL/BL 1.22, TAL/TL 0.57); musculature well developed (TMH/MTH 0.58, TMW/MBW 0.27); "V"-shaped myosepta visible along whole length of tail, most intensely at first half of tail; upper fin originates dorsally at base of tail, higher than lower fin (UF 1.97 mm, LF 1.65 mm); vent tube has medial position, emerges sagittal from abdomen between two hind limb buds, ends with short rounded opening. Oral disc (Fig. 1B) elliptical, located ventrally, size nearly half of body width (ODW/MBW 0.40); small, rounded papillae located marginal at lower jaw sheath, absent at upper labium, except most lateral part; numbers of marginal papillae range from 54 up to 69; submarginal papillae present in some individuals, can also be absent, ranging from 0 up to 14 per individual; some papillae develop labial teeth; anterior labium contains one marginal tooth row, two tooth rows on each side of mouth; outermost row shorter than inner row; posterior labium contains three tooth rows; small medial gap in innermost tooth row, rows get shorter to outside; labial tooth row formula 3(2-3)/3(1); jaw sheaths black, with serrations on edges; upper jaw sheath wider and higher than lower jaw sheath, indentation at top.

Coloration in life. Tadpoles are ashy grey at body and tail; venter is slightly paler; tail translucent with dark pigmentation; vent tube unpigmented (Fig. 1C).

Colouration in preservative. Snout and dorsum of tadpoles dark grey with most pigmentation on dorsum; light symmetrical patches barely pigmented on lateral side and anterior eyes; pigmentation decreases as well towards posterior; eyes also surrounded by dark colouration; ventral side of snout dark grey; light patches starting laterally run around whole body, sharply differentiated by roof shaped outlining of dark area starting in centre of ventral side of body, directs towards posterior; patch equally becomes much lighter towards posterior body; vent tube unpigmented; anterior tail, especially base, more pigmented than second half, tail slightly lighter; fins coloured in light grey, slightly transparent; developing hind limb buds unpigmented, whitish as well as medially oriented vent tube.

Measurements. All nine tadpoles are considered, and measurements are given in mm, as median (range): MBH 7.3 (6.7–8.2); BL 14.7 (14.1–16.3); MBW 10.0 (8.9–11.2); ED 1.5 (1.2–1.9); IND 2.3 (2.1–2.6); IOD 3.5 (3.1–4.5); UF 1.9 (1.8–2.1); LF 1.7 (1.5–1.9); MTH 5.6 (5.0–6.2); END 2.7 (2.3–3.1); ODW 3.8 (3.2–4.1); RND 2.0 (1.9–2.3); RSD 9.8 (8.7–11.2); TAL 18.7 (17.6–22.1); TL 32.3 (30.9–36.4); TMH 3.3 (2.8–3.6); TMW 2.8 (2.3–3.6); BWE 8.6 (7.9–9.6); BWN 5.7 (5.4–6.9); RED 4.5 (4.1–5.6); MBW/BL 0.67 (0.59–0.72); RED/BL 0.31 (0.29–0.34); ED/BL 0.10 (0.09–0.13); RND/RED 0.45 (0.41–0.50); IND/IOD 0.61 (0.50–0.83); TMW/MBW 0.28 (0.23–0.36); MBH/MBW 0.74 (0.72–0.77); TAL/BL 1.23 (1.20–1.43); TAL/TL 0.57 (0.56–0.61); TMH/MTH 0.56 (0.52–0.60); ODW/MBW 0.38 (0.36–0.41); RSD/BL 0.67 (0.60–0.69); BWN/BWE 0.68 (0.63–0.78); marginal papillae 64 (53–69); submarginal papillae 5 (0–14).

Comparison to other *Theloderma* tadpoles. Tadpoles from north-eastern Thailand described by Chunskul *et al*. (2021) are very similar to tadpoles in this study, which were assigned to specimens originating from northern Vietnam. Body shape and colour are alike, and minor differences regarding LTRF [some Thai individuals showed LTRF 2(2)/2(1)] can be interpreted as ontogenetic variations. The comparison of body measurements suggests that, although most proportions are similar, northern Vietnamese tadpoles have shorter tails (TAL/BL about 10% smaller than in Thai tadpoles) and wider oral discs (ODW/MBW about 10% larger in tadpoles here studied).

Table 1 shows summarized descriptions of tadpoles of 15 *Theloderma* species. All *Theloderma* tadpoles share a robust appearance with a distinctly flattened body, dorsally oriented eyes and nostrils, and dark body colouration ranging

from dark brown, grey, blue to black (except for *T. laeve*, which is mainly unpigmented, fleshy, pinkish). Furthermore, they show similar spiracles, vent tubes, oral disc positions and shapes of lower and upper jaw sheaths. Most tadpoles are easily distinguishable from those of *T. albopunctatum* by their LTRF. Twelve species (including *T. vietnamense* where the lowest LTRF corresponds to young tadpoles) have high LTRF with 4 or 5 upper tooth rows. Only three species share the LTRF 3/3 of *T. albopunctatum*. Among them, tadpoles of *T. bicolor* are larger at comparable stages (almost twice the total length of Thai *T. albopunctatum* tadpoles), while tadpoles of *T. lateriticum* are slightly smaller. The features analysed do not allow to distinguish tadpoles of *T. asperum* from those here studied.

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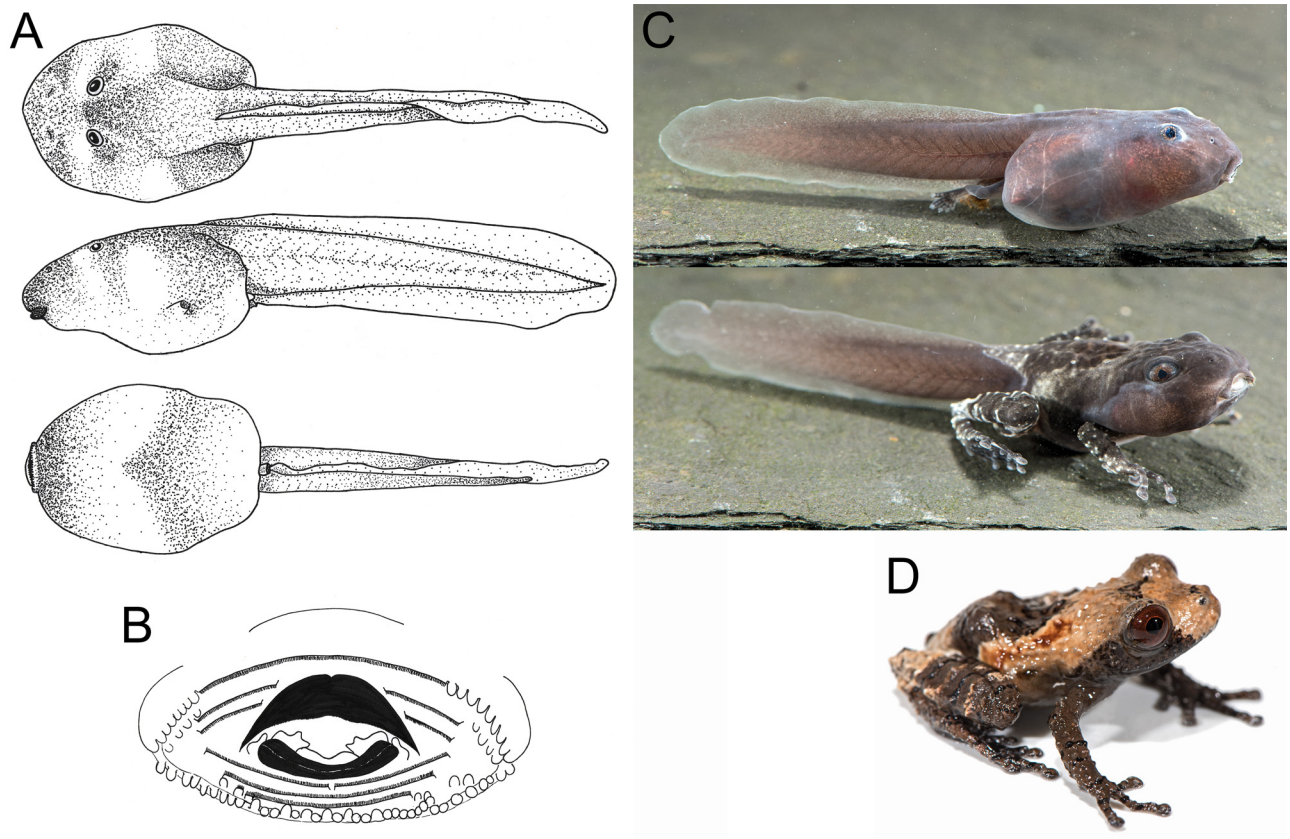

FIGURE 1. *Theloderma albopunctatum* from northern Vietnam: (A) tadpole at Stage 31 (ZFMK 103953) in dorsal, lateral, and ventral views (hind limb buds excluded to show the vent tube), (B) oral disc, frontal view, (C) tadpoles at stages 37 and 42 in life, (D) juvenile frog in life. Photographed by Morris Flecks and drawn by Lisa M. Schmitz.

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