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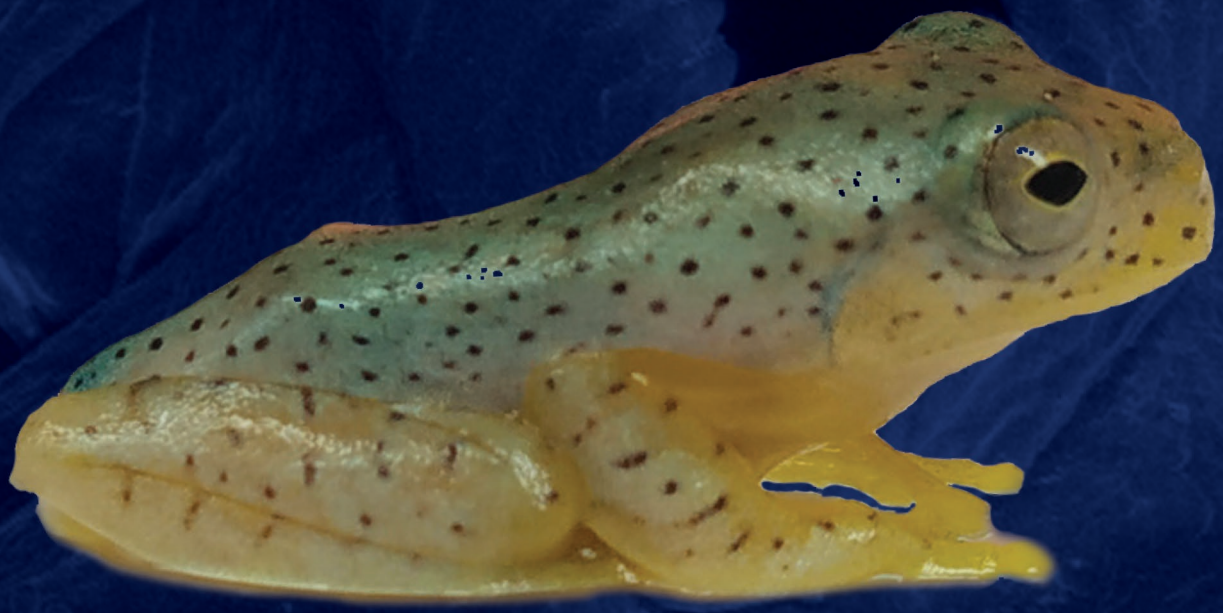

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Ontogenetic systematic characterisation of an endemic frog *Rhacophorus malabaricus* Jerdon, 1870 (Anura: Rhacophoridae) from Western Ghats, Kerala, India

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ABSTRACT
In the present study, the complete description of the development and metamorphosis of an endemic frog *Rhacophorus malabaricus*, Jerdon, 1870, is documented in its natural habitat from the Southern part of Western Ghats (Peppara Wildlife Sanctuary, India). A brief illustration of each Gosner stage (fertilized egg to metamorphosed froglet) is given based on the direct monitoring and characterisation of morphological and morphometric variations. The identity of the tadpoles was confirmed by gene sequencing. Fertilisation and early development (cleavage, blastulation gastrulation and neurulation) take place inside the foam at 21.4 ± 0.1°C. The motile stage begins at 3rd day, after hatching and then eventually dropping into the water body. Hind limbs start differentiation first on the 15th day. The larva attains maximum size (TL 48.70 ± 0.22 mm) at Gosner stages 42. The morphometric measurements are significantly correlated with Gosner stages especially from 26 to 39. Oral disc features are described with LTRF of 7(3-7)/3 and KRF of 2:5+5/3. The morphological and morphometrical data of *R. malabaricus* larva is compared with other known *Rhacophorus* Kuhl & Van Hasselt, 1822 members. The present study shows that the relative lengths of TL, SVL, BH and TAL with stages are significant morphometric characters for taxonomy. Morphological features (limb bud development, pigmentation, oral disc features, etc.) are potentially useful characters for tadpole based anuran taxonomy at stage 26 or later.

KEY WORDS
Rhacophoridae,
Western Ghats,
ontogenetic
development,
morphometric
correlation,
tadpole description,
oral disc.

RÉSUMÉ

Caractérisation ontogénétique systématique d'une grenouille endémique Rhacophorus malabaricus *Jerdon, 1870 (Anura: Rhacophoridae) des Ghâts occidentaux, Kerala, Inde.*

Dans cette étude, une description complète du développement et de la métamorphose d'une grenouille endémique de la partie sud des Ghâts occidentaux, *Rhacophorus malabaricus* (Jerdon 1870), est documentée dans leur habitat naturel (Peppara Wildlife Sanctuary, Inde). Chaque stade de Gosner (de l'œuf fécondé jusqu'à la grenouille métamorphosée) est illustré sur la base d'un suivi direct et de la caractérisation des variations morphologiques et morphométriques. L'identité des têtards a été confirmée par séquençage des gènes. La fécondation et les premiers stades de développement (clivage, blastulation, gastrulation et neurulation) se déroulent à l'intérieur de l'écume à 21.4 ± 0.1 °C. Le stade mobile commence au 3e jour, quand le têtard a éclos puis est finalement tombé dans le plan d'eau. Ses membres postérieurs se forment à partir du 15e jour. La larve atteint sa taille maximale (TL 48,70 ± 0,22 mm) aux stades Gosner 42. Les mesures morphométriques sont significativement corrélées avec les stades Gosner en particulier entre les stades 26 à 39. Les caractéristiques du disque oral sont décrites avec LTRF de 7 (3-7) / 3 et KRF de 2: 5 + 5/3. Les données morphologiques et morphométriques de la larve de *R. malabaricus* sont comparées à celles d'autres membres connus de *Rhacophorus* Kuhl & Van Hasselt, 1822. La présente étude montre que la longueur relative de TL, SVL, BH et TAL des différents stades sont des caractères morphométriques significatifs pour la taxonomie. Les caractéristiques morphologiques (développement des bourgeons des membres, pigmentation, caractéristiques du disque buccal, etc.) sont des caractères potentiellement utiles pour la taxonomie des anoures basée sur les têtards à partir du stade 26.

MOTS CLÉS
Rhacophoridae, Ghâts occidentaux, développement ontogénétique, corrélation morphométrique, description du têtard, disque oral.

INTRODUCTION

The family Rhacophoridae Hoffman, 1932 (1858) (tree frogs) consisting of 440 species attributed to 23 genera is one of the species rich families among anurans (AmphibiaWeb 2021 accessed on 14 July 2021). The Western Ghats, a distinct biogeographic zone in India is recognised as a primary centre for radiation of Rhacophoridae (Inger 1986, 1999; Yu *et al.* 2009; Abraham *et al.* 2013; Biju *et al.* 2013; Jiang *et al.* 2019). Earlier reports on biodiversity of Rhacophoridae indicate that more species from the family are yet to be discovered (Glaw & Köohler 1998; Vasudevan & Dutta 2000; Biju *et al.* 2013; Vijayakumar *et al.* 2014; Mo *et al.* 2016; Priti *et al.* 2016; Garg *et al.* 2021).

The conventional systematics, principally rooted in morphological and morphometric data is often fragmentary and does not reconcile anuran phylogenetics. Homoplasy of morphological variations and similarity of long established morphological characters among anurans are the rationale underling this convolution. Henceforth, oral morphology with larval characters can be used as an alternative approach. Many herpetologist had discussed the phylogenetic potential of larval morphological characters. Furthermore, studies centred on oral morphology had produced concordant results with resumed adult morphological evidence (Grillitsch *et al.* 1993; Haas 2003; Grosjean 2005; Raharivololoniaina *et al.* 2006; Laurin & Germain 2011; Randrianiaina *et al.* 2011; Wolfe & Hegna 2014). The developmental stages of many frog species are not brought to light so far. Descriptions of precise developmental variations among different species are often too concise to be a part of taxonomic and systematic studies. Ontogenetic characterisation of obtainable species is a pertinent redressment in ontogenetic systematics.

Recent studies have been reported on the development of Rhacophoridae family by Mohanty-Hejmadi & Dutta (1988); Hendrix *et al.* (2007); Dehling (2008); Wildenhues *et al.* (2010); Haas *et al.* (2012); Amit (2013); Biju *et al.* (2013); Vassilieva *et al.* (2016). Larval description of *Rhacophorus helenae* Rowley, Tran, Hoang & Le, 2012b (Vassilieva *et al.* 2016), *Leptomantis rufipes* (as *Rhacophorus rufipes*) Inger, 1966, *Leptomantis penanorum* Dehling, 2008 (as *Rhacophorus penanorum*), *Zhangixalus dulitensis* Boulenger, 1892 (as *Rhacophorus dulitensis*) (Haas *et al.* 2012), *Vampyrius vampyrus* (as *Rhacophorus vampyrus*) Rowley, Le, Thi, Stuart & Hoang, 2010 (Vassilieva *et al.* 2013), *Leptomantis orlovi* (as *Rhacophorus orlovi*) Ziegler & Köhler, 2001 (Wildenhues *et al.* 2011) were reported. Generalized larval morphology, morphometry and oral descriptions of *Rhacophorus kio* Ohler & Delorme, 2006, *Rhacophorus rhodopus* Liu & Hu, 1960 (Grosjean & Inthara 2016), *Zhangixalus smaragdinus* Blyth, 1852 (as *Rhacophorus maximus*) (Wildenhues *et al.* 2010), *L. rufipes*, *L. penanorum*, *Z. dulitensis* (Haas *et al.* 2012), *R. vampyrus* (Vassilieva *et al.* 2013), *R. orlovi* (Wildenhues *et al.* 2011) were accounted.

Kadadevaru & Kanamadi (2000) and Amit (2013) gave a peripheral overview that provides data on courtship and nesting behaviour of *R. malabaricus* Jerdon, 1870. Biju *et al.* (2013) also mentioned the ontogenetic colour changes along with reproductive behaviour. Even though some elucidations are available for developmental stages, these are often in abbreviated forms. So far no serious attempt has been made to study the complete monitoring of development and metamorphosis of the Malabar gliding frog or Malabar flying frog, *Rhacophorus malabaricus* (endemic to Western Ghats). Detailed descriptions on larval biology of *R. malabaricus* from their natural habitat are limited. The present study describes ontogenetic

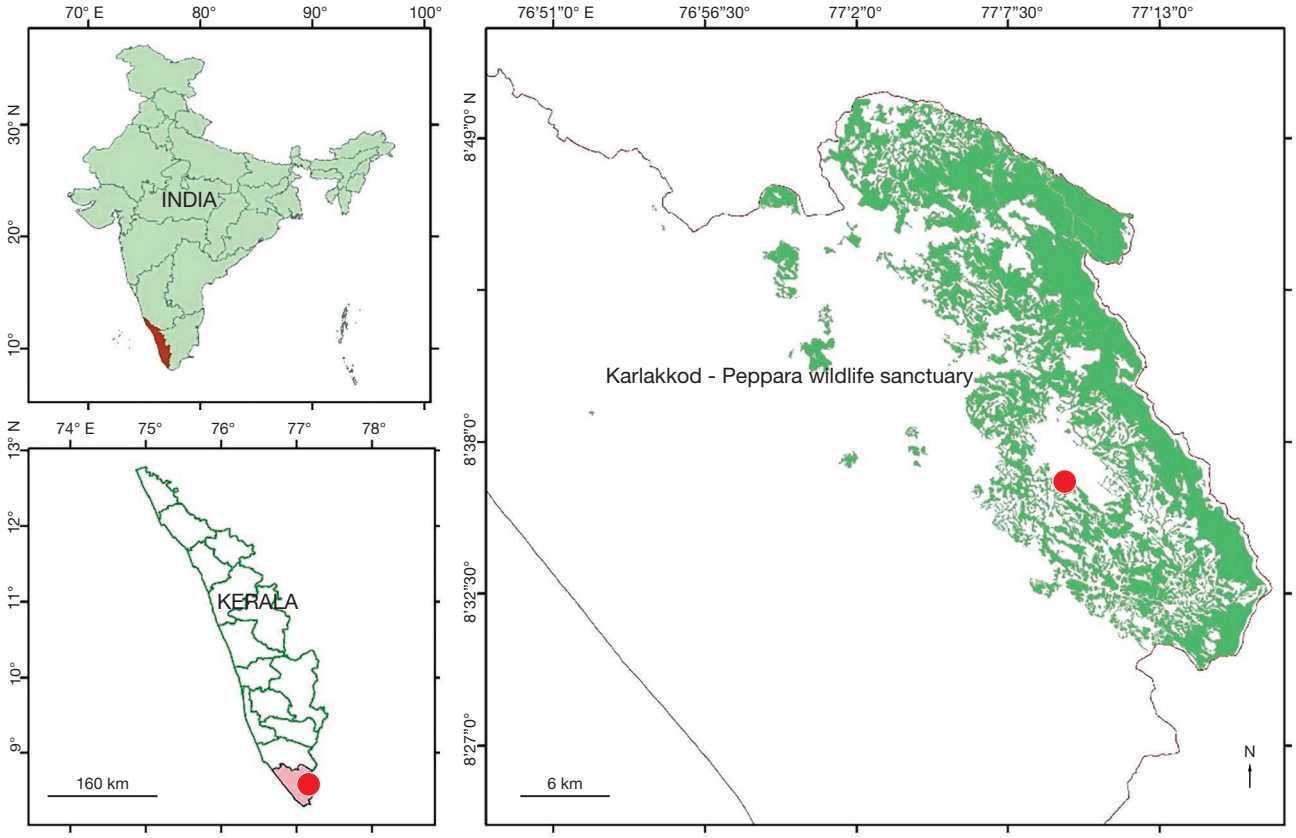

Fig. 1. — Map of study area (Karlakkod, Peppara Wildlife Sanctuary, Thiruvananthapuram, Kerala, India). Source: Survey of India Topographical Map.

variations in morphologic, morphometric and oral disc features of *R. malabaricus* tadpoles from the natural habitats of Peppara Wildlife Sanctuary, Western Ghats, Kerala, India.

MATERIAL AND METHODS

Direct observation, identification and collection

Foam nest of *R. malabaricus* were found during field survey from Karlakkod, Peppara Wildlife Sanctuary (8°36'31.4"N, 77°09'38.5"E) Western Ghats, Kerala, India, on 18 July 2019. The average atmospheric temperature during development was 26-28°C. The nest was suspended on the shrub branch above stagnant portion of water stream (2 m above from water level) which is the tributary part of River Karamanayar. The stream was about 1-1.5 m wide and its depth ranged from few centimetres to 1 m. The bottom of water body had rocks, gravel and clay covered by dead leaves and other plant debris. Eggs and embryo in early developmental stages were collected, preserved (70% ethanol), monitored and morphometric measurements were taken with graduated ocular micrometre in stereo-microscope (Olympus Ch20i). The hatched tadpoles that frequently fall into the water, were randomly collected (n = 2-6), photographed and released back after immediate recording of 16 morphometric measurements with Vernier callipers. Tadpoles were staged according to Gosner (1960). Morphological terminology followed in the present study was according to Altig & Johnston (1989), Altig & McDiarmid (1999) and Altig (2007). Data were analysed with software SPSS 10.1. Pearson correlation analysis was also done to express the variation of morphometric parameters (Delaugerre & Dubois 1985). Tadpoles were collected (Gosner stage 21-40) and preserved (30% ethanol) for oral disc analysis. The specimen preparation for SEM examination was prepared by passing over in dehydration series (30%, 50%, 75%, 95% and 100%) of ethanol followed by drying and mounting on stub.

The tadpole identification was further substantiated by 16S rRNA gene sequencing. Genomic DNA of tadpole was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. The 16S rRNA sequence were amplified using universal primer, Forward(CGCCTGTTTATCAAAAACAT) and reverse (CCGGTCTGAACTCAGATCACGT) (Palumbi *et al.* 1991).The PCR amplification was done in thermal cycler (Eppendorf) in a total volume of 10 µL, containing 2 µL of 5× PCR buffer, 0.2 µL of dNTP (2 mM), 0.5 µL of each primer (10 mM), 0.2 µL of Phire Taq DNA polymerase (Applied Biosystems, Foster City, CA), 3.5-5.5 µL of ddH2O and 1-3 µL of template DNA (10-20 ng). The cycling condition as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 55°C for 40 s, 72°C for 90 s, and then followed by final extension step at 72°C for 5 min. 5 µl of PCR product is mixed with

2 µl of ExoSAP-IT and incubated at 37°C for 30 minutes followed by enzyme inactivation at 80°C for 15 minutes to purify PCR products. Sequencing reaction was performed in an ABI 3730 capillary sequencer using Big Dye Terminator V.3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA). Taxonomic identity was authenticated by the 16S sequence similarity search using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). 23 sequences used in the present study were retrieved from Genbank (Table 1) and sequence of *Polypedates pseudocruciger* Das & Ravichandran, 1998 was chosen as out-group. Multiple sequence alignment was carried out using MUSCLE in MEGA X 10.1.8 (Kumar *et al.* 2018). The phylogenetic tree was constructed in Mr. Bayes 3.2.6 (Ronquist *et al.* 2012) program. Bayesian analysis was done selecting the GTR+G+I model as best fitting model and two independent runs were performed for 2 × 10^6 generations sampling per 100 generations. The first 25% of the trees acquired were discarded as burn-in, and a 50% majority-rule consensus tree with posterior probability (PP) values was generated from the leftover trees. The phylogenetic tree was constructed using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Abbreviations

TL	Total length (from the tip of the snout to the tip of the tail);
SVL	snout-vent length (from the tip of the snout to the junction of the posterior body and the tail musculature);
TAL	tail length (from the junction of the posterior body and the tail musculature to the tip of the tail);
BW	body width (the highest width of the body);
BH	body height (the highest height of the body);
HW	head width at level of eyes;
TMH	tail muscle height at base of tail;
UTF	upper tail fin height (the highest height of the upper fin, from the upper margin of the tail musculature to the upper margin of the upper fin);
LTF	lower tail fin height (the highest height of the lower fin, from the lower margin of the lower fin to the lower margin of the tail musculature);
TMW	tail muscle width at base of tail;
END	eye-naris distance (from the anterior corner of the eye to the posterior margin of the naris [nostril]);
NSD	naris-snout distance (from the anterior margin of the naris to the tip of the snout);
IND	internarial distance (the distance between the median margins of the nares);
IOD	interorbital distance (the distance between the median margins of the orbits);
ED	eye diameter (the greatest length of the orbit from the anterior margin to the posterior margin of the eye);
MTH	maximum height of tail (the highest height of the tail).

RESULT

Family Rachophoridae Hoffman, 1932 (1858)
Genus *Rhacophorus* Kuhl & Van Hasselt, 1822

Rhacophorus malabaricus Jerdon, 1870

Rhacophorus malabaricus Jerdon, 1870: 66.

Molecular identification

The 16S rRNA gene similarity search using BLAST confirmed the organism as *Rhacophorus malabaricus*. The sequences obtained were submitted to GenBank (Accession No.MW130836 and MW130837). Phylogenetic analysis was done based on a dataset of 26 sequences from the family Rhacophoridae including four sequences of *R. malabaricus* (two from present study and two obtained from GenBank). All the four sequences of *R. malabaricus* were recovered in a single cluster. The genetic distance calculated was significantly low (≤ 1.8%) which was in congruence with the obtained tree topology. The closest relative inferred for *R. malabaricus* from the BI tree was *R. pseudomalabaricus* Vasudevan & Dutta, 2000 as anticipated (genetic distance ≤ 7.61 %). The other sister species which had close genetic association with *R. malabaricus* was *Rhacophorus catamitus* Harvey, Pemberton & Smith, 2002 (genetic distance ≤ 13.2%; Fig. 12).

Development of tadpole

We observed that the life cycle of *R. malabaricus* was completed in 44 days. The suspended foam nests of *R. malabaricus* were formed by connecting leaves (Fig. 2). Nest was observed with approximately 235 eggs (diameters of 2.17 ± 0.41, n = 15). Gosner stage 1- 21 was completed within the foam nest. The average temperature within the nest was 21.4 ± 0.1°C. They dropped down into the water body at Gosner stage 22, and their aquatic life then lasted up to Gosner stage 43. Metamorphosis was completed at Gosner stage 46 on 44 day during which the larva got adapted to terrestrial habitat.

The following description of different stages of tadpoles was based on the age, size and external morphological characters. Development and metamorphosis of *Rhacophorus malabaricus* has been briefly recorded below:

Fertilized egg (Gosner stage 1)

The spherical shaped fertilized egg (0 hr) measured was about 2.1 ± 0.2 mm diameter (n = 6). The animal pole was dark brown coloured and vegetal pole yellowish white coloured (Fig. 3A). The eggs were macrolecithal.

Cleavage and blastulation (Gosner stage 2 to 12)

Gosner stages 2 to 12 were completed within 10.30 hrs. After fertilisation, the first cell division took place in about 1 hr. The diameter was about 2.26 ± 0.11 mm (n = 6). The embryo became 64 to 128 celled morula in 8 hours (Gosner stage 8) and later reached at blastula stage by the repeated cleavage in 8.20 hrs. The pigmented region on the animal pole slightly extended to vegetal pole. Later, the embryo entered gastrulation (Gosner stage 10) in 9 hrs with a diameter of about 3.2 ± 0.4 mm (n = 4). The crescent shaped dorsal lip was visible. The pigmented area was extending to the vegetal pole and the unpigmented area was highly reduced. Yolk plug appeared at the end of 10.30 hrs (Gosner stage 12).

Neuralation (Gosner stage 13 to 16)

The duration for neuralation was 11 hrs to 2 days. The neural plate appeared in Gosner stage 13 (Fig. 3B) which showed a distinct broader cerebral region, followed by a narrow spinal

FIG. 2. — Leafs hiding a foam nest of *Rhacophorus malabaricus* Jerdon, 1870.

TABLE 1. — Details of species included here in phylogenetic analysis.

Species	Genbank AN	Country	Locality	Reference
Polypedates pseudocruciger Das & Ravichandran, 1998	KU169984	India	Kerala	Biju *et al.* 2016
Rhacophorus annamensis Smith, 1924	KX139179	Vietnam	Dak Lak Province, Chu Yang Sin	Vassilieva *et al.* 2016
R. baluensis Inger, 1954	KC961089	Malaysia	Sabah	Hertwig *et al.* 2013
R. bipunctatus Ahl, 1927	LC010569	Malaysia	Pahang	Tao *et al.* 2014
R. borneensis Matsui, Shimada & Sudin, 2013	AB781693	Malaysia	Sabah	Matsui *et al*. 2013
R. catamitus Harvey, Pemberton & Smith, 2002	JF748392	Indonesia	Sumatra	Streicher *et al*. 2012
R. helenae Rowley, Tran, Hoang & Le, 2012	JQ288088	Vietnam	Dong Nai Province, Tan Phu	Vassilieva *et al.* 2016
R. kio Ohler & Delorme, 2006	JQ288093	Laos	Houaphan Province	Vassilieva *et al.* 2016
R. lateralis Boulenger, 1883	AB530548	India	Mudigere, Karnataka	Hasan *et al.* (2014)
R. malabaricus Jerdon, 1870	AB530549	India	Madikeri, Karnataka	Hasan *et al.* (2014)
R. malabaricus	MW130836	India	Thiruvananthapuram, Kerala	present study
R. malabaricus	MW130837	India	Thiruvananthapuram, Kerala	present study
R. malabaricus	KU170014.1	India	Wayanad	Xu *et al* 2020
R. nigropalmatus Boulenger, 1895	AB781701	Malaysia	Sarawak	Vassilieva *et al.* 2016
R. norhayatii Chan & Grismer, 2010	AB728191	Malaysia	Johor	Vassilieva *et al.* 2016
R. orlovi Ziegler & Köhler, 2001	LC010597	Vietnam	Thanh Hoa	Tao *et al.* 2014
R. pardalis Günther, 1858	JN377368	Malaysia	Sarawak	Haas *et al*. 2012
R. pseudomalabaricus Vasudevan & Dutta, 2000	KC593855	India	Idukki, Kerala	Biju, *et al.* 2013
R. reinwardtii Schlegel, 1840	JN377364	Malaysia	Sarawak, Batang Ai	Vassilieva *et al.* 2016
R. rhodopus Liu & Hu, 1960	LC386573	China	Jinghong	Matsui *et al.* 2019
R. translineatus Wu, 1977	MW111521	India	Himalaya	Xu *et al.* 2020

code region. The rest of the embryo was light brown coloured except the neural plate which was sandy yellow coloured. The diameter of the embryo in Gosner stage 15 was 4.0 ± 0.31 mm (n = 5; Fig. 3C). Neural tube was formed by the fusion of neural fold in both cerebral and spinal code regions. Gosner stage 16 was completed on the 2nd day.

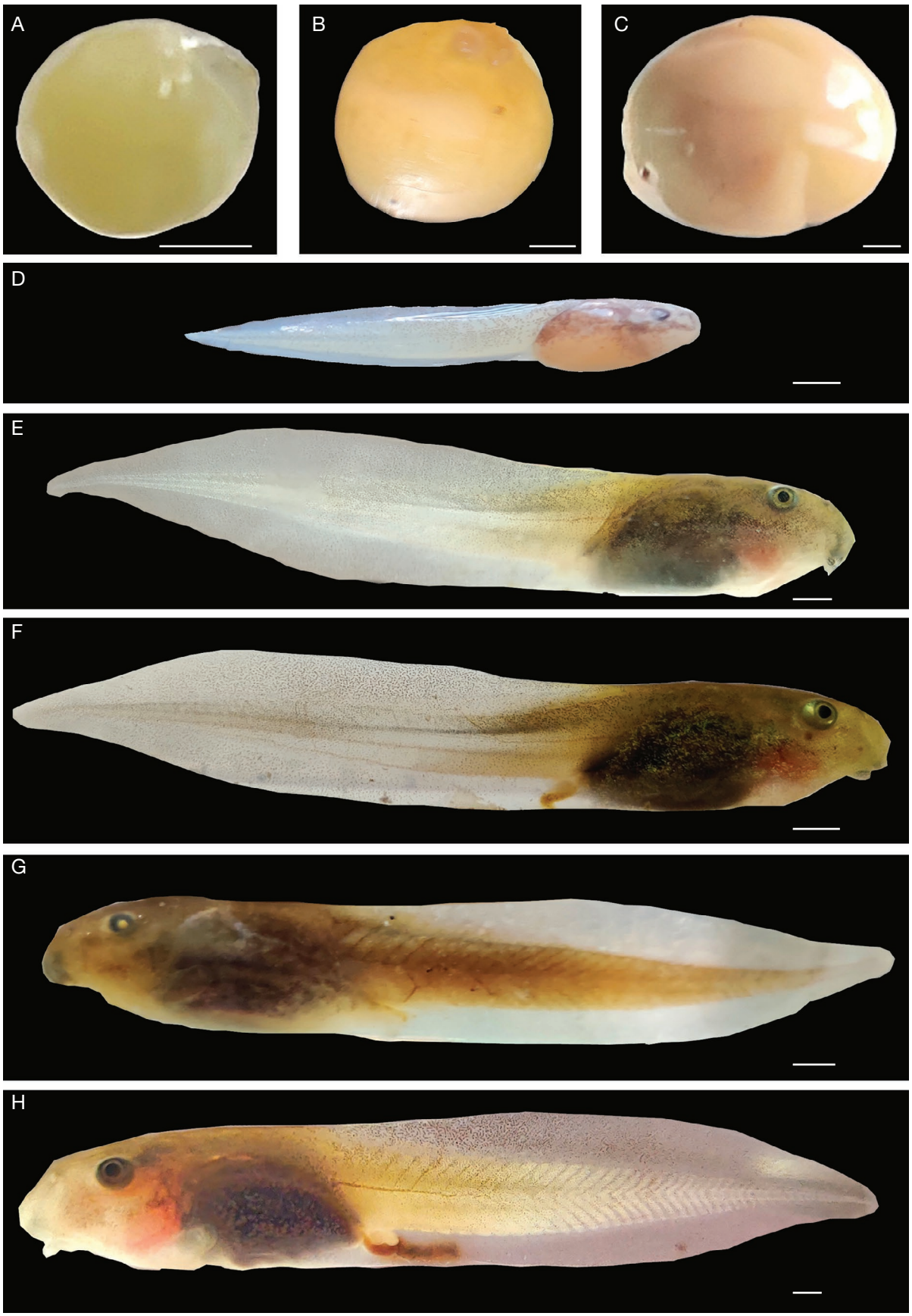

Fig. 3. — Developmental stages of *Rhacophorus malabaricus* Jerdon, 1870: **A**, Gosner stage 1; **B**, Gosner stage 13; **C**, Gosner stage 15; **D**, Gosner stage 22; **E**, Gosner stage 26; **F**, Gosner stage 27; **G**, Gosner stage 29; **H**, Gosner stage 31. Scale bars: 1 mm.

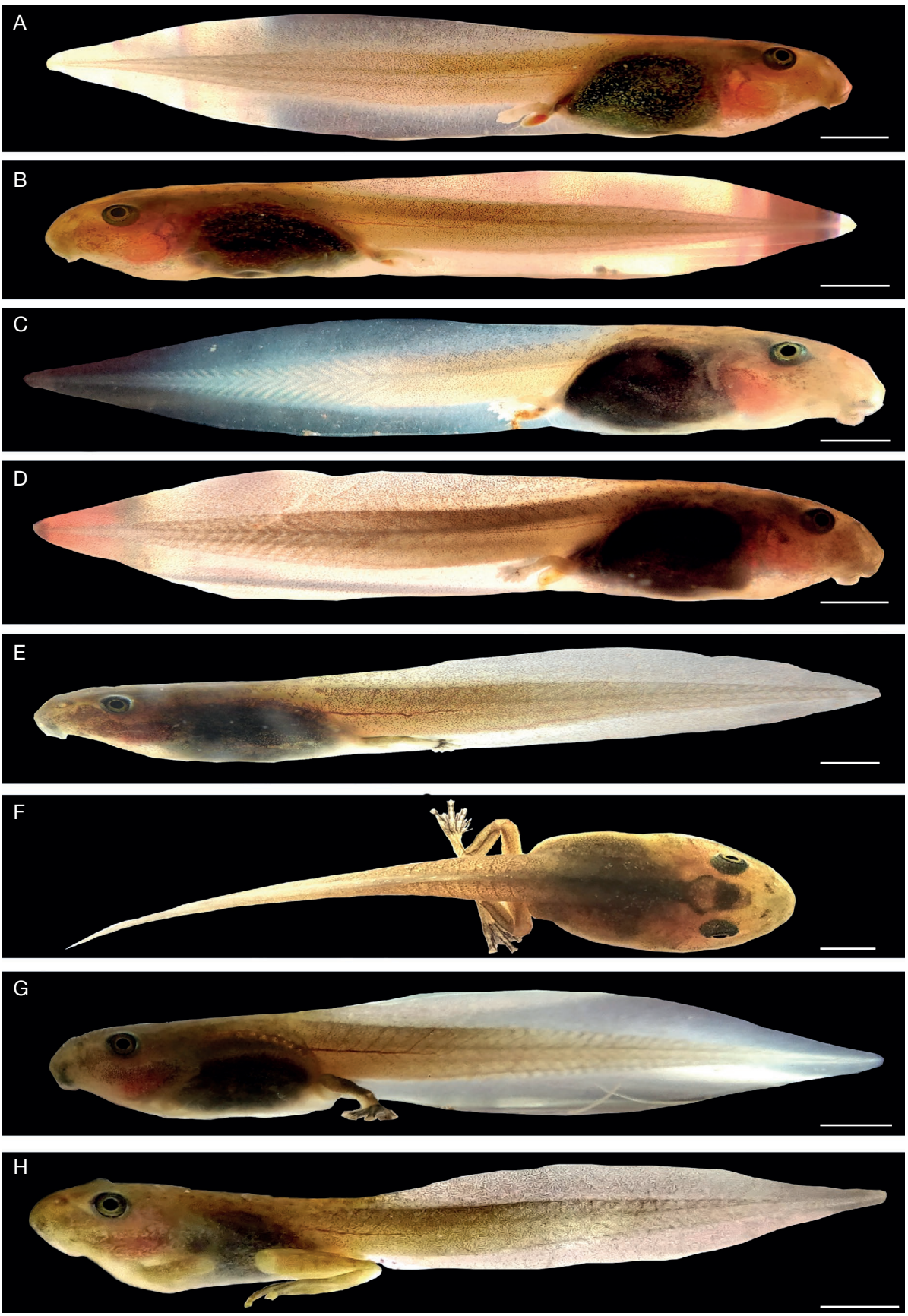

FIG. 4. — Developmental stages of *Rhacophorus malabaricus* Jerdon, 1870: **A**, Gosner stage 34; **B**, Gosner stage 35; **C**, Gosner stage 36; **D**, Gosner stage 37; **E**, Gosner stage 38; **F**, Gosner stage 39; **G**, Gosner stage 40; **H**, Gosner stage 41. Scale bars : A-E, 3 mm; F-H, 4 mm.

TABLE 2. — Morphometric data of *Rhacophorus malabaricus* Jerdon, 1870 for stages 26-39. Mean ± SD. Abbreviations: see Material and methods.

STAGE	TL	SVL	TAL	BW	BH	HW	TMH	UTF
26 (n = 4)	17.27 ± 0.09	6.12 ± 0.03	10.09 ± 0.09	3.47 ± 0.31	2.21 ± 0.41	3.42 ± 0.00	1.90 ± 0.00	0.92 ± 0.01
27 (n = 4)	19.20 ± 0.02	6.69 ± 0.24	11.49 ± 0.25	4.30 ± 0.10	2.49 ± 0.08	3.40 ± 0.28	2.11 ± 0.15	1.24 ± 0.25
28 (n = 3)	21.33 ± 0.08	7.44 ± 0.35	13.23 ± 0.19	4.62 ± 0.25	3.33 ± 0.43	3.60 ± 0.51	2.38 ± 0.33	1.67 ± 0.22
29 (n = 4)	24.61 ± 0.20	8.50 ± 0.38	14.38 ± 0.26	4.46 ± 0.53	3.33 ± 0.45	3.52 ± 0.32	2.44 ± 0.29	1.71 ± 0.03
30 (n = 3)	28.36 ± 0.45	11.62 ± 0.46	17.65 ± 0.41	5.25 ± 0.26	4.38 ± 0.38	4.33 ± 0.42	3.39 ± 0.46	1.92 ± 0.07
31 (n = 5)	31.69 ± 0.25	12.57 ± 0.37	19.70 ± 0.24	6.26 ± 0.31	4.59 ± 0.38	4.47 ± 0.41	3.52 ± 0.24	1.66 ± 0.27
32 (n = 6)	32.50 ± 0.31	12.52 ± 0.20	19.58 ± 0.40	6.64 ± 0.32	4.66 ± 0.22	4.57 ± 0.25	3.74 ± 0.23	1.73 ± 0.26
33 (n = 3)	33.66 ± 0.21	11.82 ± 0.16	19.62 ± 0.29	6.53 ± 0.44	4.47 ± 0.46	5.65 ± 0.56	3.64 ± 0.22	1.82 ± 0.11
34 (n = 4)	34.46 ± 0.36	12.37 ± 0.44	20.67 ± 0.45	6.35 ± 0.39	4.65 ± 0.32	6.61 ± 0.40	3.61 ± 0.25	1.86 ± 0.09
35 (n = 3)	37.70 ± 0.36	13.66 ± 0.32	23.78 ± 0.25	7.52 ± 0.41	5.58 ± 0.28	6.55 ± 0.48	4.63 ± 0.23	1.81 ± 0.04
36 (n = 3)	39.48 ± 0.45	14.73 ± 0.17	25.59 ± 0.40	8.49 ± 0.36	5.45 ± 0.33	7.49 ± 0.38	4.40 ± 0.41	1.74 ± 0.13
37 (n = 4)	40.71 ± 0.28	14.47 ± 0.33	25.68 ± 0.44	8.47 ± 0.40	6.52 ± 0.37	7.53 ± 0.29	6.34 ± 0.43	1.77 ± 0.12
38 (n = 4)	43.49 ± 0.33	16.36 ± 0.24	27.36 ± 0.37	8.58 ± 0.35	6.59 ± 0.44	8.59 ± 0.27	7.13 ± 0.10	1.77 ± 0.25
39 (n = 3)	44.49 ± 0.39	19.49 ± 0.39	28.43 ± 0.37	11.41 ± 0.44	9.48 ± 0.39	9.55 ± 0.41	7.57 ± 0.35	2.44 ± 0.48
STAGE	**LTF**	**TMW**	**END**	**NSD**	**IND**	**IOD**	**ED**	**MTH**
26 (n = 4)	0.91 ± 0.01	1.66 ± 0.37	0.50 ± 0.01	0.92 ± 0.02	0.91 ± 0.00	1.64 ± 0.04	0.52 ± 0.02	3.75 ± 0.08
27 (n = 4)	1.25 ± 0.35	1.86 ± 0.04	0.68 ± 0.07	1.12 ± 0.12	0.92 ± 0.02	1.61 ± 0.23	0.53 ± 0.18	3.88 ± 0.19
28 (n = 3)	1.31 ± 0.43	1.67 ± 0.58	1.33 ± 0.50	1.56 ± 0.47	1.30 ± 0.46	1.60 ± 0.51	0.66 ± 0.07	4.45 ± 0.18
29 (n = 4)	1.41 ± 0.33	2.43 ± 0.42	0.84 ± 0.02	1.52 ± 0.24	1.40 ± 0.35	2.16 ± 0.08	0.73 ± 0.13	4.45 ± 0.27
30 (n = 3)	1.57 ± 0.37	2.51 ± 0.26	1.44 ± 0.48	1.60 ± 0.45	1.45 ± 0.44	2.34 ± 0.54	0.95 ± 0.04	4.62 ± 0.53
31 (n = 5)	1.62 ± 0.33	2.47 ± 0.29	1.43 ± 0.35	2.47 ± 0.49	1.43 ± 0.18	2.31 ± 0.29	1.52 ± 0.47	5.26 ± 0.40
32 (n = 6)	1.49 ± 0.36	2.62 ± 0.30	1.46 ± 0.41	2.50 ± 0.45	1.53 ± 0.28	2.59 ± 0.37	1.36 ± 0.42	5.33 ± 0.36
33 (n = 3)	1.44 ± 0.36	3.45 ± 0.45	1.61 ± 0.49	1.61 ± 0.51	1.76 ± 0.20	3.32 ± 0.53	1.35 ± 0.49	6.65 ± 0.51
34 (n = 4)	1.30 ± 0.37	3.39 ± 0.36	1.41 ± 0.35	1.63 ± 0.45	1.50 ± 0.31	3.44 ± 0.45	1.31 ± 0.41	7.45 ± 0.44
35 (n = 3)	1.54 ± 0.50	3.73 ± 0.23	1.60 ± 0.51	1.56 ± 0.47	1.55 ± 0.44	3.32 ± 0.00	1.41 ± 0.51	7.28 ± 0.25
36 (n = 3)	2.52 ± 0.44	3.57 ± 0.28	2.34 ± 0.53	1.64 ± 0.56	1.62 ± 0.53	3.62 ± 0.53	1.43 ± 0.40	7.43 ± 0.37
37 (n = 4)	2.49 ± 0.34	3.60 ± 0.40	2.41 ± 0.39	0.58 ± 0.19	1.72 ± 0.46	3.55 ± 0.21	1.42 ± 0.34	8.31 ± 0.34
38 (n = 4)	2.48 ± 0.32	4.72 ± 0.19	2.45 ± 0.32	1.55 ± 0.41	2.21 ± 0.16	4.38 ± 0.36	1.45 ± 0.32	8.36 ± 0.37
39 (n = 3)	2.40 ± 0.05	4.53 ± 0.46	2.72 ± 0.22	2.57 ± 0.40	2.31 ± 0.31	4.56 ± 0.29	1.67 ± 0.24	8.44 ± 0.42

Tail bud, external gills, operculum and pigmentation (Gosner stage 17 to 25)

On the 3rd day, the tail bud appeared at the posterior end (Gosner stage 17). The total length of the embryo was about 4.5 ± 0.33 (n = 5) in tail bud stage. The head developed with characteristic optic and gill plate bulges.

The embryo got elongated in Gosner stage 18 and the tail began to be curved within the vitelline membrane. The head, abdomen and tail were completely differentiated on 5th day (Gosner stage 19). The length of the embryo was about 5.2 ± 0.06 mm (n = 6). The hatching of larva started on 3rd day and the hatched motile larvae stayed submerged in the foam (Gosner stage 21). The larva is 9.16 ± 0.08 (n = 5) mm long. While hatching, the body was yellowish-brown coloured and tail was whitish-grey. At Gosner stage 22, they attained a total length of 11 ± 24 mm (n = 4) and mouth was slightly wider than internarial ditance (Fig. 3D). The external gills were covered by opercular foldings in Gosner stage 23. Pigmentation of the tail occurred on the 6th day. Operculum was closed on the right side by Gosner stage 24 (age 6 days) and closed on left side at Gosner stage 25 (age 7 days). The oral disc was well-developed and distinct. The anal tube was opened and the total length was measured as 14.5 ± 0.08 mm (n = 6).

Hind limb bud development (Gosner stage 26 to 30)

The larva reached the 26th Gosner stage on 8th day having a body length of 17.23 ± 0.02 mm (Fig. 3E). A limb bud appeared at the rear part of the body near the vent. The pigmentation extended to tail and was later spread over the translucent tail. The upper and lower tail height was more or less the same. In 27th Gosner stage (age 9 days), the hind limb bud was equal to half of its height (Fig. 3F). The tail was about 60% of the total length. The eye diameter is about 0.50 ± 0.02 mm. In Gosner stage 28 (age 10 days) the length of the larva was about 21.33 ± 0.08 mm and the tail was 62% of total body length. The length of hind limb bud is equal to 1.5 times of its height at Gosner stage 29 (age 11 days) with total body length of 24.65 ± 0.23 mm (Fig. 3G). Body length was 34.5% of its total length. In Gosner stage 30 (age 12 days, total body length 28.36 ± 0.45 mm), the length of the limb bud was equal to twice its height.

Toe differentiation (Gosner stage 31 to 39)

Foot pads were visible in Gosner stage 31 (age 13 days, total body length 31.60 ± 0.30 mm). No pigmentation was seen in limb buds and tail length was more than half of the total body length (Fig. 3H). The first indentation between the fourth and fifth toes was perceivable in Gosner stage 32 (age 14-15 days). The larva became 32.49 ± 0.37 mm long. The second indentation between the third fourth toe appeared in Gosner stage 33 (age 16-17 days, total body length 33.66 ± 0.21 mm). The head and trunk were well developed. On the 18th day (Gosner stage 34) the third indentation between the second and third toe was visible (total body length 34.51 ± 0.43 mm) (Fig. 4A). The pigmentation

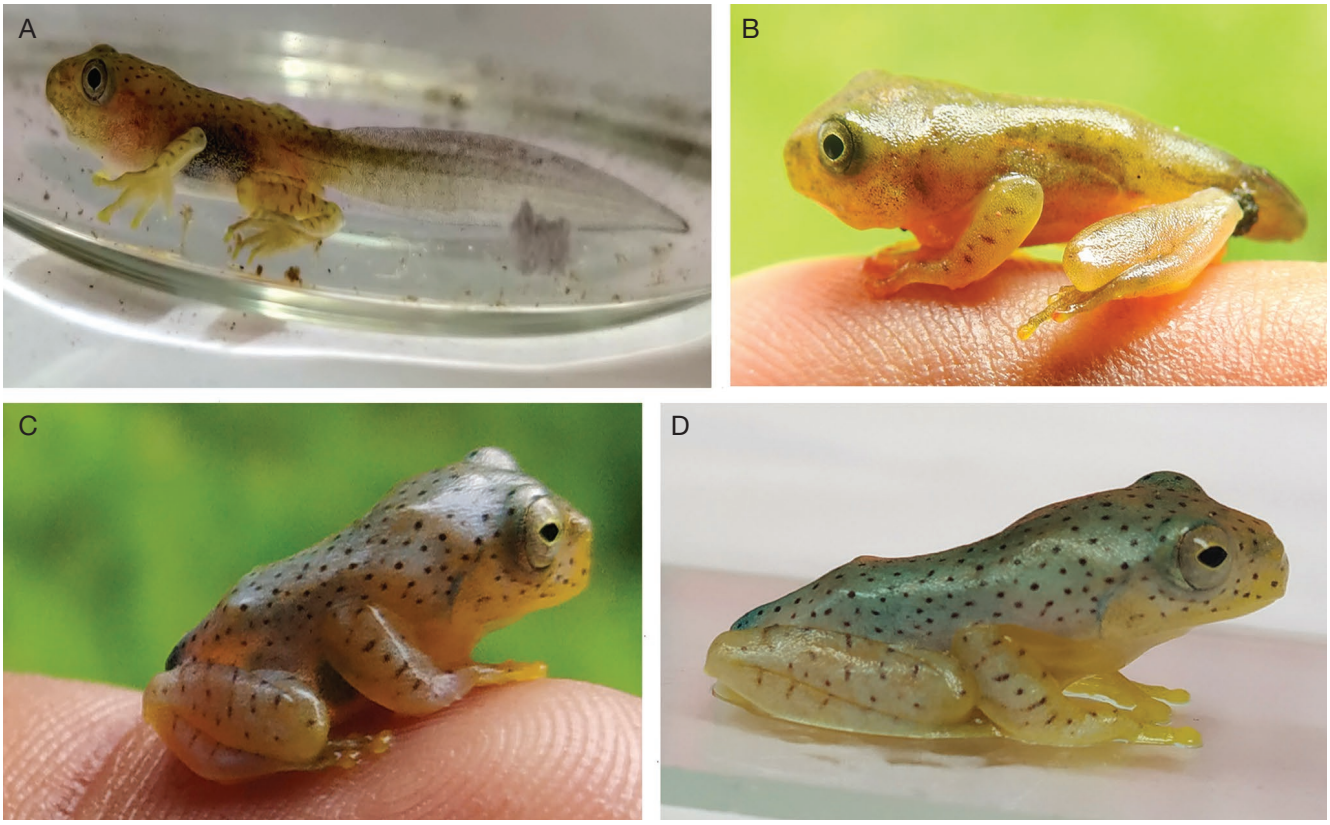

FIG. 5. — Developmental stages of *Rhacophorus malabaricus* Jerdon, 1870: **A**, Gosner stage 42; **B**, Gosner stage 43; **C**, Gosner stage 44; **D**, Gosner stage 46.

was predominant. For fourth and last indentation, first and second toes appeared in Gosner stage 35 (age 19-20 days, total body length 37.70 ± 0.36 mm) (Fig. 4B). In this stage all toes were visible but they were not separated from each other. In Gosner stage 36 (Fig. 6) (age 21-22 days, total body length 39.48 ± 0.45 mm) the third toe and the fifth toe was separated independently from fourth toe (Fig. 4C). All toes separated from each other in Gosner stage 37 (age 23-24 days, total body length 40.71 ± 0.34 mm) (Fig. 4D) and inner metatarsal tubercle appeared posterior to the first toe in Gosner stage 38 (Fig. 4D; age 25-27 days, total body length 43.44 ± 0.39 mm). The subarticular tubercles were visible in Gosner stage 39 (age 28-30 days, total body length 44.49 ± 0.39 mm; Fig. 4F). Dark coloured pigmentation was seen in hind limbs except for first and second toes.

Well-developed hind limb (Gosner stage 40 to 42)
In Gosner stage 40 (Fig. 4G; age 31-33 days, total body length 46.44 ± 0.39 mm), outer metatarsal tubercle and foot subarticular tubercles were distinct. Mouth parts gradually degenerated. The vent tube was still present. Forelimb buds appeared in Gosner stage 41 (age 34-36 days, total body length 43.44 ± 0.39 mm) and the vent tube disappeared (Fig. 4H). In Gosner stage 42 (Fig. 5A) (age 37-38, total body length 48.7 ± 0.22 mm), the fore limbs were emerged. At this stage the larva attained maximum total body length. The mouth was slightly shifted from anterior to the nostril.

Mouth restructuring and tail reabsorption (Gosner stage 43 to 44)
The atrophying of tail began in Gosner stage 43 (Fig. 5B) (age 39-41 days, total body length 27.13 ± 0.43 mm). The angle of mouth was widened and reached between nostril and eye. Both dorsal and ventral tail fins started to shrink. Truncate digital discs on both limbs were distinct. In Gosner stage 44 (Fig. 5C) (age 42 days, total body length 23.01 ± 0.42 mm), widening of mouth continued up to beneath the eye. Dorsal and ventral tailfins disappeared. Tail was greatly reduced to 12.38 ± 0.43 mm. The metamorphosing tadpoles were observed in the boundary of water and land and started to come out from water. Ventral parts of head, trunk and limbs turned to pale yellow colour. Dorsum of head, trunk and limbs were blue coloured with dark sport. The patches in the limb were jointed as small darkened striations.

Metamorphosis (Gosner stage 45)
In Gosner stage 45 (age 43 days), the mouth was extended up to the posterior margin of the eye. The dark blue-green rounded tail stump was visible at the base of cloaca. The snout vent length was reduced as 19.31 ± 0.41 mm.

Metamorphosed froglet (Gosner stage 46)
The tail completely disappeared. Hind limbs and fore limbs were well developed and metamorphosis was completed in 44 days and the juvenile froglet emerged (Fig. 5D).

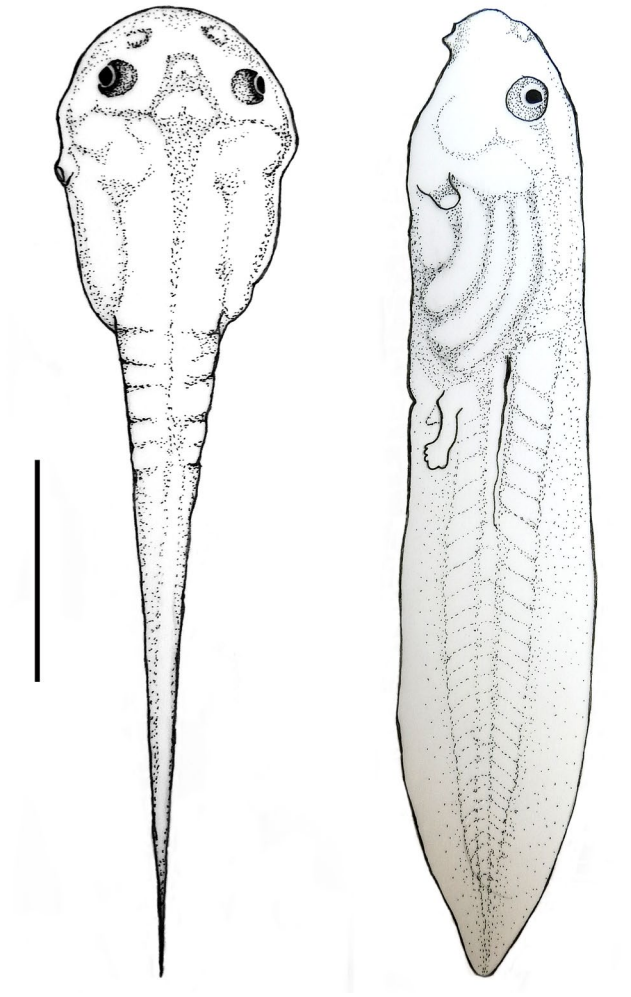

Fig. 6. — Habitus of the tadpole of *Rhacophorus malabaricus* Jerdon, 1870 stage 36: **A**, lateral view; **B**, dorsal view. Scale bar: 10 mm.

Table 3. — Regression equations, Pearson correlation between morphometric parameters. All correlation coefficients are significant at the 0.05 level. Abbreviations: see Material and methods.

	correlation between stage and parameters		correlation between total length and parameters	
	Equation	R^2	Equation	R'^2
TAL	Y = 1.398x-25.605	0.971	Y = 0.663x-1.503	0.987
SVL	Y = 0.857x-15.786	0.904	Y = 0.411x-1.157	0.94
BH	Y = 0.408x-8.452	0.843	Y = 0.190x-1.308	0.829
ED	Y = 0.083x-1.514	0.464	Y = 0.041x-0.144	0.52
TL	Y = 2.100x-36.078	0.976	-//-	-//-

Morphometric variation

The morphometric measurements of different developmental stages are shown in Table 2. The total length of larva in Gosner stage 26 was 17.27 ± 0.09 mm, gradually increased to 48.85 ± 0.01mm in Gosner stage 42. Tail length followed a similar trend up to Gosner stage 42, later declined drastically and disappeared at Gosner stage 46 (Fig. 7). Thus total length was reduced as 17.17 ± 0.06 mm. The upper and lower tail fin height was more or less the same in the beginning stages, the length of upper tail fin was slightly increased in later stages. In all graphs, the total body length and tail length showed a proportionate increase in different developmental stages (Fig. 2). Similar observations were found in the graph plotted for snout-vent length and body height as a function of the total body length. The measurement of the body height, body length and diameter of eyes were highly correlated with developmental stages (Table 3). The correlation between total body length and all parameters was significant at the level of 0.05 (Fig. 8).

Oral morphology

The following description of oral morphology is based on tadpoles at Gosner stage 32. The mouth was slightly protruding ventrally. The oral disc was elliptical. Two rows of marginal and submarginal soft unpigmented moderate sized, round-ended papillae were present on the margin of lower labium. The upper labium margin with moderate sized rounded papilla that shows a wide dorsal gap (55-65% of the width of oral disc) (Fig. 9). The upper mouth sheath was nearly shaped as an inverted 'U', with keratinised, moderately sharp and equal sized serrations. Lower mouth sheath was 'V' shaped with well keratinised sharp, equal sized serrations. The number of anterior and posterior keratodont rows were 7 (A-1-2-3-4-5-6-7) and 3 (P-1-2-3) respectively. A-1 and A-2 were entire, A-3 was divided medially by a conspicuous gap. A-4-5-6-7 were completely separated by upper mouth sheath. Three undivided keratodont rows (P-1-2-3) were present on the lower labium (Fig. 10). All keratodont rows were biserial except A6 and A7 (uniserial). The order of relative lengths of anterior and posterior tooth rows were A2>A1>A3>A4>A5>A6>A7 and P3>P2>P1 respectively. Labial Tooth Row Formula (LTRF) 7(3-7)/3, Keratodont Row Formula (KRF), 2:5+5/3. Keratodonts (17-20 µm long) with spatulate apex bearing 8-10 sharp marginal denticles (Fig. 11).

DISCUSSION

In the present study, we observed that *Rhacophorus malabaricus* has significant resemblances in developmental durations with other rhacophoridan members. (Alcala 1962; Hendrix *et al.* 2007; Biju *et al.* 2010). The breeding of *Rhacophorus malabaricus* was surveyed during the period of 18 July to 30 August, 2019. Similar reports on Amit (2013) and George *et al.* (1996) were done on the same season. Analogous breeding seasons were found in the studies on *R. helenae*, *Rhacophorus calcadensis* Ahl, 1927, *Rhacophorus pseudomalabaricus* (Rowley *et al.* 2012b, Biju *et al.* 2013). Foam nest building is a stereotypic habit of *Rhacophorus* species (Liem 1970; Kadadevaru & Kanamadi 2000; Grosjean 2005; Biju 2009; Chakravarty *et al.* 2011; Meegaskumbura *et al.* 2010; Lalramdinfeli & Lalremsanga 2017), which is also observed in *R. malabaricus*. *Rhacophorus malabaricus* has remarkable similarity in courtship behaviour and foam nest construction with *R. pseudomalabaricus* and *R. calcadensis* (Biju *et al.*

2013). During amplexus, both parents together make foam and deposite their gamete into it. Post mating, the male departs but female nevertheless works for building nest. They exhibit less parental care after laying eggs (Amit 2013; Biju *et al.* 2013). Normally, they complete their development up to Gosner stage 21 within the foam nest and dropped into the water at stage 22. The egg diameter of *R. malabaricus* described from the present study is 2.17 ± 0.21 mm which is in the range reported for *R. pseudomalabaricus* (2.6 ± 0.9 mm), *R. calcadensis* (2.4 ± 0.6 mm) (Biju *et al.* 2013) and other *Rhacophorus* species (Vassilieva *et al.* 2013).

Tadpole description of *R. malabaricus* in the current study generally agrees with available larval descriptions of other Rhacophoridae species. Morphological features (semi-ovoid body with slight dorsoventral depression, sinistral spiracle, dorsolateral eyes, etc.) of the benthic type larva of *R. malabaricus* described within the genus are almost identical (Alcala 1962; Vasudevan & Dutta 2000; Hendrix *et al.* 2007; Biju 2009; Wildenhues *et al.* 2011; Haas *et al.* 2012; Biju *et al.* 2013). The spiracle of *R. malabaricus* is lateroventral, sinistral, short narrow tube with aperture directed posterodorsally as in *R. helenae* and *R. rhodopus* (Grosjean & Inthara 2016; Vassilieva *et al.* 2016). The spiracle opening of *R. malabaricus* is moderately larger than *Rhacophorus orlovi* Ziegler & Köhler, 2001 (Wildenhues *et al.* 2011). The vent tube of *R. malabaricus* is characterised by short dextral, attached to lower fin with oblique aperture oriented ventrocaudally as in *R. helenae* (Vassilieva *et al.* 2016). The tail is observed as moderate not tapering in proximal part as in *Rhacophorus annamensis* Smith, 1924. Tail musculature of *R. malabaricus* tadpole is parallel in proximal half then gradually tapering like *R. rhodopus* and *Rhacophorus kio* Ohler & Delorme, 2006 (Grosjean & Inthara 2016).

During metamorphosis, the ontogenetic colour change is remarkably monitored in *R. malabaricus* as reported by Biju *et al.* (2013). Studies of Jungfer & Hödl (2002) suggested

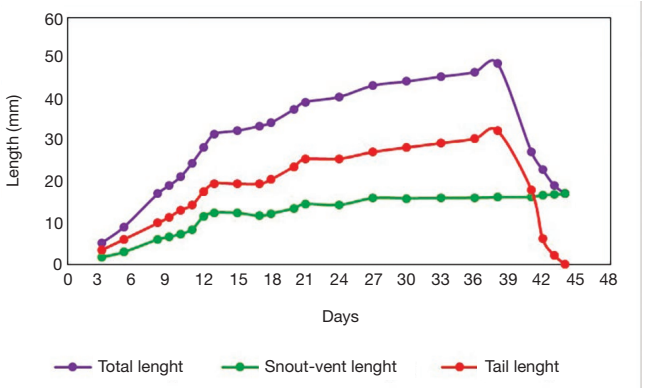

FIG. 7. — Comparison of length-age ratio of tadpole of *Rhacophorus malabaricus* Jerdon, 1870.

that ontogenetic colour change can be used as a characteristic feature in anuran taxonomy and systematics. In this study, though adult morphology of *R. calcadensis* and *R. pseudomalabaricus* have high resemblance with *R. malabaricus*, the larvae have distinct dissimilarities. The larvae of *R. malabaricus* was noticed as greenish black, pale yellowish-green or bluish green dorsum, with black spots. A slightly different larvae was observed in *R. pseudomalabaricus* with light green dorsum including both limbs of metamorph with distinctive thick zebra-like black lines and *R. calcadensis* with a uniformly green dorsum (Vasudevan & Dutta 2000; Biju *et al.* 2013). Morphometric measurements of tadpole was generally used in inter- and intra-specific comparisons. Different parameters were usually expressed as a ratio of total length, developmental stages, tail length or snout vent length (Haas 1997; Altig & McDiarmid 1999; Haas 2003; Grosjean 2005; Haas *et al.* 2012; Biju *et al.* 2013; Ninh *et al.* 2020). The present findings also give evidence for the significant correlation between morphometric measurement with developmental stages and total body

TABLE 4. — List of tadpoles of the *Rhacophorus* Kuhl & Van Hasselt, 1822 species with Labial Tooth Row Formula (**LTRF**) and Keratodont Row Formula (**KRF**).

Species	LTRF	KRF	Gosner Stage	Reference
R. annamensis Smith, 1924	7(3-7)/3	2:5+5/3	41	Hendrix *et al.* 2007
R. baluensis Inger, 1954	7(2-7)/3(1)	1:6+6/1+1:2	–	Inger *et al.* 2005
R. bipunctatus Ahl, 1927	6(2-6)/3 or 6(2-6)/3(1)	1:5+5/3 or 1:5+5/1+1:2	–	Fei 1999 Fei *et al.* 2009
R. borneensis Matsui, Shimada & Sudin, 2013	5(2-5)/3(1) or 6(2-6)/3(1)	1:(4+4)/1+1:2 or 1:(5+5)/1+1:2	35	Inger 1966, 1985
R. catamitus Harvey, Pemberton & Smith, 2002	8(5-8)/3	4:4+4/3	25-41	Streicher *et al.* 2012
R. helenae Rowley, Tran, Hoang & Le, 2012	5(2-5)/3	1:4+4/3	37	Vassilieva *et al.* 2016
R. kio Ohler & Delorme, 2006	5(2-5)/3	1:4+4/3	36	Grosjean & Inthara 2016
R. lateralis Boulenger, 1883	6(3-6)/3(1)	2:(4+4)/1+1:2	40	Prudhvi Raj unpublished data
R. malabaricus Jerdon, 1870	7(3-7)/3	2:5+5/3	32	Present study
R. nigropalmatus Boulenger, 1895	–	1:(5+5)/1+1:2 or 2:4-4/1+1:2	36-40	Inger 1966, 1985
R. norhayatii Chan & Grismer, 2010	–	1:(5+5)-(6+6)/1+1:1-2 or (5-5)-(6-6)/1+1:2	–	Berry 1972 (as *R. nigropalmatus*)
R. orlovi Ziegler and Köhler, 2001	5(2-5)/3(1)	1:4+4/1+1:2	40	Wildenhues *et al.* 2011
R. pardalis Günther, 1858	7(3-7)/3	2:5+5/3	37	Inger 1966
R. reinwardtii Schlegel, 1840	6(2-6)/3	1:5+5/3	–	Iskandar 1998
R. rhodopus Liu and Hu, 1960	6(2-6)/3(1)	1:5+5/1+1:2	36	Grosjean & Inthara 2016
R. translineatus Wu, 1977	6(2-6)/3(1)	1:5+5/1+1:2	43	Fei *et al.* 2009

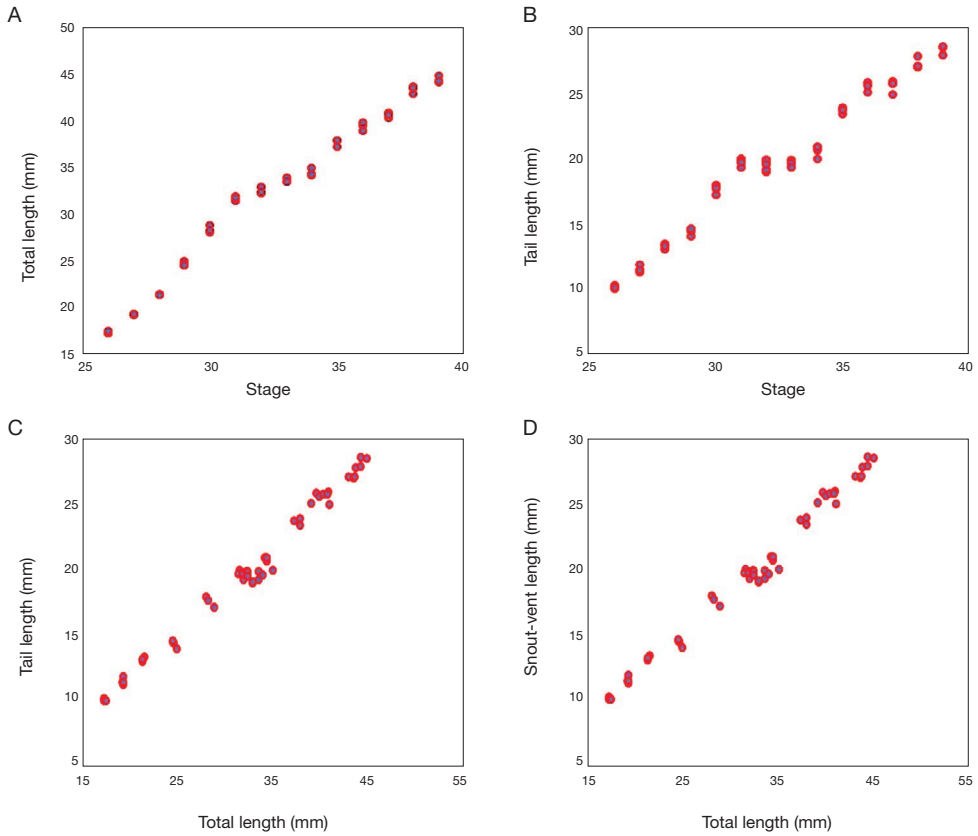

FIG. 8. — Correlation between morphometric parameters (total length and tail length) and stages **A**, and **B**, correlation between morphometric parameters (tail length and snout-vent length) and stages **C**, and **D**.

length. Amit (2013) and George *et al.* (1996) independently reported the maximum total body length (MTBL) of unspecified Gosner stage of *R. malabaricus* from different states of India. Amit (2013) observed *R. malabaricus* with MTBL of 42.50 mm from Amboli, Sindhudurg, State Maharashtra, and George *et al.* (1996) reported a *R. malabaricus* with MTBL of 41.12 mm from Pindimedu, Ernakulam district of State Kerala. Present observations reveal the total body length of larvae as 48.85 ± 0.01mm in Gosner stage 42. This change of growth rate, size and body weight of same tadpoles reported from different places could be related to various factors like environmental temperature, food availability, dissolved oxygen of water, density and kinship (Wilbur 1977; Dash & Hota 1980; Saidapur 2001).

Compared with other Rhacophorid tadpoles at Gosner stages 40/41 the larva of *R. malabaricus* (TL 46.44 ± 0.39 mm) is similar to total length of *R. helenae* and moderately larger than that of *R. orlovi* (TL 24.45 mm) (Wildenhues *et al.* 2011), *Rhacophorus borneensis* Matsui, Shimada & Sudin, 2013 (TL 42.7 mm) (Inger 1985), *Rhacophorus pardalis* Günther, 1858 (TL 43.2 mm) (Inger 1966) and *R. annamensis* (TL 34.37-41.69 mm) (Hendrix *et al.* 2007). The total length of *R. malabaricus* is slightly lower than *R. kio* (48.9 ± 2.71 mm) Grosjean & Inthara (2016) and *Rhacophorus translineatus* Wu, 1977 (48.4 mm). The descriptions of *Rhacophorus baluensis* Inger, 1954 has 61.35% greater total length (75 mm) than *R. malabaricus* (Malkmus *et al.* 2002; Inger *et al.* 2005)

The oral disc of different species in the Rhacophoridae family showed significant diversity (Table 4). Oral disc morphology of *R. malabaricus* is subterminal and not visible from dorsal part of the body as in *R. helenae* (Vassilieva *et al.* 2016). The oral apparatus of *R. malabaricus* has same general organisation with keratinised jaw sheath and oral disc with keratinised tooth rows of *Zhangixalus smaragdinus* Blyth, 1852 and *R. helenae* (Wildenhues *et al.* 2010). *Rhacophorus malabaricus* showed typical generalized rhacophoridan oral disc as in *R. baluensis*, *R. annamensis* and *Kurixalus appendiculatus* Günther, 1858, and those with cup-like oral disc as in *Leptomantis angulirostris* Ahl, 1927, *Leptomantis cyanopunctatus* Manthey & Steiof, 1998. *Rhacophorus malabaricus* marginal papillae of the anterior labium have a large dorsal gap with a fleshy labium like *R. orlovi*, *R. helenae* and *R. annamensis* (Wildenhues *et al.* 2011; Vassilieva *et al.* 2016). Like most of the rhacophoridan larvae, *R. malabaricus* have uninterrupted double layer of posterior marginal papillae (single uninterrupted marginal papillae visible in *Rhacophorus georgii* Roux, 1904 (Gillespie *et al.* 2007) while the marginal papillae of the posterior labium is continuous without gap as in *Zhangixalus dulitensis*, Boulenger, 1892,

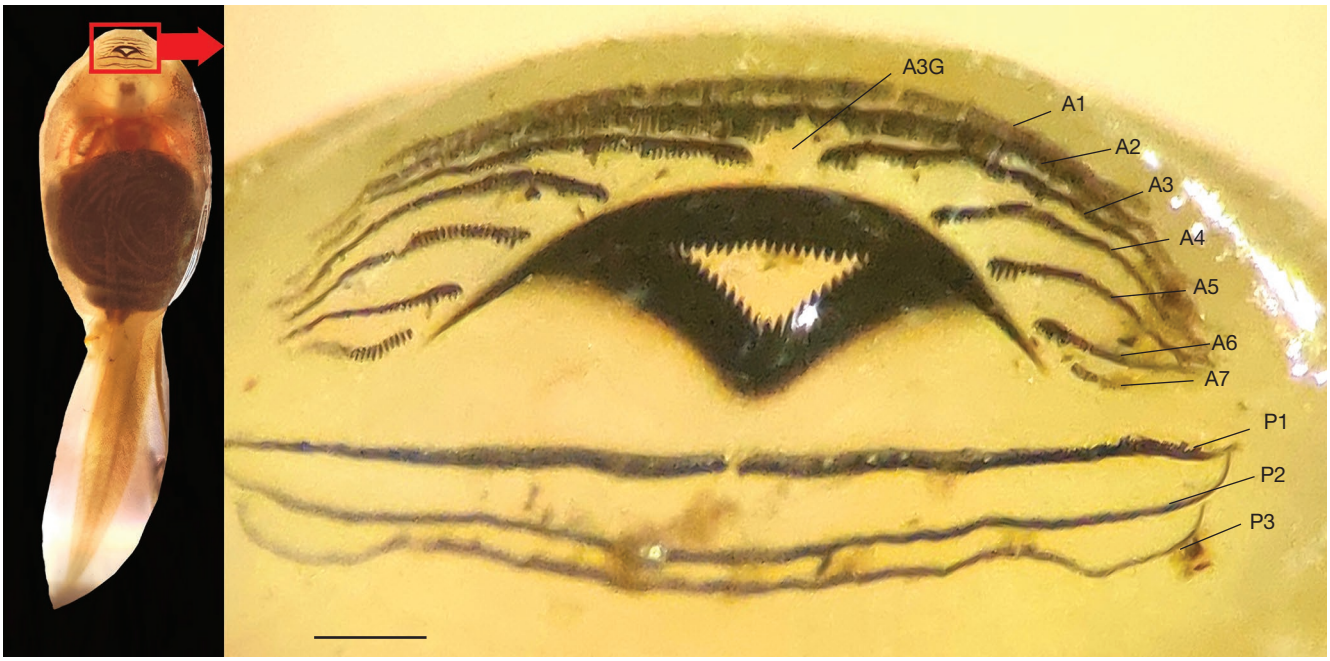

FIG. 9. — Oral disc morphology of *Rhacophorus malabaricus* Jerdon, 1870 (Gosner stage 32). Abbreviations: **A3G**, third anterior tooth gap; **A1** to **A7** anterior tooth rows 1 to 7; **P1** to **P3** posterior tooth rows 1 to 3. Scale bar: 100 µm.

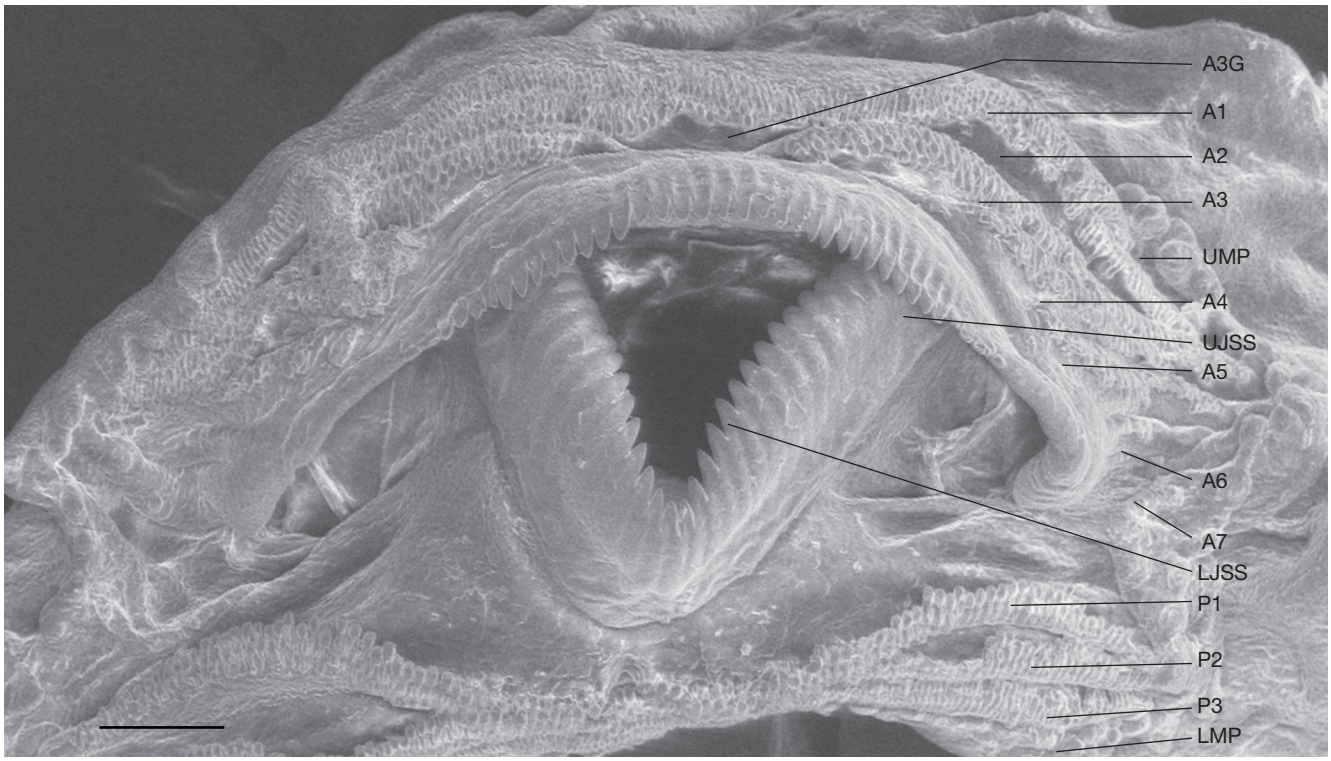

FIG. 10. — SEM image of oral disc morphology of *Rhacophorus malabaricus* Jerdon, 1870 (Gosner stage 32). Abbreviations: **A3G**, third anterior tooth gap; **A1** to **A7**, anterior tooth rows 1 to 7; **LJSS**, lower jaw sheath serration; **LMP**, lower marginal papillae; **P1** to **P3**, posterior tooth rows 1 to 3; **UJSS**, upper jaw sheath serration; **UMP**, upper marginal papillae. Scale bar: 100 µm.

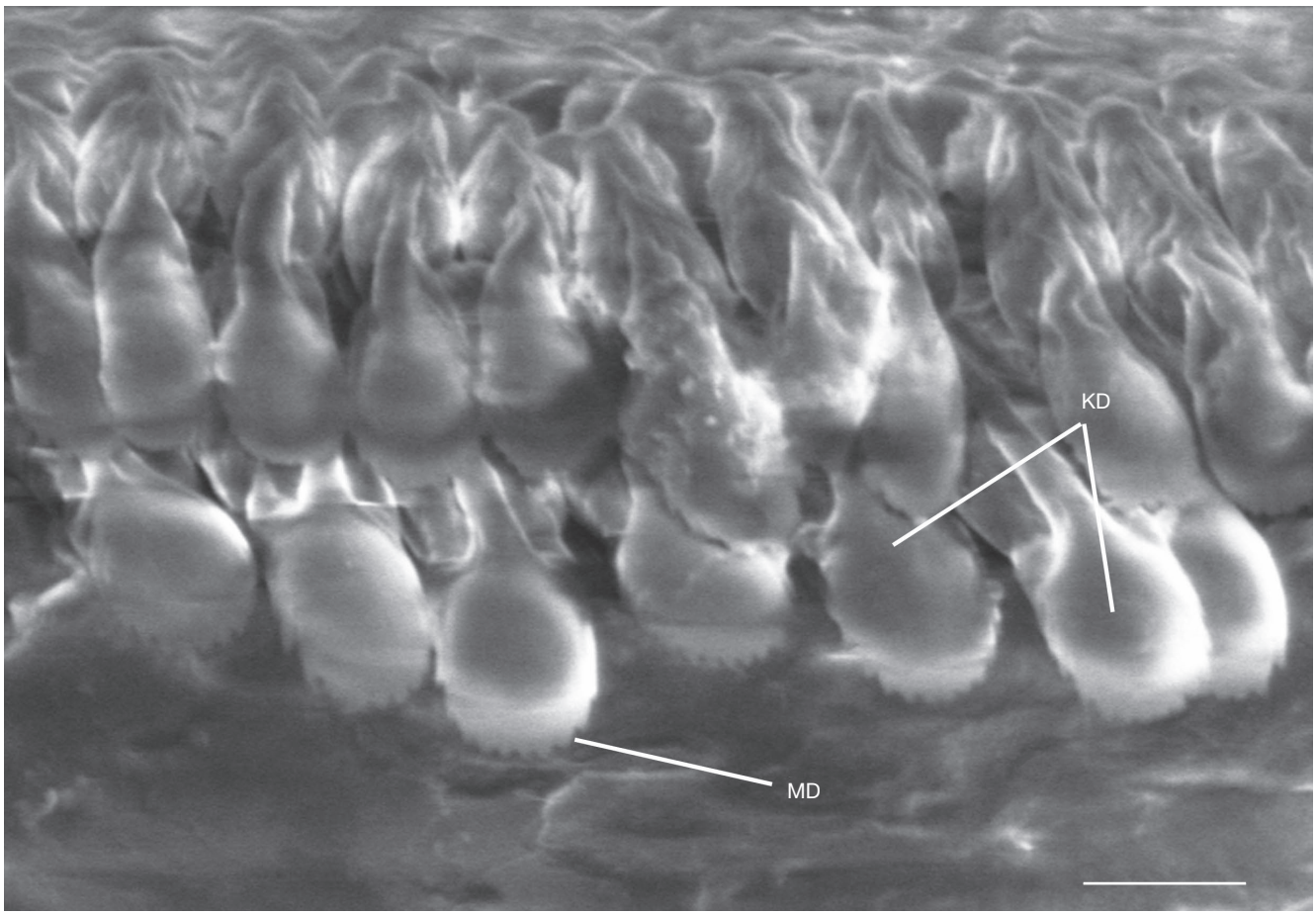

FIG. 11. — SEM image of keratodonts of second anterior tooth row (**A2**) of *Rhacophorus malabaricus* Jerdon, 1870 (Gosner stage 32). Abbreviations: **KD**, keratodonts; **MD**, marginal denticles. Scale bar: 10 µm.

R. kio, *R. orlovi*, *R. helenae* and *R. annamensis* (Wildenhues *et al.* 2011; Grosjean & Inthara 2016; Vassilieva *et al.* 2016).

Chan *et al.* (2018), Jiang *et al.* (2019), Xu *et al.* (2020) and Garg *et al.* (2021) have recently presented a resolved phylogeny of Rhacophoridae family constituting a dataset of 43 species. The same phylogenetic position for *R. malabaricus* was recovered in the present phylogenetic analysis. *Rhacophorus reinwardtii* Schlegel, 1840 species group was found to be monophyletic in the current study in consonances with the study of Hasan *et al.* (2014) and Jiang *et al.* (2019). As always *R. nigropalmatus* was a separate clade as in all previous reports. The close relationship between *R. malabaricus* and *R. pseudomalabaricus* recovered from the tree consonance with similarity of both in reproductive biology (Biju *et al.* 2013; Jiang *et al.* 2019). *Rhacophorus malabaricus*, *R. pseudomalabaricus* and *Rhacophorus catamitus* Harvey, Pemberton & Smith (2002) were obtained in the same clade, similar to the studies of Jiang *et al.* (2019). Haas (2003) has proved oral morphology as efficacious in anuran phylogenetics while considering 136 larval characters. In the current investigation, the closely related species in phylogenetic tree based on molecular data exhibited homogenous pattern of oral morphology. This data supports the applicability of oral morphology in anuran systematics advocated by Haas (2003). The keratodont rows of *R. malabaricus* is 7 on upper lip and 3 on lower lip. Comparisons to the larvae of *R. malabaricus* with related species revealed that some obvious similarities in the structure and orientation of the oral disc of *R. annamensis* with Labial Tooth Row Formula (LTRF) of 7(3-7)/3) and *R. baluensis* with LTRF of 7(2-7)/3(1) (Inger *et al.* 2005; Hendrix *et al.* 2007). In the *reinwardtii* clade, closely related species *Rhacophorus bipunctatus* Ahl, 1927, *Rhacophorus norhayatii* Chan & Grismer, 2010, and *R. rhodopus* have LTRF of 6(2-6)/3(1) (Fei 1999; Fei *et al.* 2009; Berry 1972, Grosjean & Inthara 2016). Another LTRF similarity of 5(2-5)/3 observed in the sister taxa of both *R. helenae* and *R. kio* (Grosjean & Inthara 2016; Vassilieva *et al.* 2016). Similar resemblance could also be visible in Keratodont Row Formula (KRF) of above mentioned species. KRF of *R. malabaricus* (2:5+5/3) is the same as in *R. annamensis*. Grosjean & Inthara (2016) referred to the unpublished data of Prudhvi Raj, which includes the KRF of *R. malabaricus*, 2:(4+4)-(6+6)/3 which slightly varies from our observation, maybe because tadpoles were illustrated in early developmental stages. Henceforth the arrangement and number of labial tooth rows succeeding to Gosner stages 26 are more or less stable as species-specific (Altig & McDiarmid 1999).

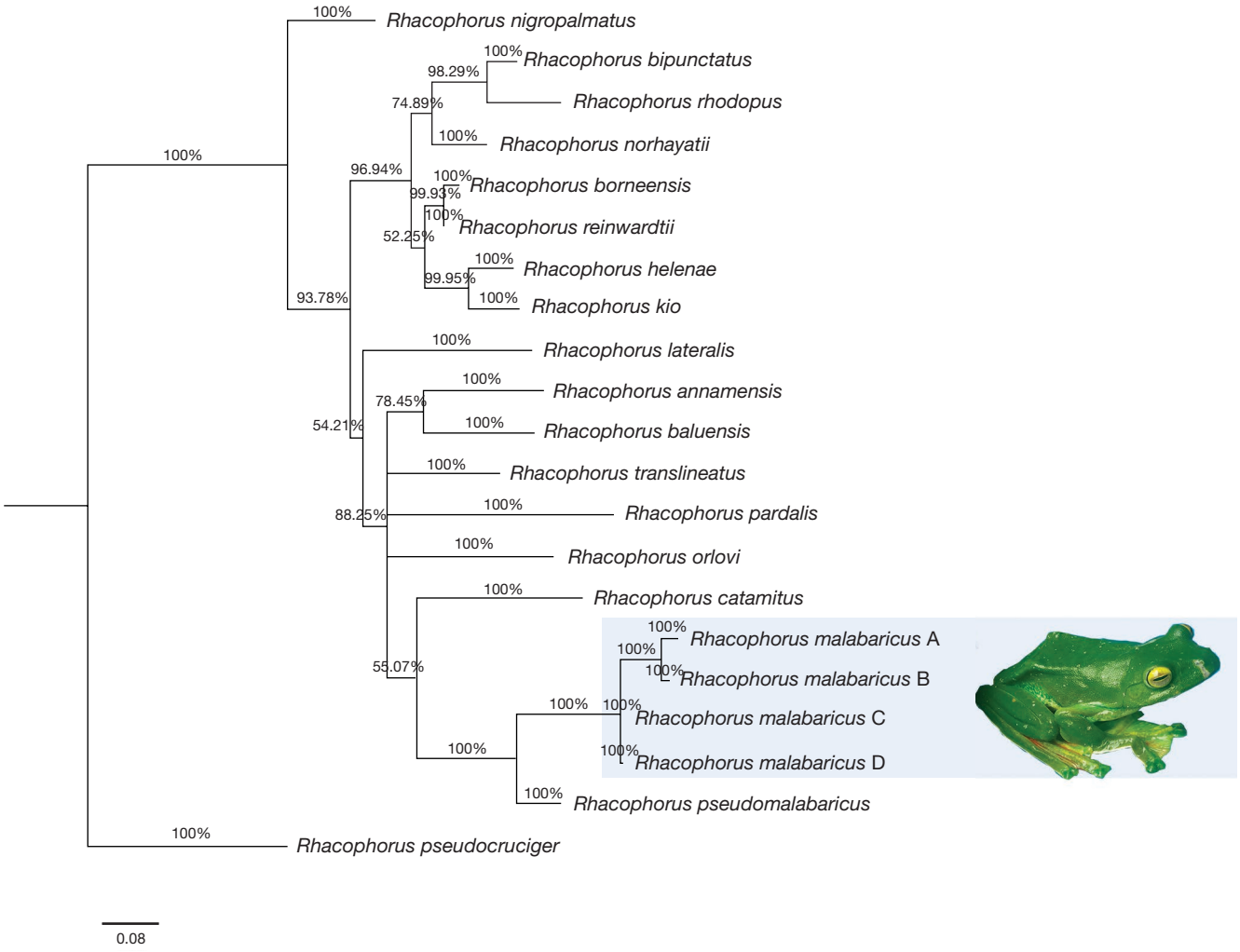

FIG. 12. — Bayesian inference tree of selected species of Rhacophoridae Hoffman, 1932 (1858). For *Rhacophorus malabaricus* Jerdon, 1870, the sequences are newly generated (**A**, **B**), or from GenBank (**C**, **D**).

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