



Research article

Larval description of Fea's horned frog *Brachytarsophrys feae* (Boulenger, 1887) (Anura: Megophryidae) from northern Thailand

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Abstract

Importance of the work: Existing knowledge lacks intensive description of tadpoles of *Brachytarsophrys feae*. The present work fulfils this knowledge gap by providing an intensive description of external and buccal anatomy.

Objectives: To describe the external morphology and buccal anatomy of tadpoles of *B. feae*.

Materials & Methods: Tadpoles were described based on two specimens (THNHM 28693.1–2) collected in Doi Mae Tum, Chiang Rai province, northern Thailand. Identification was based on comparisons with published taxonomic works. External morphological description was based on THNHM 28693.1. Buccal description was based on images from scanning electron micrographs of THNHM 28693.2.

Results: The tadpoles are medium sized (total length 34.7–39.1 mm, stage 30–31), with an umbelliform oral disc, emarginated and marginal papillae absent and numerous submarginal papillae inside. The belly is separated from the chest by a white band, broad white lateral stripes along both sides of the head and body and five symmetrical white bands. Buccal features are similar to those of *B. carinense* but differing slightly by a smaller distance between choana.

Main finding: The work provided the first description of the external morphology and buccal anatomy of tadpoles of *B. feae*. This work adds knowledge on buccal anatomy, which is insufficient in members of *Brachytarsophrys*.

Introduction

Brachytarsophrys feae (family Megophryidae) was described from “East of Bhamò, Khakhyen Hills, Burma

(= Myanmar)” by Boulenger (1887). The species has been detected in China (southwestern Guangxi and southwestern and southeastern Yunnan) and northern Myanmar, Thailand, and Vietnam (Frost, 2021). Zhao et al. (2014) showed a photograph of a *B. feae* tadpole (in preservative) from China, but little information of this tadpole was reported worldwide.

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Chan-ard et al. (2007) confirmed the occurrence of *B. feae* in Thailand based on frog specimens and a photograph of a tadpole. They referred to two tadpole specimens collected from a small hill stream in Doi Mae Tum, Chiang Rai province, in February 2000. However, Chan-ard et al. (2007) noted that these specimens were missing, although a larval photograph was shown. The tadpole morphology was reported as: total length 45–50 mm, oral disc terminal and funnel shaped, head and body dark brown, ellipsoidal in shape, about as long as wide, ventral with broad black cross bands, tail fin margins with dark spots, tail tapering gradually posteriorly with a round tail tip. The tadpoles were assigned to *B. feae* based on the larval characteristics, especially the ventral patterns that corresponded with the illustration of a *B. feae* tadpole in Fei (1999). The description of *B. feae* tadpoles is incomplete. Therefore, the present work fills the gaps in the knowledge of the external morphology and buccal anatomy of *B. feae* tadpoles.

Materials and Methods

Two tadpole specimens (THNM 28693.1–2) from the collection of the Thailand Natural History Museum (THNM) were used to describe the external and buccal morphologies. These two specimens are the tadpole specimens in Chan-ard et al. (2007) according to the label on the bottle. The tadpoles were collected by Tanya Chan-ard and Kriangkrai Suwannapakdi from a location in Doi Mae Tum, Chiang Rai province, northern Thailand. The developmental stage was assigned according to Gosner (1960). The morphological terminology follows Altig and McDiarmid (1999), the oral disk terminology follows Altig (1970), and the buccal anatomy terminology follows Wassersug (1976). The measurements were performed as described by Grosjean (2005). Most measurements (Table 1) were made using digital calipers (to the nearest 0.1 mm), whereas characters smaller than 1 mm were measured using an ocular scale attached to a stereomicroscope (Nikon C-LEDs). The abbreviations used in the descriptions are: AL, anterior labium; BH, maximum body height; BL, body length; SVL, snout to vent length; BW, maximum body width; ED, maximum eye diameter; LF, maximum height of lower tail fin; MTH, maximum tail height; NN, internarial distance; ND, narial diameter; NP, naro-pupillary distance; ODW, oral disk width; PL, posterior labium; RN, rostro-narial distance; SD, spiracle diameter; SS, distance from tip of snout to opening of spiracle; TAL, tail length; TL, total length; TMH, tail muscle

height; TMW, tail muscle width; and UF, maximum height of upper tail fin.

Specimens were prepared and viewed using scanning electron microscopy (SEM) at the Faculty of Science of Khon Kaen University, Thailand. Buccopharyngeal anatomical studies require specimen preparation before SEM and details of procedures followed Chuaykern et al. (2018, 2019) and Chunskul et al. (2021). Pieces of the buccal roof and buccal floor were processed by progressive dehydration using an alcohol series (1 d each with 70%, 90% and then 100%), transferred to a 1:1 mixture of amyl acetate and absolute alcohol for 20 min and soaked in pure amyl acetate for 20 min before the specimens were critical-point dried using liquid carbon dioxide. Finally, the dry specimens were sputter-coated in gold and subjected to SEM observation.

Results

Larval description

Larval description based on specimen THNM 28693.1, stage 30; TL 39.1 mm, BL 11.6 mm. Body in lateral view slender (Fig. 1), oval in lateral view (Fig. 1). Snout rounded in dorsal view, narrowly rounded in lateral view; BW 123% of BH. Eyes of moderate size, ED 10% of BL, bulging and not visible in ventral view, positioned and directed dorsolaterally. Nares oval, moderately large, rimmed, positioned and directed anterolaterally, closer to pupils than to tip of snout, RN 160% of NP. Spiracle single, sinistral, tube shape, small size, at midpoint of the body; positioned ventrolaterally, directed posteriorly. Tail musculature moderately strong, TMH 71% of BH and 59% of MTH, runs parallel in proximal half, gradually tapering to tail tip. Tail fins moderate size, same as body height in proximal half, small arch in distal half; UF 31% of MTH, LF 35% of MTH; upper fin slightly arched in distal half, tapering gradually in distal half, upper fin not extending onto body; lower fin shallow in proximal half, tapering gradually in distal half, maximum tail fin at middle, tail tip rounded. Vent tube (Fig. 1) narrowly conical, small, medial and entirely attached directly to lower fin, opening directed posteriorly. Oral disc (Figs. 1 and 2) medium, umbelliform (funnel shape), positioned and directed anterodorsally, not emarginated. Jaw sheaths small, upper jaw sheath thin, semicircular, serrated, lower jaw sheath thick, semicircular, serrated. Marginal papillae absent. Submarginal papillae 30, long. Denticulate papillae absent.

Labial teeth absent. Pineal ocellus absent. Lateral line absent. In preservative, dorsal and lateral head and body brownish; ventral surface of head and body white background, with several dark bands, six bands on belly and a large trapezoid on chin; two short longitudinal white stripes along sides of body, each stripe separated by a dark stripe. Dark ventrolateral stripe extending from mid-body through lower tail to a position at nearly two-thirds of tail length. Oral disc whitening gradually from edge into inside the mouth. Upper and lower fine white.



Fig. 1 *Brachytarsophrys feae* tadpole in preservative (THNHM 28693.1) at stage 30 (total length 39.1 mm) showing external morphology in the lateral (top), dorsal (middle) and ventral (bottom) views



Fig. 2 Photograph showing the oral disc morphology of a *Brachytarsophrys feae* larva in preservative (THNHM 28693.1, stage 30; total length 39.1 mm, oral disk width 2.7 mm)

In life (based on **Fig. 3** in Chan-ard et al., 2007), dorsal and lateral head and body brownish; ventral surface of head and body white background with several dark bands, six bands on belly and a large trapezoid on chin; dark ventrolateral stripe extending from mid-body through lower tail to a position at nearly two-thirds of tail length. Oral disc whitening gradually from edge into inside the mouth. Upper and lower fine white.

Buccal description

Buccal description based on specimen THNHM 28693.2, stage 31, TL 34.7 mm, BL 11.1 mm.

Buccal roof (**Fig. 3**) has prenarial arena with two median projections, small, top of projection smooth and round. Prenarial papillae: two pairs of projections, bulging triangular, round tip, attached to buccal roof, in body axis direction. Choanae: narrow round, horizontal, without wall, internarial distance approximately one-fourth length of choanae. Narial valve projection: single pair, round tip, attached to buccal roof, in body axis direction. Postnarial arena small area. Postnarial papillae: absent. Lateral ridge papillae: single pair, bulging, pointed tip, parallel to median ridge, anterior direction. Median ridge: single ridge, very large, pagoda shaped, middle position, closer to upper jaw sheath than dorsal velum, anterior direction. Buccal roof arena: large, oval shape, smooth. Buccal roof arena papilla: single pair, round tip, attached to buccal roof, anterior direction. Lateral roof papillae: absent. Glandular zone: smooth. Dorsal velum: continuous and small cushion.

Buccal floor (**Fig. 4**) has buccal floor arena hexagonal shape, small horizontal cushion at anterior part. Tongue anlage: absent. Infralabial papillae: single pair, medium, bulging, round tip, inside of lower jaw sheath, anterior direction. Lingual papillae: single pair, medium, round, behind infralabial papillae, dorsal position. Buccal floor arena papillae: absent. Prepocket papillae: absent. Buccal pockets: horizontal slit, closer to ventral velum than lower jaw sheath. Ventral velum: continuous, margin smooth. Branchial baskets: nearly vertical, longer than wide, four filter plates on each side, the second filter plate length approximately one-half of buccal floor arena length. Median notch: medially. Glottis: medium size.

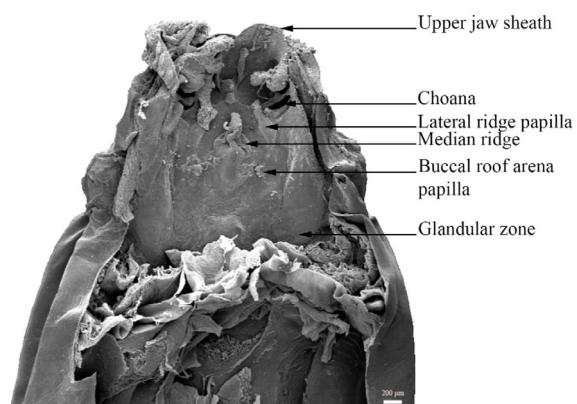


Fig. 3 Scanning electron micrograph showing roof of buccal cavity of *Brachytarsophrys feae* tadpole (THNHM 28693.2, stage 31)

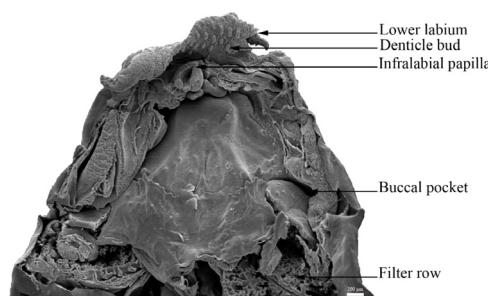


Fig. 4 Scanning electron micrograph showing floor of buccal cavity of *Brachytarsophrys feae* tadpole (THNHM 28693.2, stage 31)

Discussion

Taylor (1962) included *B. feae* (as *Megophrys feae*) in the amphibian fauna of Thailand but failed to confirm a specimen from this country. He mentioned that the species was reported from Thailand, perhaps incorrectly, but probably occurs in the mountains of northern Thailand. Chan-ard et al. (2007) confirmed its occurrence, based on a specimen (FMNH 20963) collected from Doi Ang-Ga (Chiang Mai province, northern Thailand), a photograph of a live frog from Doi Inthanon (Chiang Mai province) and two tadpoles collected from Doi Mae Tum (Chiang Rai province, northern Thailand). Chan-ard et al. (2007) included a tadpole photograph (in lateral view), but they did not indicate whether the tadpole was photographed while it was alive or after preservation. These two tadpole specimens could not be found in the collection of the Thailand Natural History Museum according to Chan-ard et al. (2007). The two tadpoles described in the present work are

the specimens in Chan-ard et al. (2007), according to the label on the bottle. Chan-ard et al. (2007) assigned these tadpoles to *B. feae* based on their external characteristics, which were consistent with the illustration in Fei (1999). Similar to Chan-ard et al. (2007), the current study assigned the tadpoles to *B. feae* based on comparisons with relevant taxonomic works (Fei, 1999; Zhao et al., 2014; Fei and Ye, 2016; Li et al., 2020). The tadpoles from Thailand have coloration on the ventral part of the head and body with six light transverse bands.

The tadpoles (TL 39.1 mm and BL 11.6 mm, at stage 30) seemed smaller than those of *B. carinense* (TL 40.3 mm and BL 14.4 mm, at stage 30), as shown in Tables 1 and 2. The ventral part of the head and body has six light transverse bands in *B. feae* (Fig. 1), while it is reticulate in *B. carinense* (Fig. 5). The white streak running from the snout along the lateral head and body of *B. carinense* (Fig. 5) is absent in

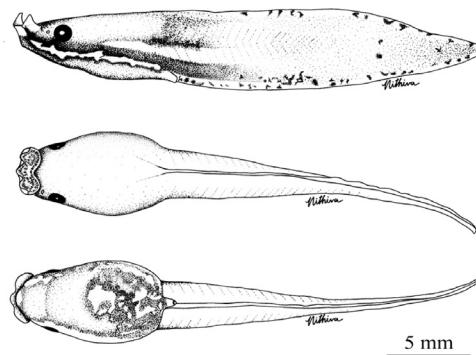


Fig. 5 Illustration of *Brachytarsophrys carinense* tadpole (KKUC 01071.1) at stage 26 (total length 41.0 mm) showing external morphology in the lateral (top), dorsal (middle) and ventral (bottom) views (drawn by Nithina Kaewtongkum)

Table 1 Measurements (in millimeters) of tadpoles of *Brachytarsophrys feae*

Measurement	THNHM 28693.1, Stage 30	THNHM 28693.2, Stage 31	Measurement	THNHM 28693.1, Stage 30	THNHM 28693.2, Stage 31
TL	39.1	34.7	TAL	26.4	23.3
BL	11.6	11.1	UF	2.1	1.5
SVL	12.7	11.4	LF	2.3	1.8
SS	7.0	6.0	MTH	6.7	5.1
BH	5.3	6.0	TMH	3.8	4.5
BW	6.5	6.4	TMW	2.7	3.1
NN	3.4	3.6	ODW	2.7	1.4
RN	1.6	1.1	AL	0.8	0.5
NP	1.0	0.9	PL	1.9	0.9
ND	0.3	0.3	SD	1.7	1.1
ED	1.2	1.2			

TL = total length; BL = body length; SVL = snout to vent length; SS = distance from tip of snout to opening of spiracle; BH = maximum body height; BW = maximum body width; NN = internarial distance; RN = rostro-narial distance; NP = naro-pupillary distance; ND = narial diameter; ED = maximum eye diameter; TAL = tail length; UF = maximum height of upper tail fin; LF = maximum height of lower tail fin; MTH = maximum tail height; TMH = tail muscle height; TMW = tail muscle width; ODW = oral disk width; AL = anterior labium; PL = posterior labium; SD = spiracle diameter

B. feae (Fig. 1). In *B. feae*, the eye position is dorsal, while it is dorsolateral in *B. carinense*. The buccal anatomy is quite similar in both these tadpole species. Lateral ridge papillae and median ridge (stage 31) are less developed in *B. feae* than in *B. carinense* (stage 37), perhaps due to growth of the tadpoles. In *Sylvirana nigrovittata* (Ranidae), the main morphological characters in the buccal cavity are present from stage 26, although the numbers and size of papillae and pustules continue to increase during the subsequent stages (Grosjean, 2005). As in *Lithobates catesbeiana* (Ranidae), the shape of the tongue anlage varies throughout larval development (Hamerman, 1969). *B. feae* (stage 31) has several papillae in the lateral area of the buccal floor arena that are absent in *B. carinense* (stage 37). This feature should differentiate the larval species because the papillae occur earlier in development. Wassersug (1976) observed earlier stabilization of the characters in *Pseudacris regilla* (Hylidae). He found that numerous characters (infrabial papillae, lingual papillae, buccal floor arena papillae, ventral velum projections) on the buccal floor were stabilized by developmental stage 26.

Current knowledge of the diversity of amphibian species is growing rapidly (Frost, 2021). Worldwide, 8,395 species have been recognized, of which approximately 2.6% (218 species according to Frost, 2021) are known from Thailand. However, as the genus-level recognition of monophyletic short-legged toads (*Brachytarsophrys*) was recently drawn into the taxonomic debate on *Megophrys* sensu lato, Li et al. (2020) regarded *Brachytarsophrys* as a distinct genus based on its marked morphological differentiation and recent molecular analyses. They revalidated the species *B. platyparietus* and described *B. orientalis* as new to science. According to Li et al. (2020), seven *Brachytarsophrys* species are recognized and have been separated into two species groups based on their morphological differences and phylogenetic relationships: the groups *B. carinense* (*B. carinense* and *B. intermedia*) and *B. feae* (*B. chuannanensis*, *B. feae*, *B. orientalis*, *B. platyparietus*, and *B. popei*). Unfortunately, current knowledge of tadpole morphology is still incomplete and there may be many more cryptic species in Thailand (Sheridan and Stuart, 2018). Studies have revealed previously unreported species diversity in Thailand (Sheridan and Stuart, 2018; Suwannapoom et al., 2018), highlighting the need for further research on amphibians, particularly amphibian tadpoles. The present work has completed a gap in the knowledge regarding descriptions of the external and buccopharyngeal parts of *Brachytarsophrys* tadpoles from Thailand.

Table 2 Comparison of tadpole morphology of *Brachytarsophrys feae* with its congeners

Character	<i>B. carinense</i>	<i>B. chuannanensis</i>	<i>B. feae</i>	<i>B. orientalis</i>	<i>B. popei</i>
Size	BL 14.4 mm; TL 40.3 mm; Stage 30	-	BL 11.1–11.6 mm; TL 34.7–39.1 mm; Stage 30–31	BL 12.3 mm; TL 27.6 mm; Stage 36	No data
Dorsum pattern	Brownish, uniform	-	Brownish, uniform	No data	Brownish, uniform
White band separating chest and belly	Absent ¹	Present	Present	Absent	Present
Chest pattern	Uniform	Dark background mixed; narrow oblique white lateral stripes along both sides of chest	Dark background medially; broad white lateral stripes along both sides running across chest as a transverse band	Dark background mixed with white spots; narrow white lateral stripes along both sides of chest	Dark background with mottles; broad white lateral stripes along both sides of chest
Belly pattern	A symmetrical ventral pattern	Dark background with numerous white blotches ²	Dark background with five symmetrical white bands, the first band very narrow or indistinct ^{2,3,4}	Dark background with numerous white blotches ²	Dark background with white reticulated pattern ²

Table 2 Continued

Ventrolateral white-stripe	Present	Absent	Absent	Absent	Absent	Absent
Tail pattern	White stripe along lower part of tail; dark stripe along lower edge of tail	Dark stripe between upper and lower parts of tail; white stripe along lower part of tail; dark stripe along lower edge of tail	White stripe along lower part of tail, dark stripe along lower edge of tail	Dark stripe along lower edge of tail	Dark stripe along lower edge of tail	Absent
Buccal roof (prenarial arena)	Small with large U-shape projection, top of projection smooth and round; two prenarial papillae, large, bulging, triangular, round tip, attached to buccal roof, into body axis direction; Choanae narrow round, horizontal, without wall, internarial distance about choanae length; single pair narial valve projections, round tip, attached to buccal roof, in body axis direction.	No data	Small with two median projections, top of projection smooth and round; two prenarial papillae, round tip, attached to buccal roof, into body axis direction; Choanae: narrow round, horizontal, without wall, internarial distance ~1/4 length of choanae; Single pair narial valve projection, round tip, attached to buccal roof, in body axis direction.	No data	No data	No data
Buccal roof (prenarial arena)	Very small area; postnarial papillae absent; single pair lateral ridge papillae, bulging, triangular, pointed tip, parallel median ridge, anterior direction; single median ridge, very large, four level pagodas, middle position, closer to upper jaw sheath than dorsal velum, anterior direction.	No data	Small area; postnarial papillae absent; single pair lateral ridge papillae, bulging, pointed tip, parallel to median ridge, anterior direction; single median ridge, very large, pagoda shaped, middle position, closer to upper jaw sheath than dorsal velum, anterior direction.	No data	No data	No data
Buccal roof (buccal roof arena)	Large size, oval shape, smooth; single pair buccal roof arena papilla, round tip, attached to buccal roof, anterior position, anterior direction; lateral roof papillae absent; glandular zone smooth; dorsal velum continuous and small cushion.	No data	Large size, oval shape, smooth; single pair buccal roof arena papilla, round tip, attached to buccal roof, anterior direction; lateral roof papillae absent; glandular zone smooth; dorsal velum continuous and small cushion.	No data	No data	No data

Table 2 Continued

Ventrolateral white-stripe	Present	Absent	Absent	Absent	Absent	Absent
Buccal floor	Hexagon shape, small horizontal cushion at half, tongue anlage absent; single pair infralabial papillae, medium, bulging, round tip, attached to lower jaw sheath, inside of lower jaw sheath, anterior direction; single pair lingual papillae, medium, round, behind infralabial papillae, dorsal position; buccal floor arena papillae absent; preocket papillae absent; buccal pockets: horizontal slit, closer to ventral velum than lower jaw sheath; ventral velum: continuous, margin smooth; branchial baskets nearly vertical, longer than wide, four filter plates on each side, the second filter plate length $\sim 1/2$ of buccal floor arena length; median notch moderated size; glottis: moderate size.	No data	Hexagonal shape, small horizontal cushion at half, tongue anlage absent; single pair infralabial papillae, medium, bulging, round tip, inside of lower jaw sheath, anterior direction; single pair lingual papillae, medium, round, behind infralabial papillae, dorsal position; buccal floor arena papillae absent; preocket papillae absent; buccal pockets: horizontal slit, closer to ventral velum than lower jaw sheath; ventral velum: continuous, margin smooth; branchial baskets nearly vertical, longer than wide, four filter plates on each side, the second filter plate length $\sim 1/2$ of buccal floor arena length; median notch moderated size; glottis moderate size.	No data	No data	No data
Information source	Kaewtongkum et al. (2014), Kaewtongkum (2016)	Li et al. (2020)	Li et al. (2020), Chan-and et al. (2007), this study	Li et al. (2020)	Li et al. (2020)	Li et al. (2020)
BL = body length; TL = total length						

Conflict of Interest

The authors declare that there are no conflicts of interest.

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