

**AN EXTENDED TADPOLE DESCRIPTION OF *Rhacophorus helenae*
(Rowley, Tran, Hoang, and Le, 2012) (ANURA: RHACOPHORIDAE)
FROM DONG NAI CULTURE AND NATURE RESERVE,
AN ENDEMIC SPECIES OF VIETNAM**

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Abstract. *Rhacophorus helenae* Rowley, Tran, Hoang, and Le, 2012 was recently described from is southern Vietnam, listed as Endangered in the IUCN Red List of Threatened Species (IUCN SSC Amphibian Specialist Group 2020). The larval morphology in stages 35, 37-39, 41 of this species was provided by Vassilieva et al., 2016. We describe the external morphology of the tadpoles of the species *R. helenae* collected from Dong Nai Culture and Nature Reserve, Dong Nai Province, Southern Vietnam. The morphology of 23 tadpole specimens (Gosner stages 26, 28, 29, 32, 34, 35, 39, 41, and 43) of this species are also provided. We provide an updated development stage and discussion of the tadpole morphology different of the genus *Rhacophorus*.

Keywords: *Rhacophorus helenae*, tadpole, morphology, endemic, Dong Nai Culture and Nature Reserve.

1. Introduction

The genus *Rhacophorus* Kuhl and Van Hasselt, 1822 contains 43 species distributed from Southern India to the Philippines (Frost, 2020 [1]). In Vietnam, many species are widely distributed (Frost, 2020 [1]), one remarkable exception is *R. helenae*, which is a lowland forest in South Vietnam (Rowley et al., 2012 [2]; Vassilieva et al., 2016 [3]). *R. helenae* was described from two localities in the Nui Ong Nature Reserve, Binh Thuan Province, and Tan Phu Protection Forest, Dong Nai Province (Rowley et al., 2012 [2]). Vassilieva et al. (2016) [3] reported the species from the Binh Chau-Phuoc Buu Nature Reserve, Ba Ria-Vung Tau Province, and the Ma Da Forest (recently embedded in the Dong Nai Culture and Nature Reserve), Dong Nai Province (southern Vietnam). The species inhabits limited isolated areas of the lowland monsoon forests and is listed as Endangered in the IUCN Red List of Threatened Species (IUCN SSC Amphibian Specialist Group 2020 [4]).

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R. helenae was originally described solely based on adults (Rowley *et al.*, 2012 [2]) and tadpoles were morphological detail and drawings in stages 35, 37-39, 41 (Vassilieva *et al.*, 2016 [3]). Based on tadpoles collected in Dong Nai Culture and Nature Reserve, Dong Nai Province, southern Vietnam, which were matched with adult specimens of *R. helenae* and compared with the previous study, we herein provide the extended tadpole description of this species.

2. Content

2.1. Methods

* Sampling

Five adult specimens and 23 tadpole specimens of *R. helenae* were collected during fieldwork in Ma Da sector, Dong Nai Culture and Nature Reserve (Figure 1) on October 9, 2019 (11°11.400'N, 107°02.579'E, a.s.l. 16 m). Specimens were collected from 19:00 to 24:00.

After photographing the specimen alive, it was then euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons, 2002 [5]), fixed in 85% ethanol for four hours, and subsequently transferred into 70% ethanol for permanent storage, tadpole specimens were fixed in 4% formalin. All tadpoles are treated immediately after collection. The preserved specimen was deposited in the collection of the Museum of Biology, Hanoi National University of Education, Hanoi, Vietnam.

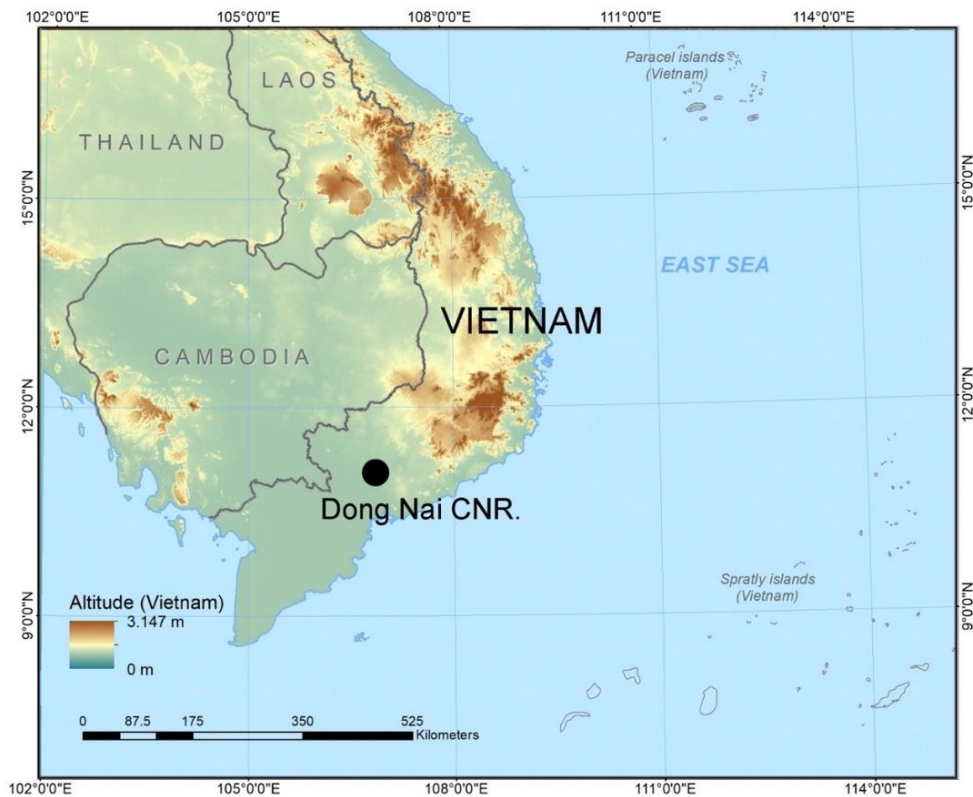


Figure 1. The Dong Nai Culture and Nature Reserve in Southern Vietnam (black circle)

*** Morphological analysis of adults**

Measurements were taken with a digital calliper to the nearest 0.1 mm; a.s.l., above sea level; for webbing formula, we followed Glaw & Vences (2007) [7].

*** Morphological analysis of tadpoles**

Morphological characters were measured with an electronic clamp with an accuracy of 0.1 mm under a stereoscopic electronic magnifier. Abbreviations are as follows BL: body length; BH: maximum body height; BW: maximum body width; ED: maximum diameter of eye (horizontal); HT: maximum tail height; LF: maximum height of lower tail fin; NN: internarial distance (measured between centers of narial apertures); NP: nario-pupilar distance (measured between the center of the nostril and the center of the pupilla); ODW: oral disc width; PP: interpupilar distance (measured between centers of pupils); RN: rostronarial distance (measured between the tip of the snout and the center of the nostril); SS: distance from tip of snout to opening of spiracle; SU: distance from the tip of snout to insertion of upper tail fin; TL: total length; TAL: tail length; UF: maximum height of upper tail fin; VT: distance from vent to tip of tail; TMH: height of the tail musculature at base; TMW: width of tail musculature at base; FL: forelimb length; HL: hindlimb length; SVL: Snout-vent length; Tooth formulas LTRF was determined according to McDiarmid & Altig (1999) [8] and for general larval types see Orton (1953) [9]. Terminology for morphometric data and abbreviations followed McDiarmid & Altig (1999) [8] and Grosjean (2005) [10]. Tadpoles were staged according to Gosner (1960) [11].

2.2. Results and discussion

*** Taxonomic account of *Rhacophorus helenae* Rowley, Tran, Hoang, and Le, 2012 /Helen's tree frog**

Specimens examined. Tadpole specimens (Fiel numbers: HNUE DN.T.2019.1-HNUE DN.T.2019.23, n = 23) were collected in Dong Nai Culture and Nature Reserve, Dong Nai Province on were collected during fieldwork in Ma Da sector, Dong Nai Culture and Nature Reserve by Pham Quang Tien, Luong Mai Anh on 9 October 2019 (11°11.400'N, 107° 02.579'E, a.s.l. 16 m). The tadpoles were collected throughout night time in a water catchment on small streams. Adult specimens (HNUE DN.A.2019.5-7, n = 3) were collected at night in the primary forest, near the water catchment of tadpoles.

Adult description. The adult males (HNUE DN.A.2019.5-7, n = 3) agreed well with the descriptions of *R. helenae* provided by Rowley *et al.* (2012) [2]: large body size (males 72.3 - 85.5 mm; females 89.4-90.7 mm), pupil horizontal, tympanum prominent externally, weak supratympanic fold; vomerine teeth present, touching the anterior edge of choanae. Forelimbs relatively robust, relative length of fingers I<II<IV<III; tips of all fingers with well-developed disks with distinct circummarginal grooves, webbing formula $I1\frac{1}{2}-1\frac{1}{2}II0-0III0-0IV$; subarticular tubercles prominent, rounded, formula 1, 1, 2, 2; palmar tubercle absent. The relative length of toes $I < II < III < V < IV$; tips of toes with well-developed disks with distinct circummarginal grooves; disks smaller than those of fingers; webbing complete, $I0-0II0-0III0-0IV-0V$; subarticular tubercles distinct, rounded, formula 1, 1, 2, 3, 2. *Skin:* Dorsal skin smooth, ventral surface of thighs and belly coarsely granular, chest and throat smooth; wide, smooth-edged dermal fringe

along the outer edge of forearm, narrower along with tarsus. *Coloration*: Dorsal surface uniformly green; flanks flecked with pale yellow; large inky black patch in the axilla; dermal appendages on arms and legs including tibiotarsal projection and supracloacal dermal ridge lined with white; webbing between fingers pale green; webbing between all toes proximally black and distally greenish; ventral surface immaculate white, slight pinkish tinge posteriolaterally; ventral surfaces of hands and feet pinkish. Iris yellowish-gold with a sparse network of faint, dark-gold reticulations; iris periphery black; sclera white.

Distribution. This species has been only from Dong Nai and Binh Thuan provinces in South Vietnam (Frost, 2020 [1]).

* *Tadpoles*

Diagnosis: Front and bottom mouths, middle size, thin fleshy spines, flanking the sides and under the mouth; sheath fragment; LTRE: 5(2-5)/3. The body is slightly flat, seen from above with ellipse, yellow-brown in body, tail with white lines; breathing hole on the left, located closer to the muzzle of the mouth than the opening of the anal canal. Tail medium, caudal fin higher than the caudal fin lower, the tip of tail rounded.

Morphological description. In this document, *R. helenae* tadpoles are described based on 23 specimens (stages 26, 28, 29, 32, 34, 35, 39, 41, and 43) from the Ma Da forest sector in the Dong Nai Culture and Nature Reserve. Morphological features of the tadpoles from Dong Nai Province agreed with the diagnosis of the tadpoles in the species *R. helenae* in the following characters: Body elliptical, slightly depressed dorsoventrally; snout broad, rounded; eyes moderately large, pupils oriented laterally; nostrils positioned closer to snout tip than to eyes; tail moderately long; upper and lower fins reaching their maximum height in the posterior third of the tail length, upper fin higher than lower fin; spiracle sinistral, aperture directed posterodorsally; spiracle opening margins even; vent tube dextral, attached to lower fin, short, with oblique aperture oriented ventrocaudally; LTRF 5(2-5)/3 (Vassilieva *et al.*, 2016 [3]). In the following, we provide a detailed description of two tadpoles of *R. helenae* at stage 41.



Figure 2. *Rhacophorus helenae* in life from Dong Nai Culture and Nature Reserve: A) Dorsal view, B) Ventral view, and C) supracloacal dermal ridge. Photos: Le Trung Dung

Dorsal view: Body of tadpole reaches a length of 43.3 - 44.7 mm in stage 41, body elliptical (BW/BL 0.64 - 0.98); snout broad, rounded; Eyes moderately large (ED/BL 0.19 - 0.24), eyes on the body; the distance between the two noses is less than the distance between the two eye sockets (NN/PP 0.49 - 0.77); Tail moderately long, the maximum width of tail musculature as half of body width (TMW/BW 0.34 - 0.56).

Lateral view: Tail length folds 4 longer (TAIL/HT 3.35 - 4.8), tails suck. Tail muscle height equals 0.55 the body height (TMH/BH 0.48 - 0.64) and 0.57 the tail height (TMH/HT 0.44 - 0.73). Caudal fin thin, caudal fin protruding, high in upper fins with high caudal fin. The height of the caudal fold is 0.31 the tail height (UF/HT 0.24 - 0.39) and the fold on the caudal fin near the base of the tail, the distance from the muzzle sucking to the caudal fold is 1.23 the body length (SU/BL 0.77 - 1.84). The maximum tail height is 0.94 the body height (HT/BH 0.6 - 1.12). The anal canal opens on the right side, facing backward. At stage 41 spiracle opening no longer exists.

Oral disc: Front and bottom mouths, medium size; The width of the oral disc is 0.13 the body length and 0.26 the width body width (ODW/BL 0.22 - 0.33; ODW/BW 0.23 - 0.34). Thorny flesh is thin, unpigmented conical papillae. bordering the sides and bottom of the mouth disc. Upper spines are equal to the length of the top row of teeth. Mouth sheaths black, thin covering and with finely serrated cutting edges; inclusion on curved and wide; implied under a V-shape, narrower than above. On the upper labium, row A-1 along the labium margin always entire, row A-2 divided medially by a narrow, rows A-3-4-5 divided and completely separated by upper mouth sheaths. On the lower labium, row P-1-2-3 margin always entire.

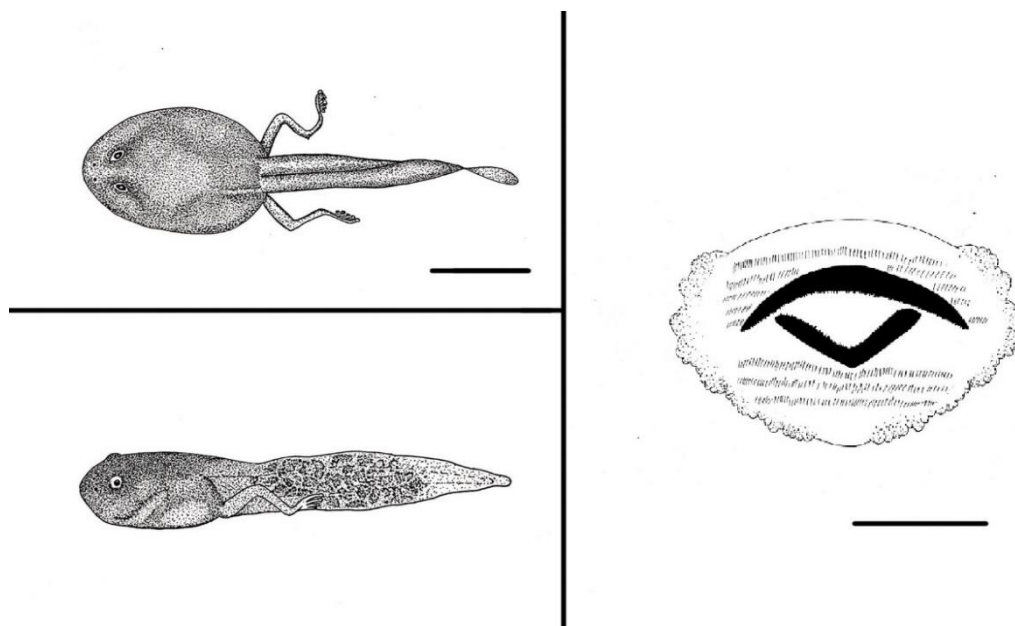


Figure 3. Drawings of the preserved tadpoles of *Rhacophorus helenae* from Dong Nai Culture and Nature Reserve: Gosner stage 41: (A) lateral view, (B) dorsal view (scale bar = 10 mm), (C) oral apparatus (scale bar = 0.5mm) (Drawing: Nguyen Thi Loc)



Figure 4. Tadpole (HNUE DN.T.2019.14, stage 41) of *Rhacophorus helenae* in life from Dong Nai Culture and Nature Reserve: (A) dorsal view, (B) ventral view, (C) mouth view (Photos: Le Trung Dung)

Colouration in life: Live samples are uniformly yellow-brown throughout the body, the tail has irregular white spots and the body is round, fin caudal approximately the height of the body.

Coloration in preservative: Under preserved conditions color in preservative rather uniform, yellowish-grey, ventral and ribs side transparent, tail fins pigmented with fine marbled pattern. Lines of neuromasts faintly visible on the dorsal and lateral surfaces of the head and trunk.

*** Comparison**

Tadpoles of *R. helenae* can be distinguished from the following species by the difference in LTRF, which is $5(2-5)/3$, instead of $7(3-7)/3$ for *R. annamensis* and *R. catamitus* [12, 13]; $4(3-4)/4-5$ for *R. Angulirostris* [15]; $9(5-9)/3(1)$ for *R. cyanopunctatus* [16]; $5(2-4)/3(1)$ for *R. dennysi* and *R. orlovi* [12, 17]; $5(2-5)/3(1)$ for *R. minimus* and *R. smaragdinus* [18, 19]; $6(2-6)/3(1)$ for *R. rhodopus* and $6(2-6)/3$ for *R. prominans* [16, 20]; $4(2-4)/3(1)$ for *R. nigropunctatus* [21]; $4(2-4)/3$ or $5(2-4)/3$ for *R. rufies* [22]; $5(2-5)/2(1)$ for *R. georgi* [23]; $4(4)/7$ for *R. penanorum* [22]; $7(2-5)$ for *R. pardalis* [24]; $2(1)/3$ for *R. vampyrus* [25].

The comparison of the tadpoles of *R. helenae* and *R. kio* is particularly relevant as they are closely related phylogenetically. The species *R. helenae* would have historically been assigned to *R. reinwardtii* or *R. kio* (Stuart *et al.*, 2005 [26]). Differences are tiny and the most obvious character for discrimination is the coloration marbled pattern which is in *R. helenae* but yellow in *R. kio*. *R. kio* is slightly larger than *R. helenae* and has a higher and wider caudal muscle (resulting in broader dorsalis trunci muscles onto the body) and a higher tail relative to body height, relatively larger and more laterally directed eyes. The maximum body height of *R. helenae* is greater than that *R. kio* (BH = 8.2 mm, *R. helenae*; BH = 7.7 - 7.8 mm, *R. kio*). Tadpole *R. helenae* can be differentiated from tadpoles of *R. kio* by having tail irregular white spots and the body is round, fin caudal approximately the height of the body; by having tail well developed (vs. musculature indistinct in *R. kio*) (Vassilieva *et al.*, 2016 [3] and this study).

Table 1. Measurements (in mm) of the four collected tadpoles of *Rhacophorus helenae* from Dong Nai Culture and Nature Reserve, Dong Nai Province (for abbreviations see material & methods)

	Stage 26 (n=1)	Stage 28 (n=1)	Stage 29 (n=1)	Stage 32 (n=1)	Stage 34 (n=1)	Stage 35 (n=1)	Stage 39 (n=2)	Stage 41 n=6)	Stage 43 (n=4)
TL	18-23	24.9	31.6	31.8- 37.4	32.7	33.9	39.8- 44.5	43.3- 44.7	31.6- 34.8
TAL	9.9-14	13.1	17.1	20- 23.1	24.8	22.1	25.3- 30.3	23.3- 34.3	20.3- 22.4
BL	8.1-10	11.8	14.5	10.3- 14.3	17.9	11.8	14.2- 14.5	10.4- 11.1	9.7- 14.5
BH	3.4- 5.2	5.7	7.1	7.9- 9.1	8.2	8.3	7-10.7	7.3- 10	5.5-6.7
BW	4.3- 5.7	7.3	8.1	8.4- 9.9	9.7	10.1	8.1-9.6	7.9- 10	7.5-8.4
SU	6.4-8. 8	11.7	13.2	12.8- 16.4	16.5	14.1	13.7- 18.6	8.6- 20.1	12.3- 17.5
SVL	3-3.6	5.1	5.7	4.6- 7.1	5.9	6.4	6.1-8	7- 10.8	8.3-9.1
ODW	1-1.9	2.1	1.8	1.9-2	2.6	2	2-3.1	2.2- 2.7	4.6-5.3
ED	0.9	1.2	1.1	1.4- 1.6	1.6	1.5	2-2.3	2.2- 2.7	2.3-2.7
NN	0.9- 1.4	1.3	1.8	1.6-2	1.8	1.8	2.3	2.2-3	2.2-2.6
PP	1.7- 2.2	2.6	3.3	2.9- 3.7	4	3.5	4.5-4.8	3.5- 5.1	3.5-5.3
NP	1-1.2	1.5	1.5	1.5- 1.9	1.7	1.9	2.3-2.4	2-3.6	1.8-2.3
RN	1.2- 1.4	1.7	2.1	2.1- 2.7	2	2.1	1.9-3.1	2-2.8	1.4-1.8
SS	5.4- 7.3	8	9.8	7-9.7	9.6	9.7	9.7- 10.1		
VT	9.8- 14.4	13.2	18.4	17.2- 22.3	26.8	19.8	26.1- 25.9	23.3- 34.7	22.9- 26.5

HT	3.4-4.8	5.4	7.4	5.7-7	6.5	5.8	7.4-9	6.7-9.7	4.8-5.6
LF	0.8	1.8	1.3	1.2-2	1.9	2	1.8-2.1	1.7-2.8	1-1.6
UF	0.6-1.3	1.1	1.4	1.2-2.3	2.4	2.4	1.9-2.4	2.4-2.6	1-1.9
TMH	2.8-2.9	4.1	1.2	4.2-5.5	5	4.7	3.4-5	5.3-6.2	3.3-4.6
TMW	1.5-1.8	2.3	2.6	3.2-4.1	4.4	3.7	3.4-5	4.3-4.9	2.5-3
HL	-	1.2	1.4	1.6-2	2.3	2.2	9-12.4	11.8-18.9	24.2-25.8
FL	-	-	-	-	-	-	-	-	11.2-12.9

Table 2. Measurements (in mm) of the two collected tadpoles of *Rhacophorus helenae* from Dong Nai Culture and Nature Reserve and *Rhacophorus kio* from Northwestern Thailand (for abbreviations see Material & Methods)

	<i>Rhacophorus helenae</i>		<i>Rhacophorus kio</i>	
	<i>Stage 34 (n = 1)</i>	<i>Stage 35 (n = 1)</i>	<i>Stage 34 (n = 2)</i>	<i>Stage 35 (n = 2)</i>
BH	8.2	8.3	7.7-7.8	7.1-8.9
BW	16.5	10.1	8.6-9.4	7.8-9.6
NN	1.8	1.8	2.8-2.9	2.3-2.9
NP	1.7	1.9	2.6-2.9	2.3-2.8
PP	4	3.5	5.5-6.2	5.1-6.2
RN	2	2.1	1.7-1.9	1.2-2.0
LF	1.9	2	2.0-2.8	1.7-2.6
ED	1.6	1.5	1.5-1.7	1.7
SU	16.5	14.1	14.1-16.0	13.1-15.8
UF	2.4	2.4	2.6-3.2	2.3-3.3
MTH	6.5	5.8	7.8-9.6	6.8-9.3
TMH	5	4.7	5.5-6.2	4.8-6.0
TMW	4.4	3.7	4.6-5.4	4.8-5.5

Table 3. Morphological comparisons between tadpoles *Rhacophorus helenae* of with other members of *Rhacophorus*

Species	LIFR	TL	Sources
<i>R. annamensis</i> (stage 41, n = 4)	7(3-7)/3	41.22	Hendrix <i>et al.</i> , 2007 [13]
<i>R. angulirostris</i>	4(3-4)/4-5		Malkmus <i>et al.</i> , 2002 [14]
<i>R. calcaneus</i>	7(2-7)/3(1)		Ninh <i>et al.</i> , 2020 [15]
<i>R. cyanopunctatus</i>	9(5-9)/3(1)		Leong, 2004 [16]
<i>R. dennysi</i>	5(2-4)/3(1)		Hendrix <i>et al.</i> , 2007 [13]
<i>R. dulitensi</i>	5(2-5)/3 or 6(2-6)/3		Haas <i>et al.</i> , 2012 [17]
<i>R. feae</i>	5(2-5)/3		Kane <i>et al.</i> , 2018 [18]
<i>R. georgi</i>	5(2-5)/2(1)		Gillespie <i>et al.</i> , 2007 [19]
<i>R. helenae</i>	5(2-5)/3		This study
<i>R. kio</i> (stage 36)	5(2-5)/3		Grosjean & Inthara, 2016 [20]
<i>R. mininus</i> (stage 35, n=1)	5(2-5)/3(1)	25.5	Rao <i>et al.</i> , 2006 [21]
<i>R. nigropalmatus</i>	5(3-5)/2(1)	?	Inger 1985 [22]
<i>R. orlori</i> (stage 40, n=1)	5(2-4)/3(1)	24.5	Wildenhues <i>et al.</i> , 2011 [23]
<i>R. rhodopus</i> (stage 36, n=8)	6(2-6)/3(1)	45.7	Grosjean & Inthara, 2016 [20]
<i>R. rufipes</i> (Stages 33–34)	4(2-3)/3 or 5(2-4)/3		Haas <i>et al.</i> , 2012 [17]
<i>R. penanorum</i> (Stages 26–28)	4(4)/7		Haas <i>et al.</i> , 2012 [17]
<i>R. pardalis</i> (stage 37)	7(2-5)		Inger, 1966 [24]
<i>R. prominanus</i>	6(2-6)/3		Berry 1972 [25]; Manthey & Grossmann 1997 [26]; Leong 2004 [16]
<i>R. smaragdinus</i> (stage 35, n=1)	5(2-5)/3(1)	39.3	Wildenhues <i>et al.</i> , 2010 [27]
<i>R. vampyrus</i> (stage 41, n=2)	2(1)/3	32.4–33.5	Rowley <i>et al.</i> , 2012 [2]

3. Conclusions

The *R. helenae* species is most similar to *R. kio*. In contrast to the widely distributed *R. kio*, the species *R. helenae* was known only from two fragments of disturbed. The continued survival of *R. helenae* is threatened by further habitat loss and degradation due to encroachment (e.g., livestock grazing and collection of forest products) and habitat isolation (Rowley *et al.*, 2012 [2]). The known distribution of *R. helenae* has been recorded in 4 localities in South Vietnam: Nui Ong Nature Reserve, Binh Thuan Province, Tan Phu Protection Forest, Dong Nai Province (Rowley *et al.*, 2012 [2]), Binh Chau - Phuoc Buu Nature Reserve, Ba Ria - Vung Tau Province, Ma Da Forest sector (Dong Nai Culture and Nature Reserve), Dong Nai Province (Vassilieva *et al.*, 2016 [3]). We describe morphology *R. helenae* from Dong Nai Culture and Nature Reserve, and supplement data on comparing between two species *R. helenae* and *R. kio* that in which the adults could be largely similar, the knowledge of the larvae and the reproductive modes could be of great help in defining and delimiting the species boundaries. This study has also provided additional data for the six developmental stages of the species *R. helenae* after the previous studies (Gosner stages 26, 28, 29, 32, 34, and 43).

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