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Molecular identification and morphological description of *Theloderma albopunctatum* tadpoles from the Phu Khiao-Nam Nao Forest Complex, northeastern Thailand

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Abstract. *Chunskul J, Thongproh P, Simmasian W, Arkajag J, Tongpun S, Kanishthajata P, Prompalad S, Duangjai S, Duengkae P, Phochayavanich R, Chuaynkern C, Chuaynkern Y. 2021. Molecular identification and morphological description of* Theloderma albopunctatum *tadpoles from the Phu Khiao-Nam Nao Forest Complex, northeastern Thailand. Biodiversitas 22: 5145-5161.* Presently, tadpole description of an external morphology and internal oral features of *Theloderma albopunctatum* (Anura, Rhacophoridae) is unknown. Therefore, this study aimed to describe tadpoles of *T. albopunctatum* from the Phu Khiao-Nam Nao Forest Complex in northeastern Thailand based molecular and morphological characters. Tadpole identification was based on molecular analyses. The 16S and COI sequences of the *T. albopunctutatum* tadpole had a genetic distance of 0% and 0.15% with the adults from Phu Luang Wildlife Sanctuary and Nam Nao National Park, respectively. Bayesian inference analyses of 16S, 16S+COI, and BDNF + SIA + RHO + TYR strongly supported the identity of tadpole and adults *T. albopunctatum* tadpoles are of medium size, with a black to ashy gray body that turns dark brown in preservative, they also exhibit elliptical body depressions and follow the keratodont row formula 1:2+2/1+1:2 or 1:1+1/1+1:1. *T. albopunctatum* tadpoles are generally similar to *T. asperum*.

Keywords: DNA, morphology, SEM, DNA, tadpole, *Theloderma albopunctatum*

INTRODUCTION

Theloderma Tschudi 1838 (type species: *Theloderma leporosa* Tschudi 1838) is a genus in the family Rhacophoridae that is distributed from northeastern India to Myanmar, as well as from southern China southward through Indochina to Malaysia and Indonesia (Frost 2021). Morphologically, *Theloderma* species exhibit high degrees of similarity (Nguyen et al. 2014; Poyarkov et al. 2015; Nguyen et al. 2016; Dever 2017). Although many studies have attempted to delimit *Theloderma* species based on morphological characteristics (Dever 2017), none has formed a morphological synapomorphy for the genus. Therefore, the monophyly of *Theloderma* remains in question (Wilkinson and Drewes 2000; Wilkinson et al. 2002; McLeod and Norhayati 2007; Yu et al. 2007; Yu et al. 2008; Li et al. 2008; Bain et al. 2009; Li et al. 2009; Rowley et al. 2011; Li et al. 2013; Dever et al. 2015).

At present, *Theloderma* consists of 26 species, 7 species of which are known from Thailand, namely *T. albopunctatum* (Liu and Hu 1962), *T. asperum* (Boulenger 1886), *T. gordoni* Taylor, 1962, *T. horridum* (Boulenger 1903), *T. licin* McLeod and Norhayati 2007, *T. petilum* (Stuart and Heatwole 2004), and *T. stellatum* (Taylor 1962) (Frost 2021). However, Dever (2017) first listed *T. vietnamense* (Poyarkov et al. 2015) in Thailand based on the 16S rRNA gene analysis of specimen from Mae Yom (KUHE 22065). Later work, Niyomwan et al. (2019) included *T. vietnamense* in the list of amphibians in Thailand. Further, *T. phrynoderma* (Ahl, 1927) was also listed for the first time. Therefore, a total of nine *Theloderma* species had previously been reported (Taylor 1962; McLeod and Norhayati 2007; Poyarkov et al. 2015; Dever 2017; Phusaensri et al. 2018; Niyomwan et al. 2019), namely *T. albopunctatum*, *T. asperum*, *T. gordoni*, *T. horridum*, *T. licin*, *T. petilum*, *T. stellatum*, *T. vietnamense*, and *T. phrynoderma*.

Theloderma bicolor (Bourret 1937) was the first *Theloderma* (as *Rhacophorus leprosus bicolor*) described from “Chapa” (=Sa Pa), Lao Cai Province, Vietnam. Later, some new species were described as new to science (see Frost 2021). From 1938 to 1962, a total of 11 *Theloderma* were discovered. Lastly, 14 species were added during 2004 to the present. As the monophyly of the *Theloderma* still unclear and requires further intensive studies of morphology and molecular data. Nguyen et al. (2015) analyzed 2412 bp combined sequences of 12S rRNA, tRNA^{val}, and 16S rRNA. The phylogenetic relationship based on a maximum likelihood tree showed that *T. moloch* was not inside of *Theloderma*. This result is similar to Poyarkov et al. (2015) who analyzed 1987 bp combined sequences of 12S rRNA+16S rRNA. Monophyly of the *Theloderma* (excluding *T. moloch*) and *Nyctixalus* was strongly supported in Nguyen et al. (2015). By excluding *T. moloch*, *T. horridum* group (*T. horridum*, *T. stellatum*, and *T. vietnamense*) and *Nyctixalus* (as *T. pictum*), the monophyly of the clade comprising other *Theloderma* members. Poyarkov et al. (2015) reduced *Nyctixalus* to subgeneric level of *Theloderma* and established a new subgenus *Stelladerma* for *T. horridum*+*T. stellatum* group (*T. stellatum* and *T vietnamense*). The remaining *Theloderma*, clade A in Nguyen et al. (2015) was separated into two subclades (subclades A and B). Poyarkov et al. (2015) suggested that the remaining *Theloderma* consists of two major groups. The first group, namely *T. asperum* group contained *T. ryabovi*, *T. albopunctatum*, *T. petilum*, *T. licin*, and *T. asperum*. Members of this group are small to large-sized, presence of vocal sac opening and uniform reddish-brown iris and large white blotches on the rear part of the dorsum (absence for *T. petilum*) (Nguyen et al. 2015; Poyarkov et al. 2015). The second group was divided into two subgroups, namely *T. leporosum* group (*T. corticale*, *T. bicolor*, *T. rhododiscum*, *T. leporosum*, *T. gordoni*, and *T. palliatum*) and *T. laeve* group (*T. laeve*, *T. nebulosum*, *T. truongsonense*, *T.* cf. *truongsonense* [from central Vietnam], and *Theloderma* sp. [from South Vietnam]). Members of the *T. laeve* group join small-sized *Theloderma* taxa from southern and central Vietnam and two unidentified lineages allied to *T. truongsonense*. Members of *T. leporosum* join all other large-sized *Theloderma* species. However, phylogenetic constructing within *T. laeve* and *T. leporosum* groups received low support and relationships between members of these groups remain sufficiently unresolved and require further study. Therefore, Sivongxay et al. (2016) rejected this proposal and, maintained *Theloderma* and *Nyctixalus* as separate genera. In particular *T. asperum* group of Poyarkov et al. (2015), four species have been recorded in Thailand, namely *T. albopunctatum*, *T. petilum*, *T. licin*, and *T. asperum*. Among them, *T. albopunctatum* and *T. asperum* are highly similar in appearance and size (Dever 2017) and their tadpole are known only for *T. asperum* (Leong and Lim 2003).

Voucher specimens of *Theloderma* are rare in herpetological collections, sometimes consisting of a single sex or single age (Dever 2017). *Theloderma* tadpoles are virtually unknown from Thailand. They have been reported only by Wassersug et al. (1981), who described the buccal morphology of *T. stellatum* based on six tadpole specimens from Khao Soi Dao Wildlife Sanctuary in Chanthaburi Province, southeastern Thailand. To fill this gap in knowledge regarding *Theloderma* species, the present study aimed to describe *T. albopunctatum* tadpole specimens based on molecular and morphological analyses.

MATERIALS AND METHODS

Study area

The Phu Khieo-Nam Nao Forest Complex (PKNNFC) is located in the Phetchabun mountain range of northeastern Thailand (15°19'18"-17°33'00"N, 101°16'00"-102°43'50"E, altitude 150-1,571 m asl) (Figure 1) (Division of Information System on Wildlife Conservation 2020). The PKNNFC lies in the Phetchabun mountain range and covers an area of approximately 7,990.5 km^2 in Loei, Phetchabun, Khon Kaen, Chaiyaphum, Nong Bua Lam Phu, and Lop Buri Provinces. The PKNNFC contains 13 national parks and seven wildlife sanctuaries. The vegetation types are mixed deciduous forest, dry evergreen forest, dry dipterocarp forest, hill evergreen forest, pine forest, and savanna forest. The PKNNFC has a tropical monsoonal climate with distinct long rainy (mid-May to mid-October), winter (from mid-October to mid-February), and summer seasons (mid-February to mid-May). The average of annual rainfall and temperature ranges from 1,123-1,238 mm and 26.0-27.4°C, respectively. The average annual maximum temperature is between 32.0-33.2°C, while the minimum is 20.1-22.2°C (Department of National Parks, Wildlife and Plant Conservation 2014).

Field surveys were conducted in four protected areas within the PKNNFC, including Nam Nao National Park (Phetchabun Province), Phu Wiang National Park (Khon Kaen Province), Phu Luang Wildlife Sanctuary (Loei Province), and Phu Khieo Wildlife Sanctuary (Chaiyaphum Province).

Research materials

Permission to conduct research was granted by the Department of National Parks, Wildlife and Plant Conservation, Thailand (nos. 0907.4/15590 and 0907.4/22080). This research was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethics of Animal Experimentation guidelines of the National Research Council of Thailand (No. 660201.2.11/67).

Nine tadpoles (PT 2499.1-9) were collected in stagnant water of an old disused toilet in a dry evergreen forest nearby the second check point (17°20'5"N, 101°30'32"E, altitude 940 m asl). These tadpoles were euthanized by immersion in chlorobutanol. A portion of the tail was cut from each selected specimen (PT 2499.1) for molecular analysis. The specimen (the DNA voucher) was used for detailed description. The remaining parts of the tadpoles and other specimens were preserved in a solution containing equal parts 10% formalin and 70% ethanol (Chuaynkern et al. 2019).

Two adult specimens were used in this study. The first adult male (JC 02506) was collected along with the tadpoles at the same locality. The second adult female (PT 02378) was collected from the toilet at the headquarter of the Nam Nao National Park (16°44'279"N, 101°34'16"E, altitude 842 m asl). These frogs were euthanized by immersion in chlorobutanol, pieces of the liver were cut and preserved in 70% ethanol. The specimens were later fixed in 10% formalin and transferred for preservation in

70% ethanol. All larval and adult specimens were cataloged and deposited in the Thailand Natural History Museum (THNHM), National Science Museum, (Pathum Thani Province, central Thailand).

Molecular study

Tissue samples of livers (PT 02378 and JC 02506) and the tail portion (PT 02499.1) were used for DNA extraction. Total genomic DNA was extracted using the GF-1 Tissue DNA Extraction Kit (Vivantis, Inc.). Fragments of mtDNA 16S rRNA (16S), cytochrome oxidase subunit I (COI), nuDNA brain-derived neurotrophic factor (BDNF), rhodopsin (RHO), seventh-in-absentia (SIA), and tyrosinase (TYR) genes were amplified by polymerase chain reaction (PCR) as described by Dever (2017), using the same primers (Table 1) and modified PCR conditions as follows: 1 cycle initial denaturation at 94°C for 4 min, 33 cycles of denaturation at 94°C for 30 s, annealing at 72°C for 1 min, extension at 72°C for 7 min, and 1 cycle final extension at 72°C for 7 min. For COI condition: 1 cycle initial denaturation at 94°C for 45 min, 35 cycles of denaturation at 60°C for 30 s, annealing at 72°C for 45 s, extension at 72°C for 7 min, 1 cycle final extension at 72°C for 7 min. The PCR products were purified and sequenced by Macrogen Co., Ltd. (Seoul, South Korea).

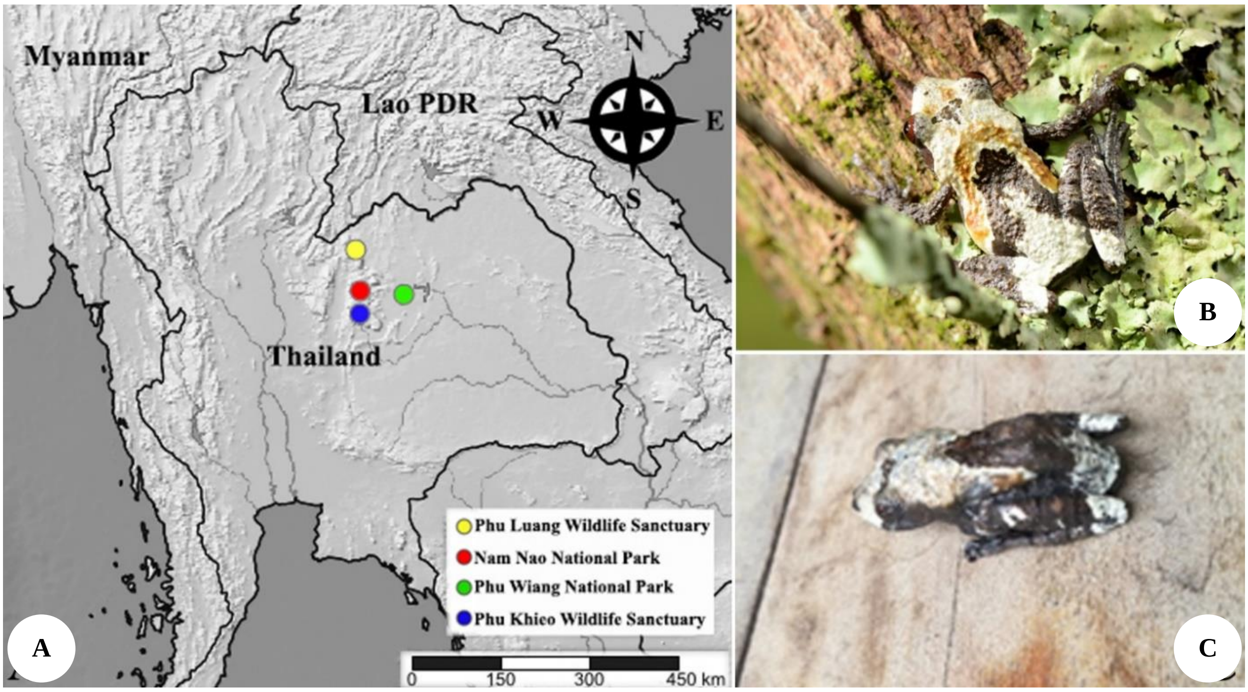

Figure 1. Location of the Phu Khieo-Nam Nao Forest complex, northeastern Thailand. A. Map showing four protected areas, B. *Theloderma albopunctatum* from Phu Luang Wildlife Sanctuary (PT JC 02506, SVL 30.3 mm), C. *Theloderma albopunctatum* from Nam Nao National Park (PT 02378, SVL 32.4 mm)

Table 1. Primers used for amplification of the markers analyzed in this study

Marker	Primer	Primer sequence (5'-3')	Source
COI	AH-CO1A-S	CTACAAYCCRCCRCCTRCTCGGCCAC	Grosjean et al. (2015)
	AH-CO1A-AS1	TADACYTCDGGRTGDCCAAARAATCA	
16S	16SaL	TADACYTCDGGRTGDCCAAARAATCA	Palumbi et al. (1991)
	16SbH	CCGGTCTGAACTCAGATCACGT	
BNDF	BNDF-F	GACCATCCTTTTCCTKACTATGGT TATTTCATACTT	Noonan and Chippindale (2006)
	BNDF-R	CTATCTTCCCCTTTTAATGGTCAGTGTACAAAC	
RHO	Rhod1A	ACCATGAACGGAACAGAAGGYCC	Bossuyt and Milinkovitch (2000)
	Rhod1D	GTAGCGAAGAARCCTTCAAMGTA	
SIA	SIA1(T3)	TCGAGTGCCCCGTGTGYTTYGAYTA	Bonacum et al. (2001)
	SIA2(T7)	GAAGTGGAAGCCGAAGCAGSWYTGCATCAT	
TYR	Tyr1A	AGGTCCTCTTRAGCAAGGAATG	Bossuyt and Milinkovitch (2000)
	Tyr1G	TGCTGGGCRTCTCTCCARTCCCA	

Morphological study

The tadpole morphological terminology used in this study follows Altig and McDiarmid (1999), the keratodont row formula (KRF) follows Dubois (1994), and the developmental stages follow Gosner (1960). Most measurements were acquired using a digital caliper; measurements smaller than 1 mm were acquired using an ocular micrometer attached to a stereomicroscope. In total, 23 measurements were used in this study: distance from tip of snout-insertion to spiracle opening (SS), distance from tip of snout-insertion to upper tail fin (SU), body length (BL), tail length (TAL), total length (TL), maximum upper fin height (UF), maximum lower fin height (LF), maximum tail height (MTH), body height (BH), maximum body width (BW), maximum tail muscle height (TMH), tail muscle width at the end of body (TMW), interpupillary distance (PP), internarial distance (NN), rostro-narial distance (RN), naro-pupillary distance (NP), maximum eye diameter (ED), nostril diameter (ND), spiracle diameter (SD), anterior labium (AL), posterior labium (PL), oral disc width (ODW), and maximum size of dorsal papilla gap (DG). Tadpole photographs were taken using a digital camera, whereas the oral disc used a digital camera attached to a stereomicroscope. Larval photographs were acquired under a stereomicroscope.

The mouthparts of selected tadpoles were prepared for scanning electron microscopy (SEM) examination at the Faculty of Science, Khon Kaen University, Khon Kaen, northeastern Thailand. The mouthparts were cut into two pieces (roof and floor) using surgical scissors. The roof and floor were wrapped with straining cloth and dehydrated by soaking in a series of ethanol solutions (70%, 90%, and 100%, 24 h per concentration). The materials were soaked for 20 minutes in a solution containing equal parts ethanol and amyl acetate, followed by soaking for 20 minutes in amyl acetate and drying in a critical-point dryer (Quorum K850, Quorum Technologies, UK). The materials were then attached to an SEM stub and coated with heavy metals for SEM photography using a DMS 950 scanning electron microscope (Zeiss, Oberkochen, Germany) at 10 kV. The SEM photographs were used for observing buccal features in detail. Terminology of the buccal features follows Wassersug (1976). The resulting images were modified using Adobe Photoshop software.

Data analysis

The sequences were edited using DNA Dragon software (Hepperle 2011) and aligned using the SeaView v4 tool (Gouy et al. 2010) or the Mesquite v3.61 tool for multiple alignments (Maddison and Maddison 2019). The available 16S and COI GenBank sequences covering all *Theloderma* species and their closest rhacophorid relatives were used as described by Dever (2017). Phylogenetic trees were determined separately for 16S, concatenated mtDNA, and concatenated nuDNA via Bayesian inference analyses using the CIPRES Science Gateway (Miller et al. 2010) and the MrBayes software, run on the XSEDE v3.2.7a platform, and Markov chain Monte Carlo analysis for 10,000,000 generations, with the initial 250,000 trees (25%) discarded as burn-in. Posterior Bayesian credibility values for each branch in a 50% majority-rule consensus tree were calculated. The evolution model was selected using the MrModeltest v2.4 software (Nylander 2004); the best-fitting mtDNA and nuDNA models were GTR+I+G and K80+I+G, respectively. Uncorrected pairwise distances (*p*) for the 16S and COI fragments were calculated using CIPRES and the results were used analyzed using a phylogenetic tree in accordance with the PAUP* method (Swofford 2019). The available sequence data for *Theloderma* species and their closest rhacophorid relatives were downloaded from GenBank. *Buergeria oxycephala* of the subfamily Buergiinae is a basal lineage of the subfamily Rhacophorinae (Wilkinson et al. 2002; Li et al. 2009) was chosen as the outgroup. Voucher specimens, localities, and GenBank numbers for all samples used in this study are presented as supplementary file (Table S1). Measurements of tadpoles were calculated an average value for the tadpoles in stage 25 (7 tadpoles) as presented in Table 3.

RESULTS AND DISCUSSION

Molecular analysis

The obtained mtDNA fragments consisted of a 1,235-bp region, and the obtained nuDNA fragments consisted of an 1,854-bp region. The 16S gene sequences contained 520 bp (46 variable and 253 informative sites), and the COI gene contained 715 bp (22 variable and 286 informative sites). Bayesian inference analyses based on the 16S matrix strongly supported the identity of PKNNFC specimens (BPP: 1) nested in group A of the *T. albopunctatum* clade (Figure 2). Based on 16S+COI analyses, PKNNFC specimens remained nested in the *T. albopunctatum* clade (Figure 3; BPP: 1). Genetic distances based on the 16S (mean, 0.07) and COI (mean, 0.10) genes between PKNNFC specimens and specimens of closely related species are shown in Table 2. Based on the BDNF+SIA+RHO+TYR matrix, PKNNFC specimens were nested in the *T. albopunctatum* clade (Figure 4; BPP: 0.88). Therefore, we assigned PKNNFC specimens to *T. albopunctatum* based on Bayesian inference analyses of mtDNA (16S and 16S+COI) and nuDNA (BDNF+SIA+RHO+TYR).

The sequences of the tadpole (PT 02499.1) and the adult specimen (JC 02506) from Phu Luang Wildlife Sanctuary had a genetic distance of 0% (16S and COI). Whereas the genetic distances between the tadpole and adult specimen from Nam Nao National Park (PT 02378) were 0% and 0.15% for 16S and COI, respectively (Table 2).

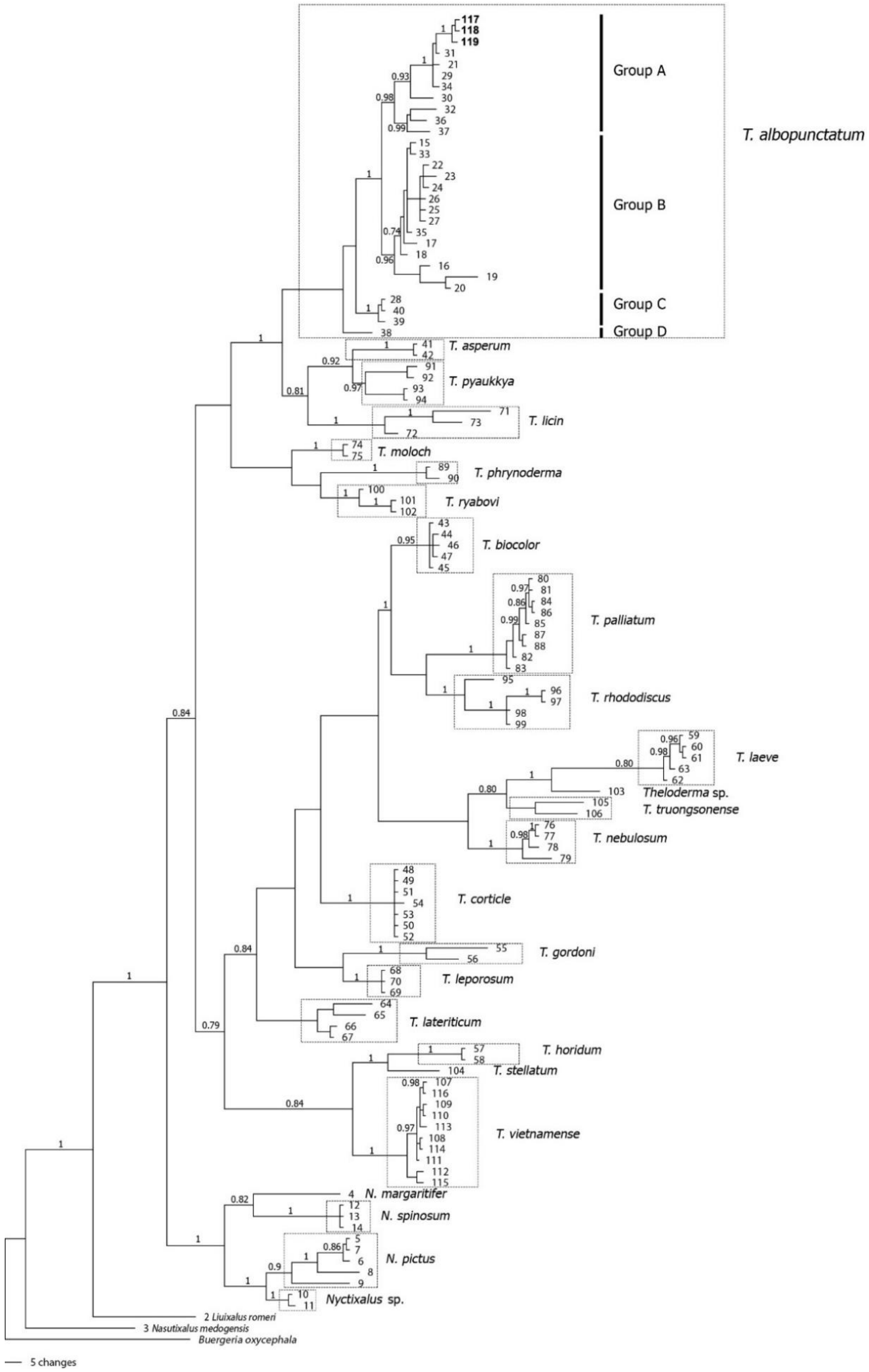

Figure 2. Phylogenetic tree constructed from partial DNA sequences of the 16S rRNA gene based on Bayesian analysis. Numbers above branches are Bayesian posterior probabilities ≥70%. Voucher specimens and GenBank accession numbers are given in the supplementary table (Table S1). Numbers of specimens (No. 1-119) correspond to those in Table S1. The bold numbers of specimens (No. 117-119) indicate new materials from this study

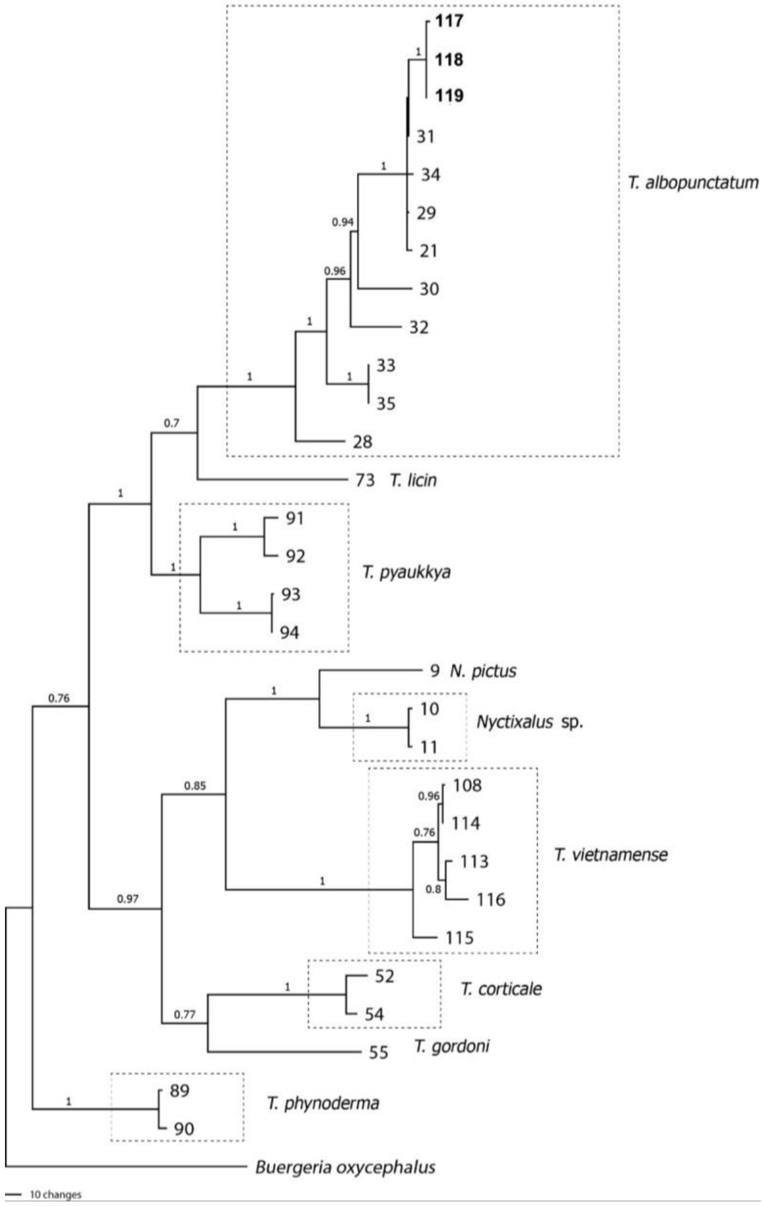

Figure 3. Phylogenetic tree constructed from partial DNA sequences of the concatenated 16S fragment and COI gene based on Bayesian analysis. Numbers above branches are Bayesian posterior probabilities ≥70%. Voucher specimens and GenBank accession numbers are given in the supplementary table (Table S1). Numbers of specimens (No. 1-119) correspond to those in Table S1. The bold numbers of specimens (No. 117-119) indicate new materials from this study

Morphological description

A total of nine tadpoles were used in the present work. Variation of these tadpoles was assessed based on three stages distributed in stages 25 (78%), 27 (11%), and 37 (11%). The BL and TAL (excluded PT02499.1) are 5.4-15.4 mm and 9.8-25.2 mm, respectively. The tadpoles of *T. albopunctatum* are medium size with a maximum total length 38.9 mm in stage 37. Different collective KRF can be found: 1:1+1/1+1:2 (78%) and 1:2+2/1+1:2 (22%). The ratios of BW 120-177% of BH; ED 4-9% of BL; RN 33-91% of NP; NN 52-94% of PP; SS 68-83% of BL; TMH 37-57% of BH; TMH 31-65% of MTH; TMW 17-37% of BW; UF 24-37% of MTH; LF 19-34% of MTH; SU 86-96% of BL; MTH 80-140% of BH; ODW 14-26% of BL; ODW 18-42% of BW; and DG 69-127% of ODW (Table 3).

Larval description

Larval description is based on *T. albopunctatum* specimen, namely PT 02499.1, Gosner stage 27, TL 13.6 mm (cut tail), BL 11.4 mm. Body in lateral view elliptical, snout oval (Figure 5A); body in dorsal view oval, snout narrow and oval (Figure 5B); BW 120% of BH. Eyes moderately large, ED 5% of BL, slightly bulging and not visible in ventral view, positioned dorsally, directed dorso-laterally. Nares small, round, rimmed, positioned and directed dorsally, closer to tip of snout than to pupils, RN 41% of NP; NN 72% of PP. Spiracle single, sinistral, square, medium-sized, at 2/3 the distance between snout and anal tube opening; in lateroventral position, oriented postero-ventrally, free from body over most of its length; SS 68% of BL; spiracle opening oval, opening situated at hind limb level. Tail musculature strong, TMH 44% of BH and 55% of MTH, parallel in anterior half and gradually tapering in posterior half, almost reaching tail tip. Tail fins large; UF 28% of MTH, 28% of MTH; upper fin extending onto body, SU 96% of BL, slightly convex; lower fin extending onto body, convex; MTH 80% of BH, tail tip rounded. Anal tube (Figure 5C) approximately conical, medial, entirely attached to lower fin, opening medial, posteriorly directed. Oral disc (Figure 5D) large, positioned and directed anteroventrally, emarginated, ODW 26% of BL and 42% of BW, semicircular without medium notch on the lower labium. Row of papillae at lateral sides of upper labium, 3-4 submarginal papillae, single papilla row on lower labium. No denticulate papillae. One large papilla gap on upper labium, no gap on lower labium, DG 73% of ODW. Keratodont row formula (KRF) of 1:2+2/1+1:2, rows of upper labium unequal, lower rows subequal. Keratodonts (Figure 10B) spoon-shaped bearing 6 large cusps. Jaw sheaths large, black, with fine serrations (Figure 5D, 10A); upper sheath shaped as reverse U, with median part dimpled; lower sheath V-shaped, very narrow. Pineal ocellus absent. Lateral line present: first lateral line beginning at margin of mouth, continuing below nares and finishing; second line continuing from midpoint of body to tail.

Coloration. In preservative: body dark brown, ventrum and tail brownish, intestinal spiral ventrally visible through skin. Hind limbs dark ashy grey on dorsum. Tail fin translucent with dark pigmentation. In life: body and tail black to ashy grey, slight pigmentation on ventrum, unpigmented on vent tube. Hindlimbs dark ashy grey on dorsum.

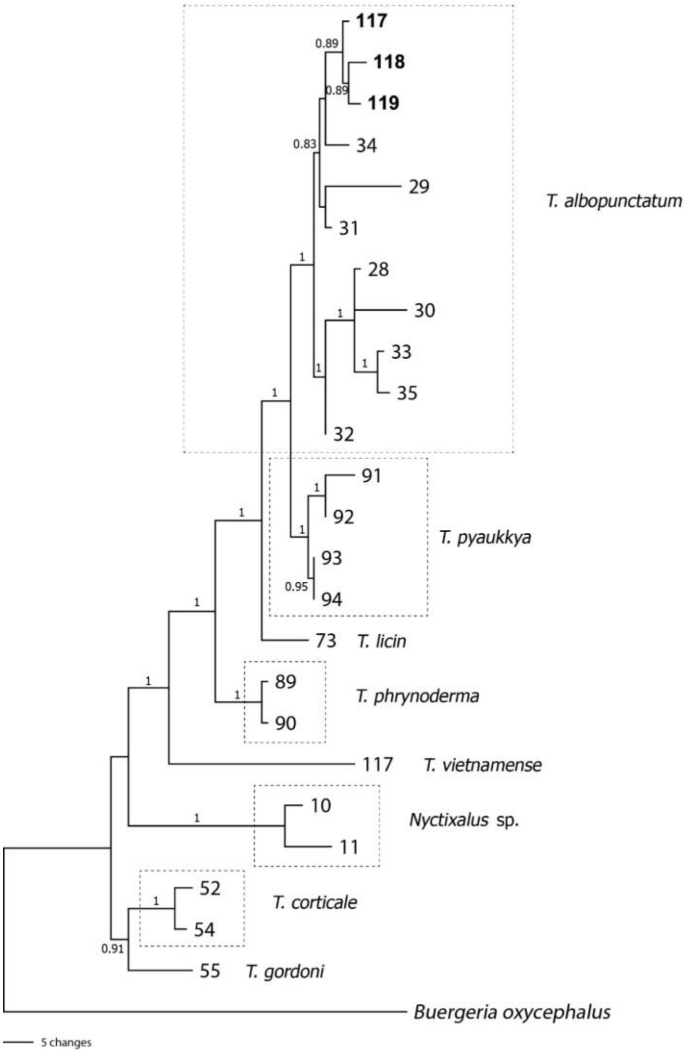

Figure 4. Phylogenetic consensus tree constructed from concatenated DNA sequences of the BDNF, SIA, RHO, and TYR nuclear genes based on Bayesian analysis. Numbers above branches are Bayesian posterior probabilities ≥70%. Asterisks indicate Bayesian posterior probabilities >0.95. Voucher specimens and GenBank accession numbers are given in the supplementary table (Table S1). Numbers of specimens (No. 1-119) correspond to those in Table S1. The bold numbers of specimens (No. 117-119) indicate new materials from this study

Table 2. Uncorrected *P*-distance (percentage) between 16S rRNA (below diagonal) and COI (above diagonal) regions of *T. albopunctatum* species

ID	Vouchers	117	118	119	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
117	PT 02378	–	0.15	0.15	–	–	–	–	–	–	1.37	–	–	–	–	–	–	10.06	1.27	8.78	1.27	8.23	8.09	1.43	8.09	–	–	–	–
118	JC 02506	0.20	–	0.00	–	–	–	–	–	–	1.21	–	–	–	–	–	–	10.19	1.10	9	1.10	8.37	8.23	1.26	8.23	–	–	–	–
119	PT 02499.1	0.00	0.00	–	–	–	–	–	–	–	1.22	–	–	–	–	–	–	10.26	1.11	8.98	1.11	8.42	8.29	1.27	8.29	–	–	–	–
15	ROM 30246	4.87	4.46	4.47	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	KIZ 060821201	5.27	4.86	4.87	2.59	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	KIZ 060821217	5.49	5.08	5.09	1.19	2.99	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18	HN0806100	5.08	4.67	4.68	0.98	2.80	1.38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	VNMN J2916	9.51	8.87	8.90	3.35	6.19	3.65	3.97	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
20	VNMN 3540	9.17	98.53	8.56	4.92	3.34	5.87	5.57	3.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
21	2006.2573	1.75	1.75	1.75	4.34	5.65	5.66	4.61	7.86	7.88	–	–	–	–	–	–	–	9.93	0.30	8.72	0.15	7.88	8.31	0.44	8.41	–	–	–	–
22	asperum-1	5.72	5.30	5.32	0.79	3.41	1.99	1.78	3.36	4.94	4.35	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
23	asperum-2	5.92	5.50	5.52	1.58	2.59	2.79	2.59	4.29	3.97	4.86	0.79	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24	asperum-3	5.71	5.29	5.30	0.79	3.40	1.98	1.78	3.35	4.92	4.34	0.00	0.79	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
25	ZMNU NAP 03557	5.93	5.52	5.53	0.98	3.62	1.78	1.58	3.05	5.26	4.61	0.20	0.99	0.20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
26	ZMNU NAP 03566	5.93	5.52	5.53	0.98	3.62	1.78	1.58	3.05	5.26	4.61	0.20	0.99	0.20	0.00	–	–	–	–	–	–	–	–	–	–	–	–	–	–
27	ZMNU NAP 03575	5.93	5.52	5.53	0.98	3.62	1.78	1.58	3.05	5.26	4.61	0.20	0.99	0.20	0.00	0.00	–	–	–	–	–	–	–	–	–	–	–	–	–
28	CAS 241559	5.64	5.64	5.64	4.31	4.97	4.31	4.09	7.56	7.56	5.36	4.75	5.64	4.74	4.75	4.75	4.75	–	10.30	10.60	10.30	10.48	8.46	9.96	8.61	–	–	–	–
29	FMNH 270722	1.29	1.29	1.29	3.05	4.40	3.95	3.06	6.74	7.12	0.26	3.95	4.40	3.95	3.95	3.95	3.95	4.88	–	8.57	0.15	7.92	8.13	0.73	8.13	–	–	–	–
30	NCSM 79167	3.84	3.84	3.84	3.40	4.72	4.28	3.41	7.88	8.24	3.42	4.28	5.17	4.28	4.28	4.28	4.28	3.85	3.05	–	8.57	8.41	7.09	8.90	7.13	–	–	–	–
31	NCSM 79445	1.05	1.05	1.05	3.21	4.08	3.64	3.21	6.51	6.87	0.52	4.09	4.53	4.08	4.09	4.09	4.09	4.54	0.21	3.20	–	7.92	8.13	0.58	8.13	–	–	–	–
32	NCSM 76483	4.50	4.50	4.50	3.86	4.72	4.30	3.85	7.54	7.88	4.23	4.75	5.64	4.74	4.75	4.75	4.75	5.20	4.17	4.06	3.87	–	7.99	7.91	8.33	–	–	–	–
33	VNMN JR2887	3.84	3.84	3.84	0.00	2.54	1.05	0.42	3.34	5.42	3.43	0.84	1.68	0.83	0.84	0.84	0.84	4.32	3.05	3.41	3.21	3.86	–	8.47	0.00	–	–	–	–
34	NCSM 79444	1.05	1.05	1.05	2.99	4.30	3.86	2.99	6.51	6.87	0.26	3.87	4.30	3.86	3.87	3.87	3.87	4.77	0.00	2.98	0.21	4.09	2.99	–	8.80	–	–	–	–
35	VNMN J2888	5.08	4.67	4.68	0.20	2.79	0.99	0.79	3.04	5.24	4.60	0.98	1.78	0.98	0.79	0.79	0.79	4.31	3.05	3.40	3.21	3.86	0.00	2.99	–	–	–	–	–
36	VNMN 4404	4.86	4.45	4.46	3.83	4.86	4.03	4.05	8.16	8.84	4.34	4.66	5.50	4.65	4.45	4.45	4.45	5.21	3.28	3.18	3.43	2.75	3.42	3.21	3.62	–	–	–	–
37	VNMN 4405	5.28	4.87	4.88	3.62	4.65	4.24	3.84	6.83	7.49	4.59	4.45	5.29	4.44	4.24	4.24	4.24	4.75	3.50	3.62	3.64	3.18	3.41	3.42	3.41	2.59			
38	VNMN 4406	7.43	7.01	7.03	4.45	5.29	5.09	4.67	7.50	6.84	7.29	4.87	5.28	4.86	5.08	5.08	5.08	4.07	5.56	5.17	5.67	6.32	4.29	5.44	4.66	6.15	5.50	–	–
39	VNMN PAE262	7.01	6.59	6.60	4.89	5.95	4.67	4.68	7.19	7.86	6.76	5.30	6.15	5.29	5.09	5.09	5.09	0.62	5.10	4.07	4.76	5.64	4.75	4.99	4.67	5.74	5.09	4.03	–
40	KUHE 23736	7.01	6.60	6.61	4.68	5.52	4.47	4.26	7.52	7.86	6.77	5.10	5.94	5.09	4.88	4.88	4.88	0.21	5.11	4.07	4.77	5.42	4.53	5.00	4.47	5.31	4.67	4.45	0.78

Table 3. Measurements (mm) of *T. albopunctatum* tadpoles from Phu Luang Wildlife Sanctuary, Loei Province, northeastern Thailand

	PT02499.1	**PT02499.2**	**PT02499.3**	**PT02499.4**	**PT02499.5**	**PT02499.6**	**PT02499.7**	**PT02499.8**	**PT02499.9**	**Average±SD (min-max)**
Stage	27	37	25	25	25	25	25	25	25	25
SS	7.7	10.4	5.6	6.4	5.5	5.6	5.3	4.2	4.5	5.3±0.74 (4.2-6.4)
SU	10.9	13.2	6.2	7.1	5.9	6.4	6.5	5.1	5.1	6.0±0.74 (5.1-7.1)
BL	11.4	15.4	6.9	7.7	6.7	7.2	7.2	5.8	5.4	6.7±0.82 (5.4-77)
TAL	2.7*	25.2	11.1	13.7	9.8	12.1	11.7	10.6	9.9	11.3±1.37 (9.8-13.7)
TL	13.6	38.9	16.9	20.3	15.8	17.9	18.0	15.2	15.1	17.0±1.87 (15.1-20.3)
UF	1.3	1.5	1.3	1.3	1.8	1.5	1.0	1.0	1.2	1.3±0.28 (1.0-1.8)
LF	1.3	1.2	1.3	1.3	1.5	1.4	0.9	1.0	1.0	1.2±0.23 (0.9-1.5)
MTH	4.7	6.2	4.0	4.1	4.9	4.1	3.3	3.2	3.3	3.8±0.62 (3.2-4.9)
BH	5.9	7.0	3.7	4.1	3.5	3.9	3.5	3.5	3.1	3.6±0.32 (3.1-4.1)
BW	7.1	12.4	5.4	6.0	5.5	5.6	5.4	4.7	4.7	5.3±0.48 (4.7-6.0)
TMH	2.6	4.0	1.7	1.8	1.5	1.5	1.5	1.3	1.3	1.5±0.19 (1.3-1.8)
TMW	2.6	4.0	0.9	1.7	1.28	1.3	1.4	1.3	1.0	1.3±0.26 (0.9-1.7)
PP	2.9	2.9	1.9	1.9	1.6	1.9	1.8	1.4	1.7	1.7±0.19 (1.4-1.9)
NN	2.1	1.5	1.4	1.4	1.5	1.4	1.4	1.0	1.2	1.3±0.17 (1.0-1.5)
RN	0.7	1.0	0.7	0.6	0.6	0.5	0.6	0.3	0.5	0.5±0.13 (0.3-0.7)
NP	1.7	1.1	1.1	1.3	1.2	1.2	1.2	0.9	1.1	1.1±0.13 (0.9-1.3)
ED	0.6	1.4	0.5	0.5	0.3	0.3	0.3	0.3	0.3	0.4±0.10 (0.3-0.5)
ND	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1±0.0 (0.1-0.1)
SD	0.8	0.6	0.6	0.8	0.6	0.5	0.6	0.4	0.5	0.6±0.13 (0.4-0.8)
AL	0.4	0.5	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.2±0.06 (0.1-0.3)
PL	0.5	0.6	0.5	0.3	0.5	0.5	0.4	0.3	0.4	0.4±0.09 (0.3-0.5)
ODW	3.0	3.1	1.8	1.1	1.7	1.6	1.7	1.3	1.3	1.5±0.26 (1.1-1.8)
DG	2.2	2.5	1.3	1.4	1.2	1.1	1.2	0.9	0.9	1.1±0.19 (0.9-1.4)
KRF	1:2+2/1+1:2	1:2+2/1+1:2	1:1+1/1+1:1	1:1+1/1+1:1	1:1+1/1+1:1	1:1+1/1+1:1	1:1+1/1+1:1	1:1+1/1+1:1	1:1+1/1+1:1	

Note: *indicates the cutting tail

Buccal description

Buccal description is based on *T. albopunctatum* specimen, namely PT 02499.2, Gosner stage 37, TL 25.2 mm, BL 15.4 mm. SEM images of the buccal roof are shown in Figures 6-7. Prenarial arena transverse, arched ridge bearing knobby projections (6 anteromedially, 7 and 2 posterolaterally) halfway from beak to nares, sides without papillae. Choanae narrow, parallel to body axis, internarial distance approximately one-third the length of choanae; anterior wall pustular; no papilla on narial valve. Postnarial arena without postnarial papillae. Median ridge transverse elliptical, wider than long, smooth with 10-11 pustolose papillae directed anteromedially, lateral ridge papilla absent. Buccal roof arena oval, rectangular, with 6 buccal roof arena papillae on each side; no papillae within arena; no papillae on anterior esophageal funnel. Posterolateral ridge without papillae. No glandular zone. Dorsal velum continuous, margin curved, medial portion curving toward esophagus.

SEM images of the buccal floor are shown in Figures 8-9. Prelingual arena square; its floor smooth except for a low ridge anterior to tongue anlage bearing two pairs of small papillae. Two pairs of infralabial papillae, anterior pair short with 6 pustules and the next pair long and large separating into two branches with 10-12 pustules. Tongue anlage large oval shaped, bearing 4 lingual papillae, the medial pair long, without pustules, and the lateral pair shorter, without pustules. Buccal floor arena square; medial part covered with small pustules; lateral and posterior parts covered with numerous short papillae; anteromedial to buccal pockets with 4 small papillae. Buccal pockets are elliptical, transverse, distance to tongue anlage larger than that of the medial end of ventral velum. Ventral velum continuous, with spicular support, highly wavy, margin with 25 projections forming a median notch medially. Glottis small. Branchial baskets oblique, longer than wide, 3 filter plates on each side, second filter plate approximately one-half the length of the floor arena.

Discussion

The results of current study confirmed the phylogeny of *Theloderma* as reported by Nguyen et al. (2015), Poyarkov et al. (2015), Dever (2017), and Poyarkov et al. (2018). The data supported the separation of *T. albopunctatum* from the *T. asperum* group, which included *T. pyaukkya*. The morphology of *T. albopunctatum* specimens from Nam Nao National Park (Phetchabun Province) showed that *T. pyaukkya* was likely to have an opening vocal sac. However, the pairwise distances between these species show deep divergences. The phylogenetic trees constructed from both mtDNA and nuDNA indicated that complexes in the *T. albopunctatum* group showed high genetic differentiation among specimens from Thailand (excluding specimens from Chiang Mai Province) and specimens from Vietnam (*p* distance, 6.41% for 16S); these complexes were more closely related to specimens from Laos (*p* distance, 1.85% for 16S) than to specimens from Myanmar (*p* distance, 2.85% for 16S).

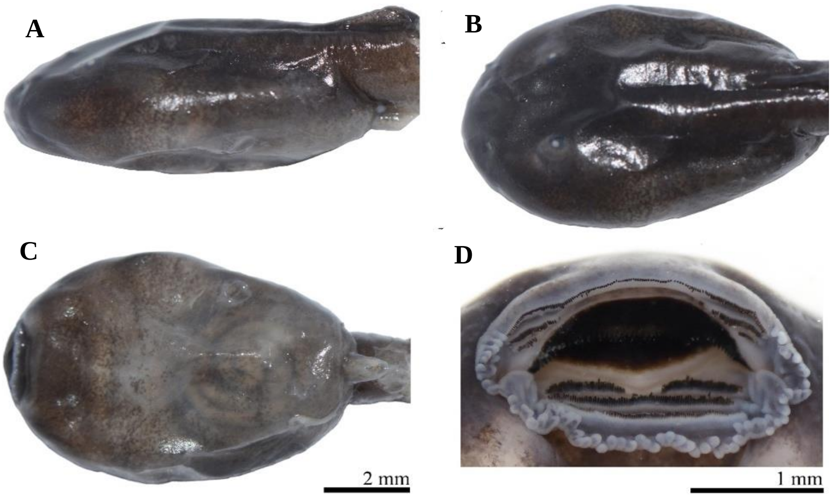

Figure 5. Photograph of *T. albopunctatum* tadpole (PT 02499.1, Gosner stage 27). A. lateral view, B. dorsal view, C. ventral view, D. oral disc. Scale bar of A, B, and C equal 2 mm

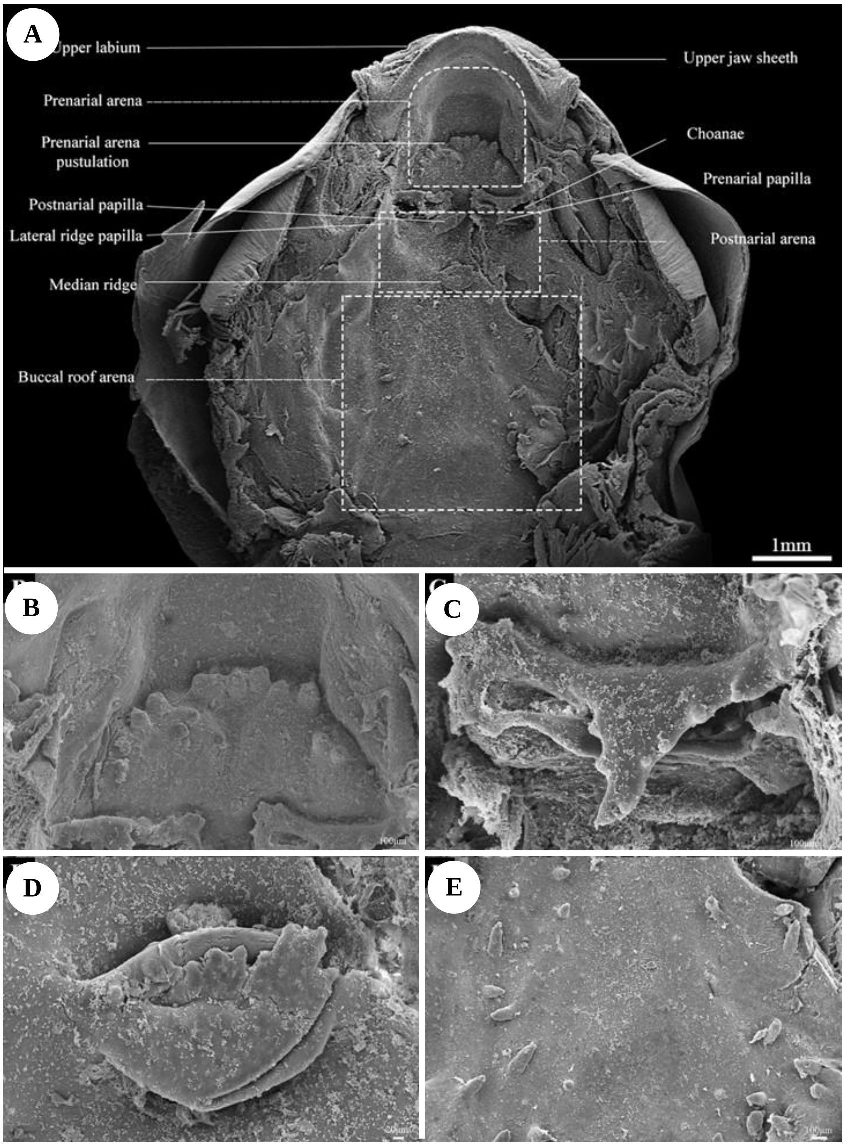

Figure 6. Buccal roof anatomy of *T. albopunctatum* tadpole (PT 02499.2, Gosner stage 37) under scanning electron microscope (SEM). A. Buccal roof, B. Prenarial arena pustulation, C. Prenarial papilla, D. Middle ridge, E. Lateral ridge papillae on buccal roof arena

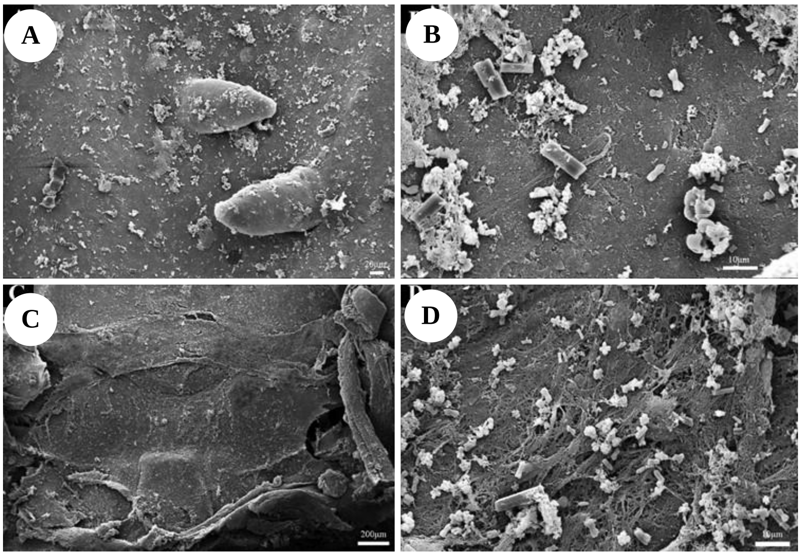

Figure 7. Buccal roof surface of *T. albopunctatum* tadpole (PT02499.2, Gosner stage 37) under SEM. A. Buccal roof arena surface at magnification 200X, B. Buccal roof arena surface at magnification 1000X, C. Glandular zone surface at magnification 46X, D. Glandular zone surface at magnification 1000X

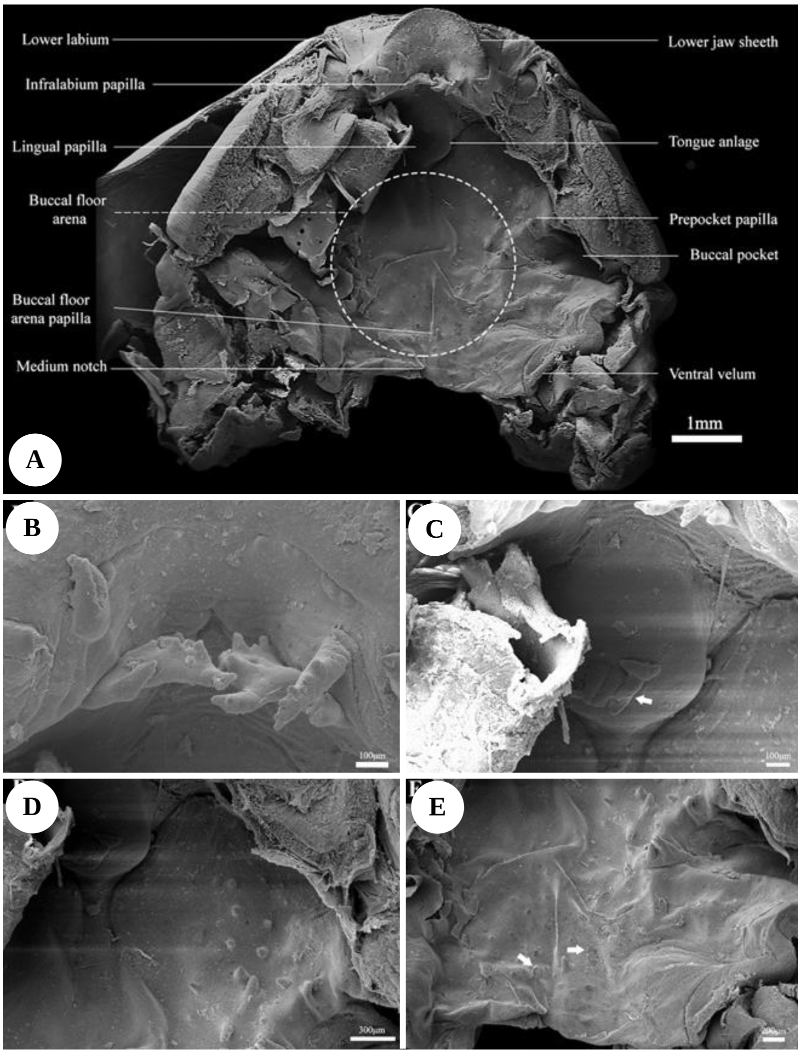

Figure 8. Buccal floor anatomy of *T. albopunctatum* tadpole (PT 02499.2, Gosner stage 37) under SEM. A. Buccal floor, B. Infralabial papillae, C. Tongue anlage and lingual papilla (white narrow), D. Buccal floor arena papillae on lateral position, E. Buccal floor arena papillae (black narrow) on posterior position

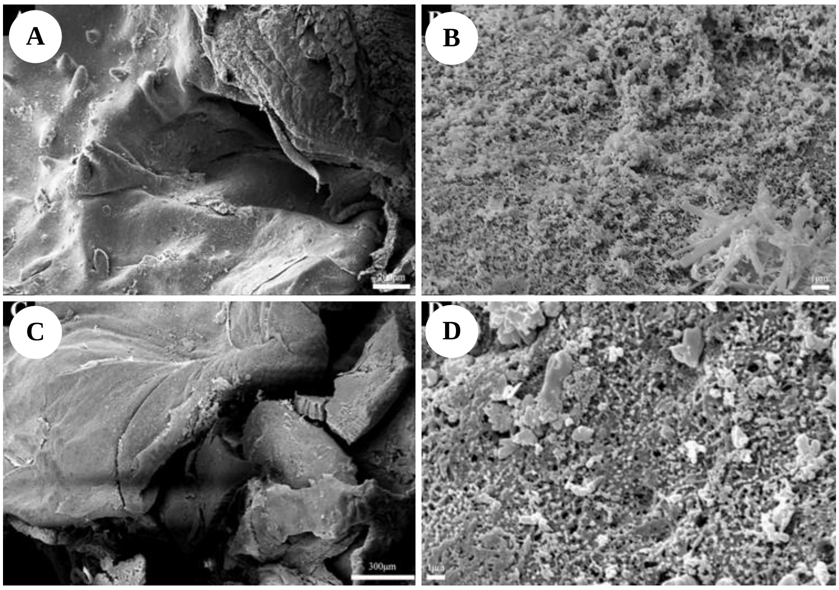

Figure 9. Buccal surface of *T. albopunctatum* (PT 02499.2, Gosner stage 37) under SEM. A. Buccal pocket surface at magnification 58X, B. Buccal pocket surface at magnification 5000X, C. Ventral velum surface at magnification 58X, D. Ventral velum surface at magnification 5000X

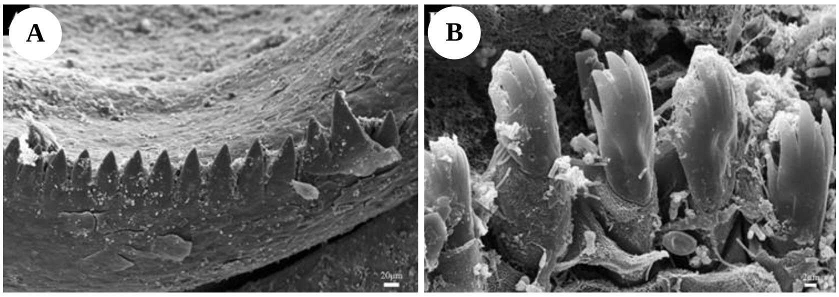

Figure 10. Jaw sheath and labial tooth shape of *T. albopunctatum* tadpole (PT02499.2, Gosner stage 37) under SEM. A. Upper jaw sheath, B. Lower labial tooth showing spoon-shaped bearing 6 large cusps

Among specimens from Thailand, one individual from Nan Province (northern Thailand) formed a clade with specimens from Loei and Phetchabun Provinces (northeastern Thailand) and from Laos, whereas specimens from Chiang Mai Province formed a clade with specimens from Myanmar and Vietnam. Thus, intraspecific genetic distances among Thailand specimens showed high divergence, with a pairwise distance of 4.42% for 16S. These results suggest the possible presence of cryptic species in this region.

Among the 26 known species of genus *Theloderma*, 12 (~46%) species have been described at the tadpole stage, namely *T. horridum* (Boulenger 1903), *T. moloch* (Annandale 1912), *T. stellatum* (Wasserug et al. 1981), *T. asperum* (Leong and Lim 2003), *T. nebulossum* (Rowley et al. 2011), *T. bambusicolum* (Orlov et al. 2012), *T. bicolor* (Gawor et al. 2012), *T. corticale* (Gawor et al. 2012), *T. auratum* (Poyarkov et al. 2018), *T. gordoni* (Kropachev et al. 2018), *T. ryabovi* (Kropachev et al. 2019), and *T. vietnamense* (Poyarkov et al. 2015). Among the eight *Theloderma* species reported to occur in Thailand, four (50%) species have been described at the tadpole stage, namely *T. asperum*, *T. horridum*, *T. stellatum*, and *T. vietnamense*, and only one of which (*T. stellatum*) was described based on specimens from Thailand.

The tadpole stage of *T. albopunctatum* is similar to the tadpole stages of 12 *Theloderma* species in terms of general characteristics, such as body shape, eyes, spiracle, vent tube position, jaw sheets shape, and oral disc position. Morphologically, *T. albopunctatum* tadpoles are most similar to *T. asperum* tadpoles. However, a single row of marginal papillae on the lower labium of *T. albopunctatum* tadpoles (stages 25, 27, and 37) was found, in contrast to single (stage 24) and double rows (stage 35) in *T. asperum* tadpoles. However, the keratodont row formula (KRF) of *T. albopunctatum* tadpoles (1:1+1/1+1:1 and 1:2+2/1+1:2) is also similar to *T. asperum* tadpoles (1:2+2/1+1:2) (Leong and Lim 2003).

The tadpoles of *T. horridum*, *T. moloch*, *T. stellatum*, *T. nebulossum*, *T. bambusicolum*, and *T. gordoni* tadpoles are similar in having numbers of KRF, which is 1:3+3/3 (Boulenger 1903; Annandale 1912; Wassersug et al. 1981;

Rowley et al. 2011; Orlov et al. 2012; Kropachev et al. 2018). Thus, the different of KRF maybe distinguished them from *T. albopunctatum*. The different of KRF also distinguished tadpoles of *T. albopunctatum* from those of *T. bicolor* of 1:2+2/3 (Gawor et al. 2012), *T. corticale* of 1:3+3/1+1:2 (Gawor et al. 2012), *T. auratum* of 2:3+3/3 (Poyarkov et al. 2018), *T. ryabovi* of 1:3+3/3, 1:3+3/1+1:2 (Kropachev et al. 2019), and *T. vietnamense* of 1:2+2/3, 1:3+3/3, 1:4+4/3 (Poyarkov et al. 2015).

Theloderma buccal anatomy has remained poorly understood since its description in *T. stellatum* based on specimens from Thailand (Wassersug et al. 1993). The buccal anatomy of *T. albopunctatum* differs from the anatomy of *T. stellatum* in terms of the following characteristics, i.e., prenarial papillae projection (absent vs. a single long papillae), postnarial arena papillae (one pair of papillae vs. two large papillae), median ridge shape (bulging vs. triangular), prepocket papillae (4 vs. 2-3), and infralabial papilla (two pairs vs. one pair).

Wassersug et al. (1993) suggested that tadpoles of *Theloderma* species feed on microscopic plankton because they have large brachial baskets, dense gill filters, and weak buccal depressors. *Theloderma* tadpoles are typically found in tree holes, as well as artificial containers in or near forest habitats (Niyomwan et al. 2019; Makchai et al. 2020). In this study, *T. albopunctatum* tadpoles were found in artificial containers covered by leaf litter or humus, which resemble tree hole environments.

In conclusion, the present work adds information on tadpole morphology and buccal anatomy to science. The sequences of the tadpole and adults of *T. albopunctutatum* from PKNNFC have genetic distance varies from 0-0.2% and 0.15% for 16S and COI, respectively. *T. albopunctatum* tadpoles are medium size, elliptical body depression, and keratodont row formula 1:2+ 2/1+1:2 or 1:1+1/1+1:1. Coloration in life is black to ashy gray and fades to dark brown in preservative. Their buccal roof bearing knobby projection on prenarial arena, and internarial distance is approximately one-third of choanae length. Postnarial and lateral papillae are absent on postnarial arena. On buccal roof arena, only six buccal roof arena papillae present on each side. Buccal floor is smooth and has two pairs of infralabial papillae. Tongue anlage is oval with four lingual papillae. Buccal floor arena covers with numerous short papillae.

Because identification of tadpoles is difficult to do in the field or with other identified methods, e.g., rearing to adults and identifying them, comparing with previous description, for instance. Some tadpole descriptions are based on species that may be later found to be a species complex and presently split into several new species. In addition, many species exhibit highly similar in the form of tadpoles and thus correct identification requires more accurate allocation (e.g., molecularly identified) and description. Such previous descriptions need to be revised and corrected, and accurate full descriptions of present species would allow their taxonomic reassessment in the future. According to the present and previous works suggested that the *T. asperum* group required further investigation.

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Table S1. Localities, voucher information, and GenBank numbers for all samples used in this study. Classification followed Frost (2021)

Species	Voucher	Localities	16S	COI	BNBF	RHO	SIA	TYR
Buergeria oxycephalus	MVZ 230425	China: Hainan	KU244359	KU244459	KU244396	KU244313	KU244427	KU244357
Liuixalus romeri	CIB<CH>20080048	China: Hong Kong	AB871412	–	–	–	–	–
Nasutixalus medogensis	6255Rao	China: Motuo, Xizang	GQ285679	–	–	–	–	–
Nyctixalus margaritifer	KUHE 26135	Indonesia: Java	LC012864	–	–	–	–	–
N. pictus	FMNH 231095	Malaysia: Sabah, Lahad Datu	DQ283133	–	–	–	–	–
N. pictus	FMNH 231094	Malaysia: Sabah, Lahad Datu	GQ204726	–	–	–	–	–
N. pictus	KUHE 53517	Malaysia: Sarawak, Bario	LC012863	–	–	–	–	–
N. pictus	AH07001	Malaysia: Sarawak, Gunung Mulu	GU154888	–	–	–	–	–
N. pictus	MVZ 239460	Indonesia: Bengkulu	KU561880	KU244454	–	–	–	–
Nyctixalus sp.	CAS 247868	Myanmar: Tanintharyi	KU244380	KU244457	KU244403	KU244314	KU244424	KU244355
Nyctixalus sp.	CAS 247498	Myanmar: Tanintharyi	KU561883	KU244456	KU561889	KU561892	KU561895	KU561898
Nyctixalus spinosum	ACA 940	Indonesia	AF458136	–	–	–	–	–
N. spinosum	Nsp1	Philippines: Mindanao	KT461916	–	–	–	–	–
N. spinosum	ACD 1043	Philippines: Mindanao	DQ283114	–	–	–	–	–
T. albopunctatum	ROM 30246	Vietnam: Tuyen Quang	AF458148	–	–	–	–	–
T. albopunctatum	KIZ 060821201	China: Yunnan	EF564521	–	–	–	–	–
T. albopunctatum	KIZ 060821217	China: Guangxi	EF564522	–	–	–	–	–
T. albopunctatum	HN0806100	China: Hainan	GQ285678	–	–	–	–	–
T. albopunctatum	VNMN J2916	Vietnam: Vinh Phuc	KJ802913	–	–	–	–	–
T. albopunctatum	VNMN 3540	Vietnam: Lao Cai	KJ802914	–	–	–	–	–
T. albopunctatum	2006.2573	Laos: Luang Phabang	KR828081	KR087994	–	–	–	–
T. albopunctatum	asperum-1	Vietnam: Kon Tum	KT461884	–	–	–	–	–
T. albopunctatum	asperum-2	Vietnam: Kon Tum	KT461908	–	–	–	–	–
T. albopunctatum	asperum-3	Vietnam: Kon Tum	KT461909	–	–	–	–	–
T. albopunctatum	ZMMU NAP 03557	Vietnam: Hai Phong	KT461910	–	–	–	–	–
T. albopunctatum	ZMMU NAP 03566	Vietnam: Hai Phong	KT461911	–	–	–	–	–
T. albopunctatum	ZMMU NAP 03575	Vietnam: Hai Phong	KT461912	–	–	–	–	–
T. albopunctatum	CAS 241559	Myanmar: Shan	KU244362	KU244441	KU244401	KU244322	KU244416	KU244352
T. albopunctatum	FMNH 270722	Thailand: Nan	KU244366	KU244440	KU244405	KU244325	KU244412	KU244345
T. albopunctatum	NCSM 79167	Laos: Savannakhet	KU244367	KU244434	KU244385	KU244324	KU244409	KU244340
T. albopunctatum	NCSM 79445	Laos: Luang Phabang	KU244369	KU244439	KU244388	KU244323	KU244415	KU244337
T. albopunctatum	NCSM 76483	Laos: Savannakhet	KU244372	KU244437	KU244386	KU244327	KU244408	KU244343
T. albopunctatum	VNMN JR2887	Vietnam: Tam Dao	KU244375	KU244431	KU244381	KU244318	KU244407	KU244338
T. albopunctatum	NCSM 79444	Laos: Luang Phabang	KU244379	KU244438	KU244387	KU244326	KU244414	KU244347
T. albopunctatum	VNMN JR2888	Vietnam: Tam Dao	LC012853	KU244432	KU244382	KU244319	KU244406	KU244351
T. albopunctatum	VNMN 4404	Vietnam: Kon Tum	LC012854	–	–	–	–	–
T. albopunctatum	VNMN 4405	Vietnam: Gia Lai	LC012855	–	–	–	–	–
T. albopunctatum	VNMN 4406	Vietnam: Than Hoa	LC012856	–	–	–	–	–
T. albopunctatum	VNMN PAE 262	Vietnam: San La	LC012857	–	–	–	–	–

T. albopunctatum	KUHE 23736	Thailand: Doi Changdao	LC012858	–	–	–	–	–	
T. asperum	ZRC1.1.9321	Malaysia: Fraser Hill	GQ204725	–	–	–	–	–	
T. asperum	pet trade	Malaysia: Perak	KT461929	–	–	–	–	–	
T. bicolor	MNHN 1999.5986	Vietnam	AY880529	–	–	–	–	–	
T. bicolor	IEBR A.20011.4	Vietnam: Lao Cai	JX046474	–	–	–	–	–	
T. bicolor	VNMN 1394	Vietnam: Lao Cai	JX046475	–	–	–	–	–	
T. bicolor	Tbic3	Vietnam: Ninhbinh	KT461899	–	–	–	–	–	
T. bicolor	Tbic2	Vietnam: Ninhbinh	KT461923	–	–	–	–	–	
T. corticale	AMNH A161499	Vietnam: Vinh Phu	DQ283050	–	–	–	–	–	
T. corticale	VNMN J2892	Vietnam: Tuyen Quang	KJ802916	–	–	–	–	–	
T. corticale	Tcort1	Vietnam: Ninhbinh	KT461885	–	–	–	–	–	
T. corticale	Tcort2	Vietnam: Ninhbinh	KT461886	–	–	–	–	–	
T. corticale	MVZ 223905	Vietnam: Tam Dao	KU244364	KU244452	KU244393	KU244316	KU244417	KU244354	
T. corticale	VNMN 3556	Vietnam: Tam Dao	LC012841	–	–	–	–	–	
T. corticale	MVZ 225131	Vietnam: Tam Dao	KU244365	KU244453	KU244394	KU244315	KU244420	KU244353	
T. gordoni	MVZ 226469	Vietnam: Tam Dao	KU244363	KU244451	KU244395	KU244334	KU244423	KU244358	
T. gordoni	VNMN 4407	Vietnam: Kon Tum	LC012852						
T. horridum	KUHE 52582	Malaysia: Negeri Sembilan, Kenaboi	LC012861	–	–	–	–	–	
T. horridum	ZMMU NAP 04015	Thailand: Satun	KT461890	–	–	–	–	–	
T. laeve	ZNMU NAP 02906	Vietnam: Binh Phuoc	KT461883	–	–	–	–	–	
T. laeve	ZNMU NAP 02907	Vietnam: Binh Phuoc	KT461905	–	–	–	–	–	
T. laeve	ZNMU NAP 02908	Vietnam: Binh Phuoc	KT461906	–	–	–	–	–	
T. laeve	ZNMU NAP 01644	Vietnam: Lam Dong	KT461907	–	–	–	–	–	
T. laeve	ZNMU NAP 01645	Vietnam: Lam Dong	KT461913	–	–	–	–	–	
T. lateriticum	AMNH 168757	Vietnam, Lao Cai, SaPa	LC012848	–	–	–	–	–	
T. lateriticum	VNMN PAE 226	Vietnam: Son La	LC012849	–	–	–	–	–	
T. lateriticum	VNMN 1215	Vietnam: Bac Giang, Yen Tu	LC012850	–	–	–	–	–	
T. lateriticum	VNMN 1216	Vietnam: Bac Giang, Yen Tu	LC012851	–	–	–	–	–	
T. leporosum	KUHE 52581	Malaysia: Kenaboi	AB847128	–	–	–	–	–	
T. leporosum	LJT W46	Malaysia	KC465841	–	–	–	–	–	
T. leporosum	Tlep1	Malaysia: Selangor	KT461922	–	–	–	–	–	
T. licin	KUHE 19426	Thailand: Nakon Sri Tamarat	LC012859	–	–	–	–	–	
T. licin	KUHE 52599	Malaysia: Selangor	KJ802920	–	–	–	–	–	
T. licin	MVZ 272184	Indonesia	KU244368	KU244447	KU244384	KU244333	KU244425	KU244346	
T. moloch	SDBDU 2011.345	Arunachal Pradesh, India	KU169993	–	–	–	–	–	
T. moloch	YPX31941	China: Medog, Tibet	KU243081	–	–	–	–	–	
T. nebulosum	AMS R 173409	Vietnam: Kon Tum	JN688168	–	–	–	–	–	
T. nebulosum	AMS R173877	Vietnam: Kon Tum	JN688169	–	–	–	–	–	
T. nebulosum	ROM 39588	Vietnam: Kon Tum	KT461887	–	–	–	–	–	
T. nebulosum	VNMN ROM 39588	Vietnam	LC012845	–	–	–	–	–	
T. palliatum	AMS R 173130	Vietnam: Lam Dong	JN688172	–	–	–	–	–	
T. palliatum	AMS R 173131	Vietnam: Lam Dong	JN688173	–	–	–	–	–	
T. palliatum	ZMMU NAP 02511	Vietnam: Lam Dong	KT461902	–	–	–	–	–	

T. palliatum	ZMMU NAP 02516	Vietnam: Lam Dong	KT461903	–	–	–	–	–
T. palliatum	ZMMU NAP 02735	Vietnam: Dak Lak	KT461926	–	–	–	–	–
T. palliatum	ZMMU NAP 02736	Vietnam: Dak Lak	KT461927	–	–	–	–	–
T. palliatum	ZMMU NAP 02756	Vietnam: Dak Lak	KT461930	–	–	–	–	–
T. palliatum	VNMN NAP 2735	Vietnam: Dak Lak	LC012843	–	–	–	–	–
T. palliatum	VNMN NAP 2736	Vietnam: Dak Lak	LCO12844	–	–	–	–	–
T. phrynoderma	CAS 243920	Myanmar: Tanintharyi	KJ128282	KU244448	KU244402	KJ128278	KU244410	KJ128276
T. phrynoderma	CAS 247910	Myanmar: Tanintharyi	KJ128283	KU244449	KU244404	KJ128279	KU244411	KJ128277
T. pyaukkya	CAS 226113	Myanmar: Kachin	KU244361	KU244443	KU244399	KU244331	KU244421	KU244339
T. pyaukkya	CAS 236133	Myanmar: Kachin	KU244360	KU244444	KU244400	KU244330	KU244419	KU24434
T. pyaukkya	CAS 234869	Myanmar: Chin	KU244370	KU244445	KU244398	KU244332	KU244418	KU244342
T. pyaukkya	CAS 234857	Myanmar: Chin	KU244371	KU244446	KU244397	KU244329	KU244426	KU244341
T. rhododiscus	AMNH A163893	Vietnam: Ha Giang, Vi Xuyen	DQ283393	–	–	–	–	–
T. rhododiscus	KIZ 060821063	China: Guangxi	DQ283393	–	–	–	–	–
T. rhododiscus	KIZ 060821170	China: Guangxi	EF564534	–	–	–	–	–
T. rhododiscus	SCUM 061192L	China: Mt. Dayao, Guangxi	EU215530	–	–	–	–	–
T. rhododiscus	CIB<CH>GX200807017	China: Guangxi	LC012842	–	–	–	–	–
T. ryabovi	VNMN 3924	Vietnam: Kon Tum, Mang Canh	LC012860	–	–	–	–	–
T. ryabovi	ryabovi-1	Vietnam: Kon Tum	KT461914	–	–	–	–	–
T. ryabovi	ryabovi-2	Vietnam: Kon Tum	KT461915	–	–	–	–	–
Theloderma sp.	VNMN 4403	Vietnam: Gia Lai	LC012846	–	–	–	–	–
T. stellatum	NAP03961	Thailand: Nakhonnayok	KT461917	–	–	–	–	–
T. truongsonense	ROM 39363	Vietnam: Quang Binh	KT461925	–	–	–	–	–
T. truongsonense	VNMN 4402	Vietnam: Khanh Hoa	LC012847	–	–	–	–	–
T. vietnamense	AMS R 173283	Vietnam: Binh Thuan	JN688170	–	–	–	–	–
T. vietnamense	AMS R 147047	Cambodia: Mondol Kiri	JN688171	KU244460	KU244391	KU244335	–	KU244356
T. vietnamense	ZMMU NAP 03724	Vietnam: Kien Giang	KT461888	–	–	–	–	–
T. vietnamense	NAP03723	Vietnam: Kien Giang	KT461919	–	–	–	–	–
T. vietnamense	ZMMU NAP 03680	Vietnam: Tay Ninh	KT461921	–	–	–	–	–
T. vietnamense	KUHE 22065	Thailand, MaeYom	LC012862	–	–	–	–	–
T. vietnamense	FMNH 267765	Cambodia: Koh Kong	KU561885	KU244462	–	–	–	–
T. vietnamense	FMNH 262786	Cambodia: Mondolkiri	KU561884	KU244461	–	–	–	–
T. vietnamense	NCSM 76490	Laos: Savannakhet	KU561886	KU244464	–	–	–	–
T. vietnamense	NCSM 80384	Vietnam: Binh Thuan	KU561887	KU244463	KU561888	KU561891	KU561894	KU561897
T. albopunctatum	PT 02378	Thailand: Phetchabun, Nam Nao NP	MW648779	MW648785	MW648776	MW648782	MW648788	MW648779
T. albopunctatum	JC 02506	Thailand: Loei, Phu Luang WS	MW648780	MW648786	MW648777	MW648783	MW648789	MW648780
T. albopunctatum	PT 02499.1	Thailand: Loei, Phu Luang WS	MW648781	MW648787	MW648778	MW648784	MW648790	MW648781

Note: 16S: 16S rRNA, COI: cytochrome oxidase subunit I, BNBF: nuDNA brain-derived neurotrophic factor, RHO: rhodopsin, SIA: seventh-in-absentia, TYR: tyrosinase