

MORPHOLOGICAL CHARACTERS, SEXUAL RATIO, TESTIS AND EGG DEVELOPMENT OF *Quasipaa verrucospinosa* (BOURRET, 1937) (AMPHIBIA: ANURA: DICROGLOSSIDAE) FROM THUA THIEN-HUE PROVINCE, CENTRAL VIETNAM

Binh Van Ngo¹ and Chung Dac Ngo²

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Quasipaa verrucospinosa (Bourret, 1937) is an endemic species of Vietnam. A total of 429 specimens (225 males, 173 females, and 31 subadults) were examined for the relevant data. This species breeds once a year and the breeding season is lasting from August to January, mainly from September to December. The ratio of male/female during breeding season is 1.3/1. The females lay a number of 483 – 968 eggs per clutch (average 795 eggs, $n = 30$). The relative reproduction is 4 – 6 eggs (average 5 ± 1) eggs per gram of the body weight.

Keywords: *Quasipaa verrucospinosa*; morphology; reproduction; Vietnam; Thua Thien-Hue; A Luoi.

INTRODUCTION

The Granular Spiny Frog was originally described by Bourret in 1937 under the name *Rana spinosa verrucospinosa* with the type specimens collected from Sa Pa and Tam Dao, Vietnam. It was subsequently upgraded to be a full species by Bourret (1939, 1942). The generic name of this species has been changed several times, i.e., *Paa* in Inger et al. (1999), *Nanorana* in Chen et al. (2005), and *Quasipaa* in Jiang et al. (2005). We follow Jiang et al. (2005) and Nguyen et al. (2009) to use the generic name *Quasipaa* for this species.

Quasipaa verrucospinosa is not only endemic to Vietnam but it is also listed in the IUCN Red List (2010) as a near threatened species. Previous studies mainly focused on taxonomy and phylogenetic relationships and no data of reproductive biology of this species has been published so far. Therefore, we herein provide some notes on reproduction of *Quasipaa verrucospinosa* in A Luoi, Thua Thien-Hue province, Central Vietnam.

MATERIAL AND METHODS

Field work was conducted in A Luoi District, Thua Thien-Hue Province, about 70 km from Hue City, with

¹ Department of Life Sciences, Institute of Biodiversity, National Cheng Kung University, No. 1 University Road, Tainan city, Taiwan, R.O.C.; e-mail: binhngodhsphue09092009@gmail.com

² College of Pedagogy, Hue University, 34 Le Loi, Hue city, Vietnam; e-mail: chunghaian@yahoo.com

the geographical co-ordinates ranges between 16°00' – 16°37' N 107°00' – 107°36' E, at the elevation above 700 m a.s.l. (Fig. 1).



Fig. 1. Map showing the study locality (red circle) in A Luoi District, Thua Thien-Hue Province, Vietnam.

TABLE 1. Weight (g), Measurements (mm), and Proportions of the Specimens of *Quasipaa verrucospinosa* from Thua Thien-Hue Province

Character	Adult males (<i>n</i> = 225)		Adult females (<i>n</i> = 173)		Subadults (<i>n</i> = 31)	
	min – max	mean ± SD	min – max	mean ± SD	min – max	mean ± SD
Measurements						
BW	14.48 – 183.40	87.43 ± 35.44	8.75 – 240.37	130.26 ± 61.05	3.45 – 18.76	5.14 ± 3.68
SVL	53 – 124	95.81 ± 15.81	56 – 137	106.48 ± 23.25	31 – 55	36.35 ± 5.93
TBL	31 – 67	54.13 ± 8.89	25 – 74	60.02 ± 9.80	19 – 29	21.42 ± 2.47
HW	21 – 50	37.16 ± 6.18	19 – 53	41.62 ± 8.72	14 – 21	16.06 ± 2.19
HL	13 – 41	30.32 ± 4.87	15 – 45	33.88 ± 7.43	10 – 18	12.81 ± 1.82
HWE	12 – 36	24.64 ± 4.02	12 – 37	27.52 ± 5.73	9 – 19	12.26 ± 2.39
SNL	9 – 22	15.66 ± 2.55	7 – 27	17.56 ± 3.82	5 – 10	6.19 ± 1.17
SND	5 – 13	9.81 ± 1.56	4 – 13	10.54 ± 2.02	3 – 6	4.13 ± 0.88
ED	7 – 16	12.11 ± 1.84	6 – 16	13.09 ± 2.41	4 – 8	5.52 ± 1.18
EW	6 – 13	10.03 ± 1.60	5 – 14	11.03 ± 2.22	3 – 7	4.06 ± 1.15
IO	7 – 22	16.63 ± 2.73	8 – 23	18.07 ± 3.53	6 – 10	7.35 ± 1.23
IN	6 – 11	9.10 ± 1.05	5 – 14	9.80 ± 1.70	4 – 6	4.52 ± 0.77
HNL	11 – 33	25.48 ± 3.99	10 – 36	28.19 ± 5.77	8 – 19	10.10 ± 2.99
FL	26 – 65	48.69 ± 7.56	21 – 69	53.82 ± 8.26	16 – 28	18.71 ± 3.57
Proportions						
HL/HW	0.59 – 0.94	0.82 ± 0.05	0.64 – 0.95	0.81 ± 0.05	0.63 – 1.00	0.80 ± 0.08
IO/HL	0.23 – 0.85	0.55 ± 0.06	0.38 – 0.69	0.54 ± 0.05	0.43 – 0.70	0.58 ± 0.08
ED/HL	0.31 – 0.69	0.40 ± 0.04	0.32 – 0.56	0.39 ± 0.04	0.27 – 0.60	0.43 ± 0.08
IN/HL	0.23 – 0.56	0.30 ± 0.04	0.24 – 0.44	0.29 ± 0.03	0.24 – 0.44	0.29 ± 0.03
HL/SVL	0.22 – 0.43	0.32 ± 0.02	0.25 – 0.38	0.32 ± 0.02	0.29 – 0.47	0.35 ± 0.04
TBL/SVL	0.38 – 0.71	0.57 ± 0.03	0.47 – 0.65	0.57 ± 0.03	0.47 – 0.68	0.59 ± 0.06

Note. SD, standard deviation; for other abbreviations see *Materials and Methods*.

Specimens were collected during a period of 13 months, from July 2008 to July 2009. A number of 30 specimens at different stages (adult and subadult) were selected for examination in each month. We investigated a total of 429 specimens, comprising 225 males, 173 females, and 31 subadults for collecting the data of reproduction. Preserved specimens were subsequently deposited at the College of Pedagogy, Hue University, Vietnam.

Morphological characters. The weight was weighed by electronic balance to the nearest 0.001 g and all the measurements were made with dial calipers to the nearest 0.01 mm. The following abbreviations are used: BW, body-weight; SVL, snout-vent length; TBL, tibia length, ankle to knee while the joints are flexed; HW, head width, width at rear of head directly above tympanum; HWE, head width at anterior margin of eye; HL, head length, from posterior margin of jaw to tip of snout; SNL, snout length, from anterior margin of eye to tip of snout; SND, distance from canthus to lower margin of upper lip; ED, eye diameter, horizontal diameter of eye; EW, eyelid width; IO, interorbital distance at narrowest point; IN, internarial distance; TD, tympanum diameter, length of the vertical axis; HNL, hand length

from proximal edge of outer palmar tubercle to tip of third finger; FL, foot length from proximal edge of inner metatarsal tubercle to tip of fourth toe.

Testes. TW, testis weight; TL, testis length; RTW, right testis weight; RTL, right testis length; RTWD, right testis width; LTW, left testis weight; LTL, left testis length; LTWD, left testis width.

The number of eggs were counted on both left and right ovaries of 30 females. According to Pravdin (1973) and Vu (2004), the relative reproduction is the ratio of the number of eggs/BW of each specimen. The weight and size of testes and eggs were measured monthly in 2008 and 2009. They were then fixed into Bowin solution for 1 – 3 days for histological study.

RESULTS AND DISCUSSION

Morphological Characters

Size. SVL of adult males 53 – 124 mm (*n* = 225), of adult females 56 – 137 mm (*n* = 173), and of subadults 31 – 55 mm (*n* = 31). In average, SVL and BW of males distinctly smaller than females; males: SVL 95.81 mm,

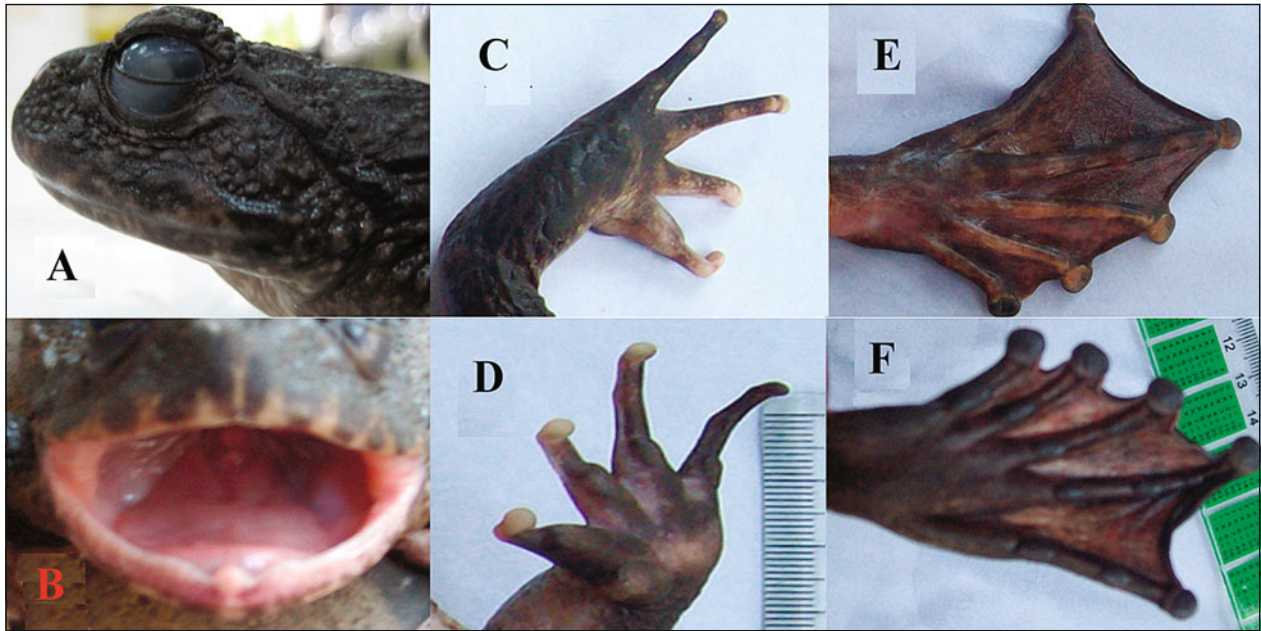


Fig. 2. Head, hand, and foot of *Quasipaa verrucospinosa*: head: (A) lateral view, (B) oral cavity; hand: (C) dorsal view, (D) ventral view; foot: (E) dorsal view, (F) ventral view.

BW 87.43 g ($n = 225$); females: SVL 106.48 mm, BW 130.26 g ($n = 173$); and subadults SVL 36.35 mm, BW = 5.13 g ($n = 31$). Other measurements (in mm): adult males ($n = 225$): TBL 54.13, HW 37.16, HL 30.32, HWE 24.64, SNL 15.66, SND 9.81, ED 12.11, EW 10.03, IO 16.63, IN 9.10, TD indistinct, HNL 25.48, FL 48.69; adult females ($n = 173$): TBL 60.02, HW 41.62, HL 33.88, HWE 27.52, SNL 17.56, SND 10.54, ED 13.09, EW 11.03, IO 18.07, IN 9.80, TD indistinct, HNL 28.19, FL 53.82; and subadults ($n = 31$): TBL 21.42, HW 16.06, HL 18.71, HWE 12.26, SNL 6.19, SND 4.13, ED 5.52, EW 4.06, IO 7.35, IN 4.52, TD indistinct, HNL 10.10, FL 12.81 (see Table 1).

Proportions. HL/HW: 82% in males, 81% in females, and 80% in subadults; IO/HL: 55% in males, 54% in females, and 58% in subadults; ED/HL: 40% in males, 39% in females, and 43% in subadults; IN/HL: 30% in males, 29% in females, and 35% in subadults; HL/SVL: 32% in males, and females, 35% in subadults; TBL/SVL: 57% in males and females, 59% in subadults. The variation of measurement ratios are given in Table 1.

A large-sized anuran frog, SVL up to 137 mm; head wider than long; tympanum indistinct; vomerine teeth present; supratympanum fold distinct, from behind eye to shoulder; adult males without vocalsacs; eye large with a vertical pupil; dorsal skin granular, back with large oval warts intermixed with small tubercles, wart with 2–5 small black spinules in breeding season; upper surface of fore and hind limbs granular; venter smooth;

relative length of fingers II < I < IV < III; fingers without webbing; relative length of toes I < II < III < V < IV; toes with completed webbing; outer palmar tubercles large,



Fig. 3. *Quasipaa verrucospinosa* in natural habitats in A Luoi District.

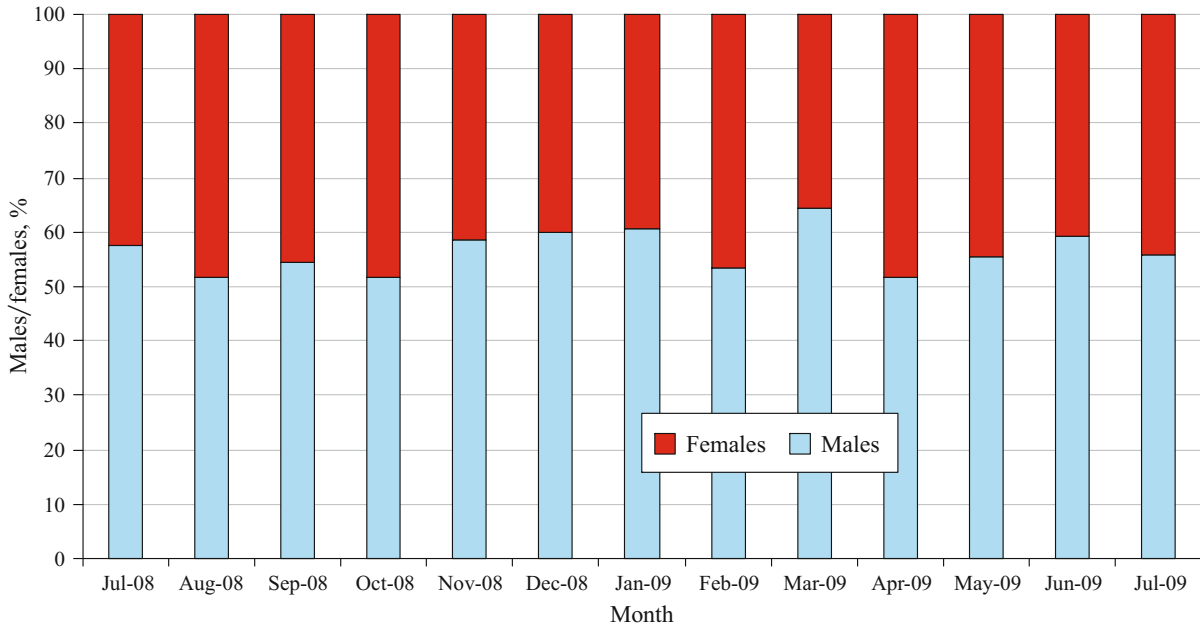


Fig. 4. Monthly sexual ratio of *Quasipaa verrucospinosa*.

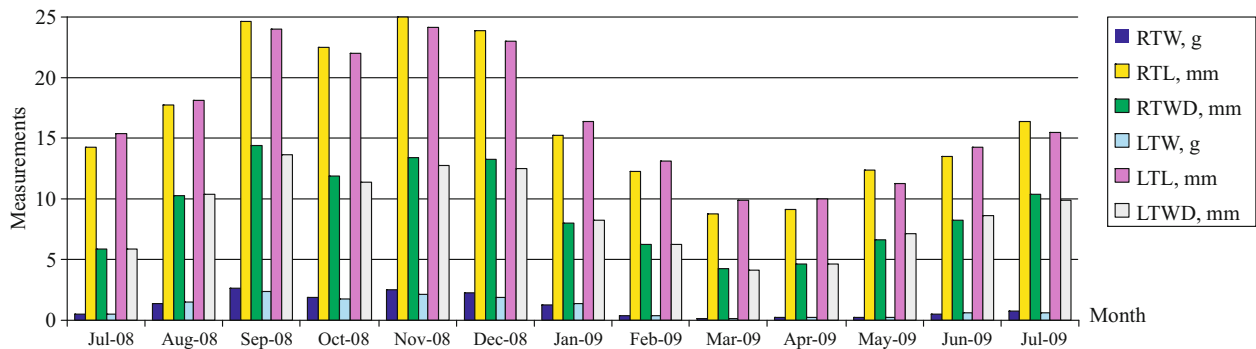


Fig. 5. Monthly development of testis of *Quasipaa verrucospinosa*.

with black spinules during breeding season, which also present on fingers I and II; toe disc rounded, slightly enlarged.

Coloration in life. Dorsal head dark brown; back gray or brownish gray in adults, reddish brown in sub-adults; lips and upper limbs with dark bars; deep-brown upper surface of limbs; throat with dark marbling, belly cream; color of webbing is dark-red (Fig. 2).

Breeding Season and Sexual Ratio

Breeding season. In A Luoi area, *Q. verrucospinosa* inhabits rocky streams in evergreen forest at elevations above 700 m a.s.l. (Fig. 3). It prefers cool temperature (16–25°C) and relatively high humidity (90–94%).

Specimens can be found near the waterfall or on the rock and they are active at night (Fig. 3). Granular Spiny Frog breeds in rainy season from August to January. The testes start developing in rainy season (July to January) and small follicles can be found in the specimens collected in dry season (February to June).

Sexual ratio. Our observations showed that *Q. verrucospinosa* is characterized by having external fertilized and population mating behavior. The annual ratio of male/female is 1.3/1. This ratio is lowest in dry season (April) and highest is in rainy season (December). This ratio is around 1/1 in August and September 2008, as well as in February and April 2009 (Fig. 4).

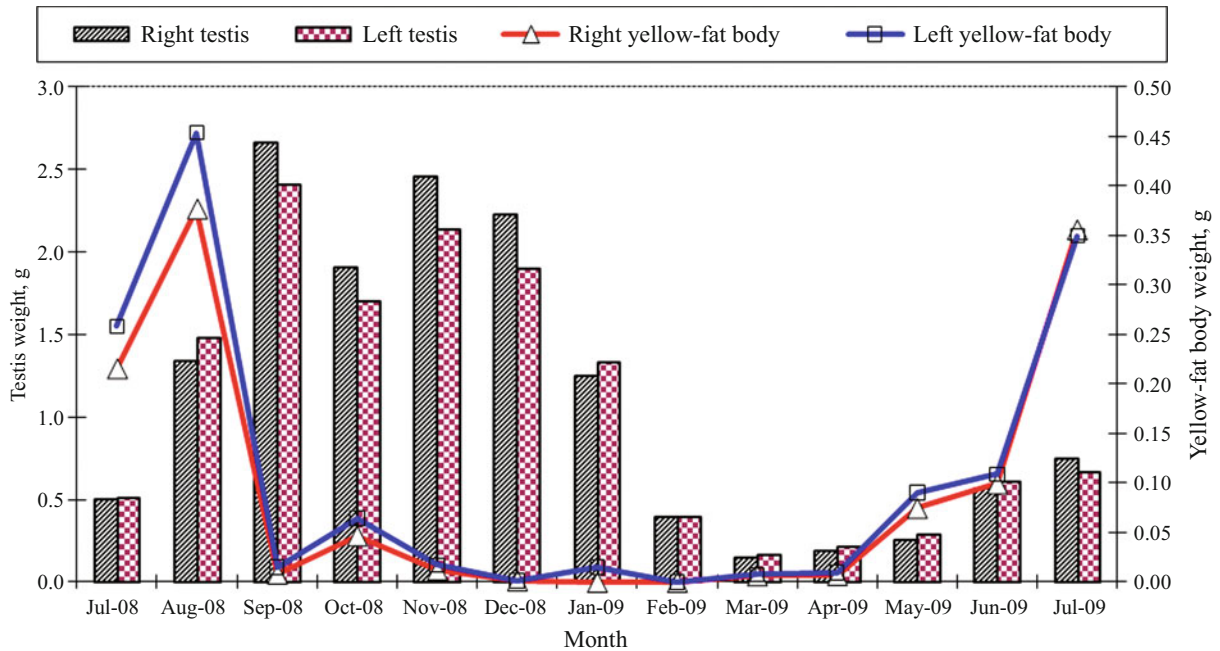


Fig. 6. Monthly weight relation between testis and yellow fat-body of *Quasipaa verrucospinosa*.

TABLE 2. Monthly Development of Testis, Weight, and Size of Specimens of *Quasipaa verrucospinosa* from Thua Thien-Hue Province

Month	n	Right testis			Left testis			BW, g	SVL, mm	TW/BW, %	TL/SVL, %
		RTW ± SD	RTL ± SD	RTWD ± SD	LTW ± SD	LTL ± SD	LTWD ± SD				
Jul-08	19	0.49 ± 0.17	14.26 ± 2.52	5.89 ± 2.49	0.50 ± 0.15	15.37 ± 2.31	5.84 ± 1.46	102.19	103.53	1.12	28.95
Aug-08	16	1.34 ± 0.89	17.75 ± 2.19	10.25 ± 3.65	1.47 ± 0.90	18.19 ± 2.17	10.38 ± 1.70	104.16	102.50	2.64	35.20
Sep-08	19	2.66 ± 0.21	24.68 ± 1.51	14.37 ± 1.28	2.40 ± 0.12	23.95 ± 1.35	13.63 ± 1.19	98.82	98.74	4.63	48.70
Oct-08	16	1.90 ± 0.51	22.50 ± 3.10	11.94 ± 2.38	1.70 ± 1.30	22.06 ± 1.26	11.44 ± 1.94	79.66	91.69	3.96	48.07
Nov-08	17	2.45 ± 1.50	24.94 ± 2.55	13.35 ± 2.31	2.13 ± 1.33	24.12 ± 2.58	12.71 ± 2.50	86.26	95.47	4.78	50.35
Dec-08	18	2.22 ± 1.41	23.89 ± 1.89	13.28 ± 3.86	1.89 ± 1.24	23.00 ± 2.24	12.56 ± 2.51	84.94	95.44	4.40	48.23
Jan-09	20	1.25 ± 0.61	15.20 ± 1.97	8.00 ± 1.52	1.33 ± 0.63	16.35 ± 2.67	8.30 ± 1.61	91.30	98.30	2.40	31.81
Feb-09	16	0.39 ± 0.01	12.25 ± 1.57	6.25 ± 1.44	0.39 ± 0.06	13.13 ± 1.54	6.25 ± 1.57	88.68	97.81	0.96	26.04
Mar-09	20	0.14 ± 0.02	8.80 ± 0.17	4.20 ± 0.25	0.16 ± 0.03	9.85 ± 0.19	4.15 ± 0.11	49.15	75.85	0.41	23.29
Apr-09	14	0.19 ± 0.05	9.07 ± 1.07	4.57 ± 1.50	0.21 ± 0.08	10.00 ± 1.11	4.57 ± 0.61	60.66	83.57	0.53	21.28
May-09	15	0.25 ± 0.07	12.33 ± 1.88	6.60 ± 1.18	0.28 ± 0.09	11.20 ± 1.65	7.13 ± 1.64	97.32	100.60	0.61	23.77
Jun-09	16	0.56 ± 0.05	13.56 ± 1.63	8.31 ± 1.62	0.60 ± 0.07	14.25 ± 1.65	8.63 ± 1.41	89.18	98.94	1.46	28.45
Jul-09	19	0.74 ± 0.05	16.37 ± 1.52	10.32 ± 2.83	0.66 ± 0.08	15.53 ± 1.39	9.84 ± 0.29	102.81	102.95	1.38	31.28
Average		1.14 ± 0.51	16.68 ± 4.80	9.08 ± 2.97	1.07 ± 0.18	16.80 ± 4.13	8.92 ± 2.56	87.32 ± 16.41	95.80 ± 8.02	2.25 ± 1.66	34.26 ± 10.79

TW, testis weight; TL, testis length.

Testis and egg development

Development of testis. In average, the right testis is a little bit larger than the left one (weight 1.14 ± 0.51 g, length 8.80 ± 0.17 mm, and width 4.20 ± 0.25 mm vs. weight 1.07 ± 0.18 g, length 9.85 ± 0.19 mm, and width 4.15 ± 0.11 mm, $n = 225$). It is noted that four male spec-

imens have the left testis only and their weight and measurement greater than the specimens with two testes collected at the same time.

Testes slowly developed between February and July, but rapidly grew up from August to January. In term of position, right testis is more close to the anus than the left one. Testes are smallest in March (right testes: weight

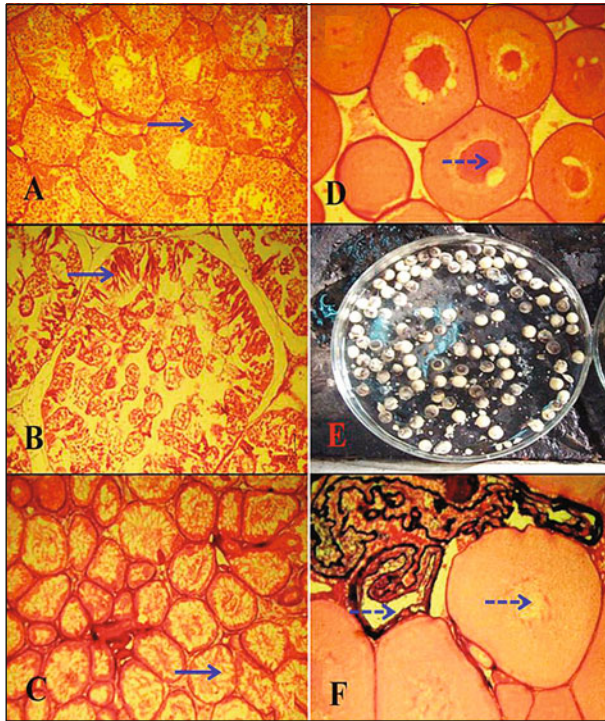


Fig. 7. Testis and ovary histology ($\times 10$) of *Quasipaa verrucospinosa*.

0.14 ± 0.02 g; length 8.80 ± 0.17 mm; width 4.20 ± 0.25 mm and left testes: weight 0.16 ± 0.03 g; length 9.85 ± 0.19 mm; width 4.15 ± 0.11 mm). This is hibernated time of frogs during the winter season. They have

largest size in September (right testes: weight 2.66 ± 0.21 g; length 24.68 ± 1.51 mm; width 13.63 ± 1.19 mm and left testes: weight 2.40 ± 0.12 g; length 23.95 ± 1.35 mm; width 23.95 ± 1.35 mm). This time is coincide with the middle of breeding season of *Q. verrucospinosa* (Table 2, Fig. 5). The ratios of TW/BW and TL/SVL are highest in September to December and lowest in February to May.

Development of yellow fat-body in males. The weight of yellow fat-body starts increasing in May (right: 0.075 ± 0.004 g and left: 0.090 ± 0.005 g) and get the biggest in August (right: 0.376 ± 0.027 g and left: 0.453 ± 0.032 g, $n = 225$). It is smallest in September (right: 0.008 ± 0.001 g and left: 0.015 ± 0.003 g, $n = 225$) (Table 2, Fig. 6).

Testis histology. Before breeding season (from June to July), genital sperm tracts in hexagonal form, ordered next to each other, embryonic germ cells separated from each other inside each genital sperm tract (Fig. 7A). During breeding season (from August to January), genital sperm tracts developed in size, desultorily arranged, more rounded in shape, embryonic germ cells separated quickly and developed into typical sperm (Fig. 7B). After breeding season (from February to May), genital sperm tracts reduced in size, circle or rounded polygonal, number of embryonic germ cells also reduced (Fig. 7C).

Development of egg. Eggs diameter with the size larger than 2 mm can be found from August to January (maximum 3.5 – 3.9 mm). In February and March, only small follicles can be found in ovary. Granular Spiny Frog reproduces only once a year, breeding season last-

TABLE 3. Monthly Development of Ovary, Yellow Fat-Body, and Egg of *Quasipaa verrucospinosa* from Thua Thien-Hue Province

Month	n	Ovary, g		Yellow fat-body, g		Total \pm SD	Number of eggs in different sizes (in mm)			
		right \pm SD	left \pm SD	right \pm SD	left \pm SD		0.5 – 1.0 \pm SD	1.1 – 1.5 \pm SD	1.6 – 2.0 \pm SD	>2.0 \pm SD
Jul-08	14	1.56 \pm 1.27	2.08 \pm 1.62	0.388 \pm 0.316	0.431 \pm 0.411	594 \pm 359	268 \pm 151	154 \pm 100	172 \pm 129	0 \pm 0
Aug-08	15	2.86 \pm 1.45	2.58 \pm 1.07	0.068 \pm 0.005	0.100 \pm 0.025	566 \pm 532	55 \pm 73	66 \pm 66	189 \pm 194	257 \pm 310
Sep-08	16	3.92 \pm 2.38	3.65 \pm 2.11	0.010 \pm 0.026	0.013 \pm 0.028	672 \pm 618	48 \pm 89	74 \pm 78	112 \pm 122	438 \pm 465
Oct-08	15	4.33 \pm 2.19	3.86 \pm 1.43	0.001 \pm 0.005	0.001 \pm 0.005	689 \pm 521	68 \pm 76	83 \pm 80	125 \pm 111	413 \pm 394
Nov-08	12	5.19 \pm 2.08	5.01 \pm 2.91	0.002 \pm 0.006	0.002 \pm 0.006	705 \pm 294	85 \pm 66	97 \pm 52	148 \pm 104	376 \pm 229
Dec-08	12	4.04 \pm 1.00	3.69 \pm 1.57	0.000 \pm 0.000	0.000 \pm 0.000	685 \pm 550	69 \pm 70	59 \pm 56	130 \pm 123	427 \pm 444
Jan-09	13	2.20 \pm 0.69	2.39 \pm 1.04	0.000 \pm 0.000	0.000 \pm 0.000	447 \pm 596	0 \pm 0	0 \pm 0	141 \pm 188	306 \pm 414
Feb-09	14	0.24 \pm 0.14	0.27 \pm 0.14	0.000 \pm 0.000	0.000 \pm 0.000	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Mar-09	11	0.04 \pm 0.01	0.06 \pm 0.02	0.011 \pm 0.016	0.011 \pm 0.015	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Apr-09	13	0.11 \pm 0.01	0.13 \pm 0.03	0.005 \pm 0.012	0.007 \pm 0.018	558 \pm 687	475 \pm 551	82 \pm 161	0 \pm 0	0 \pm 0
May-09	12	0.73 \pm 0.07	0.76 \pm 0.09	0.297 \pm 0.067	0.322 \pm 0.073	569 \pm 540	478 \pm 449	91 \pm 102	0 \pm 0	0 \pm 0
Jun-09	11	1.31 \pm 0.16	1.24 \pm 0.25	0.517 \pm 0.032	0.471 \pm 0.026	674 \pm 551	438 \pm 356	147 \pm 129	89 \pm 99	0 \pm 0
Jul-09	15	1.72 \pm 0.83	1.65 \pm 0.78	0.515 \pm 0.281	0.445 \pm 0.242	628 \pm 332	289 \pm 153	193 \pm 107	146 \pm 84	0 \pm 0
Average		2.23 \pm 1.04	2.16 \pm 0.80	0.138 \pm 0.279	0.137 \pm 0.284	522 \pm 243	175 \pm 188	80 \pm 60	96 \pm 71	171 \pm 197

ing from August to January, mainly from September to December (Table 3 and Fig. 8).

Development of yellow fat-body in females. Yellow fat-body starts developing in May (right: 0.297 ± 0.067 g and left: 0.322 ± 0.073 g), biggest in June (right: 0.517 ± 0.032 g and left: 0.471 ± 0.026 g), and rapidly decreased in August (right: 0.068 ± 0.005 g and left: 0.100 ± 0.025 g) (Table 3 and Fig. 9).

Ovary histology. Young ovary develops with inequilateral dimension oocytes (Fig. 7D), oocytes ordered next to each other, egg-membrane in center of oocytes, protoplasmic-membrane and egg-membrane having backlash with powder alkali color, middle protoplasmic-membrane and egg being colored yolk, reaching nearly all ovary volume. Eggs before producing have relatively big

average diameter (3.5 – 3.9 mm), yellow-black, circle, oocytes reaching nearly all ovary volume (Fig. 7E). Ovary after egg delivering is creasy, blood vessels are constricted, and oocytes are undeveloped (Fig. 7F).

Based on the examination of 30 females, the number of eggs varies from 483 – 968 (average 795 ± 133) per individual. The relative reproduction is 4 – 6 (average 5 ± 1) eggs per gram of body weight.

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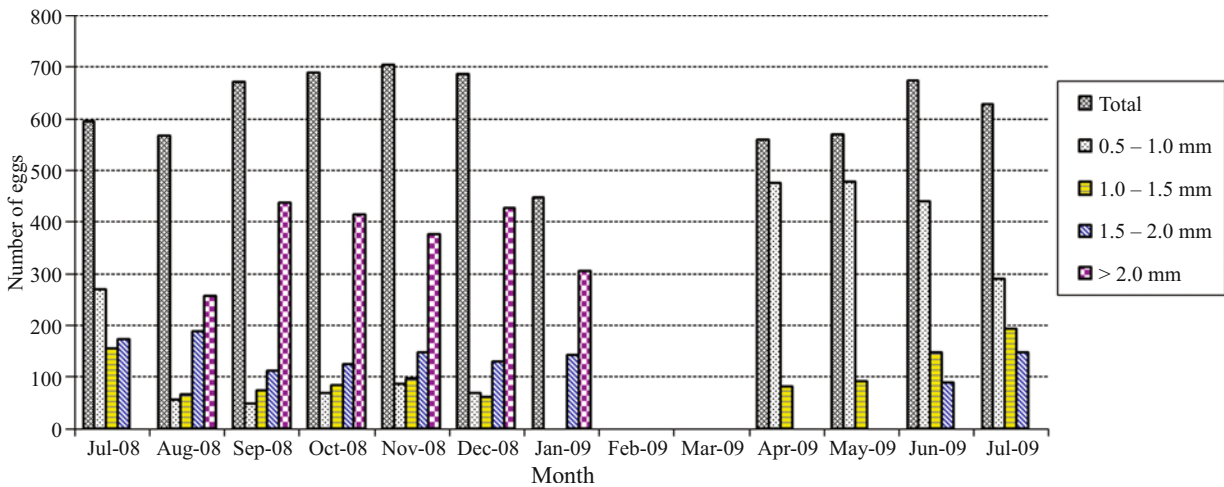


Fig. 8. Monthly number and development of eggs of *Quasipaa verrucospinosa*.

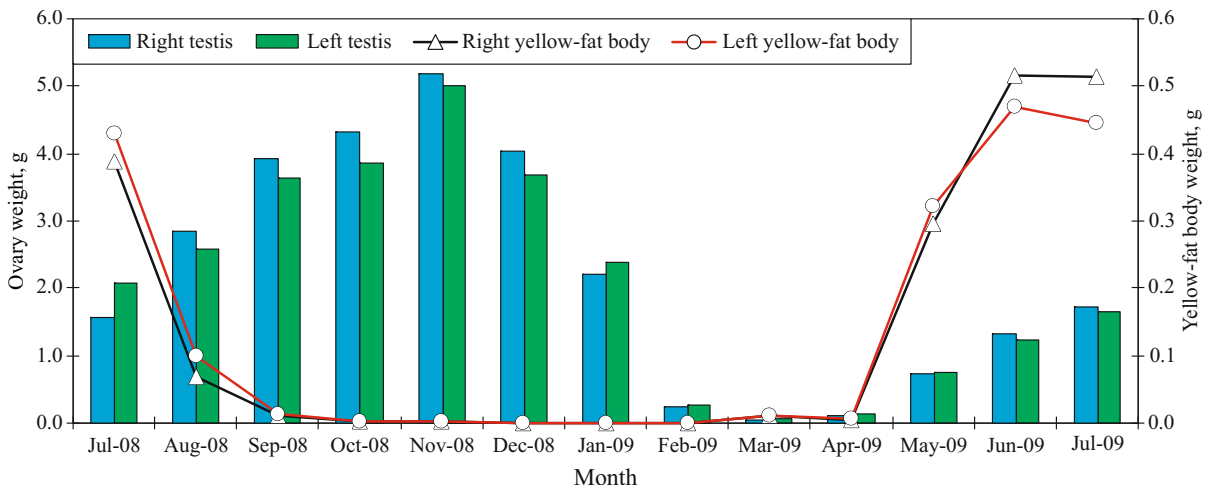


Fig. 9. Monthly weight relation between ovary and yellow fat-body of *Quasipaa verrucospinosa*.

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