

## Some Data on Reproductive Biology of Spotted Ghoul *Inimicus sinensis* (Synanceiidae)

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**Abstract**—Gonadal structure, cytological condition of oocytes, ultrastructure of egg envelope, and spermatozoa morphology are studied in the individuals of *Inimicus sinensis* 120–230 mm TL with the gonads mainly at maturity stage IV. The males possess hypertrophied urinary bladders. Based on oocytes' size distribution in the ovaries, the oogenesis is continuous and the spawning is multiple. Oocytes of the maturation period are 600–700 µm in diameter. The batch fecundity is 15380 oocytes on average. The zona radiata of oocytes that have completed growth is 10.2 µm in width on average and it consists of two layers. The narrow outermost layer of the egg envelope approximately 0.34 µm width is most likely chorion. Spermatozoon ultrastructure of *I. sinensis* does not notably differ from that in other representatives of the suborder Scorpaenoidei with external insemination. The centriolar complex is characterized by orthogonal position of centrioles. Based on ovarian structure, the representatives of the genus *Inimicus* do not differ from the majority of teleost fishes and viviparous species of the genera *Sebastes* and *Sebastes*. However, many representatives of the suborder Scorpaenoidei have unique ovarian structure with the central ovigerous stroma attached to the anterior part of the ovary. Based on the current phylogenetic schemes, this ovarian type evolved independently in several evolutionary lineages.

**Keywords:** *Inimicus sinensis*, Scorpaenoidei, oogenesis, oocyte envelope ultrastructure, spermatozoon morphology

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### INTRODUCTION

Scorpaenoid fishes (suborder Scorpaenoidei) include approximately 500 species distributed mainly in the Indo-Pacific (*Eschmeyer's Catalog...*, 2019). Almost all species of the tropical zone possess venomous fin rays, but their flesh is of a high quality, and the fishes are used for local or recreational fisheries. The species with bright coloration (*Scorpaena brasiliensis*, *Taenianotus triacanthus*, *Scorpaenopsis macrochir*, *Rhinopias frondosa*, and *Rh. eschmeyeri*) are widely used in aquarium trade. The poison of several species is valuable for medicine (Santhanam, 2019). Scorpaenoid fishes are important components of trophic chains representing their final elements in many ecosystems. The tropical species are distributed mainly in shallow coastal zones, and, therefore, they are especially vulnerable to global climate change and anthropogenic pressure, including water pollution and uncontrolled overfishing. The conservation and restoration of the scorpaenoid fish populations are impossible without the knowledge of their life histories, but the biology of the majority of species remains unknown (Kwik, 2011). Phylogenetic relationships and taxonomy of the species of the suborder Scor-

paenoidei represent a subject for intensive investigations (Smith and Wheeler, 2004; Smith et al., 2018).

The genus *Inimicus* (stingers), which includes ten species, belongs to the subfamily Choridactylinae of the family Synanceiidae (*FishBase...*, 2019). All representatives of the genus have two free anterior (lower) rays of the pectoral fin that are used for movement along the bottom. In addition, the free rays placed in front of the body are used as the support for a quick capture of prey passing above the fish. Similar free rays of the pectoral fin without notable sensory function, which are used for “walking” over the substrate, are found in several other representatives of the order Scorpaeniformes: *Choridactylus* (Synanceiidae: Choridactylinae), *Minous* (Synanceiidae: Minoinae), *Pri-onotus*, *Trigla* (Triglidae), and *Apistus* (Apistidae) (Gosline, 1994). The representatives of the genus *Inimicus* are ambush predators active at night and partly buried in the ground during the day. In the buried fish, the eyes located in the upper part of the head remain at the surface, and the pectoral fins are straightened, which allows making a quick throw to the prey. As is known, these fishes are not consumed by other animals. When disturbed, the fish will erect its dorsal spines and throw its pectoral fins forward

exposing the bright or contrasting colors on the inner surfaces of the fins as well as fanning out the colorful caudal fin. The long, needle-like spines of the dorsal fin are highly venomous. The sting is very painful, and the pain accompanied by swelling of the injured limb can hurt for several hours or even days. The fishes can be kept in comparatively small (~135 L) aquariums only one per tank to prevent aggression (Scott, 2001). The representatives of the genus *Inimicus* differ from the majority of scorpaenoid fishes with eggs developing in clutches and embedded into gelatinous matrix or from viviparous species in the release of single pelagic eggs. This spawning mode is regarded as the most generalized in the suborder Scorpaenoidei (Wourms, 1991; Pavlov and Emel'yanova, 2010, 2013).

Japanese devil *I. japonicus* is a prospective culture object in the coastal areas of China and Japan (Liu and Quan, 2005). The use of this species in intensive aquaculture is connected with valuable taste properties of the flesh as well as with comparatively large larvae (4.0 mm *TL* at hatching and 4.9 mm *TL* during the transition to exogenous feeding), which are capable to start feeding on larger organisms (rotifers) (Wang et al., 2013). The following features of *I. japonicus*'s life history have been studied: reproductive dynamics in nature (Nozaki et al., 2003); oxygen consumption of adult individuals during the day (Hirata et al., 2001); oogenesis in natural and artificial conditions (Watanabe, 2006); spermatogenesis in cultivation conditions (Watanabe, 2012); development of the embryos, larvae, and juveniles; and resistance of the progeny to salinity (Wang et al., 2013).

The object of this study is spotted ghou *I. sinensis*. It is distributed from southern China and Vietnam, southward through Java and Bali to Shark Bay, Western Australia; some records are known from the west coast of Luzon; it has also been caught in Sri Lanka and the southeast coast of India. This species is frequently caught in trawls over soft and sandy bottoms (*FAO Species...*, 1999). The fishes live in the coastal areas of tropical oceans in lagoons and on coral reefs at the depth between 5 and 90 m. The maximum body length is 260 mm *TL* (Froese and Pauly, 2019). Similar to *I. japonicus*, *I. sinensis* can be regarded as a potential aquaculture object in Southeastern Asia, but its reproductive characteristics are still unknown.

The goal of this study is to describe some features of *I. sinensis* reproductive biology, including gonadal structure and sex cell morphology.

## MATERIALS AND METHODS

Fresh exemplars of *I. sinensis* were obtained at fish markets of Nha Trang (southern Central Vietnam) from 2015 to 2017. The initial treatment and fixation of the material were conducted at the Coastal Department of the Russian–Vietnamese Tropical Research

and Technological Center (Nha Trang). The following parameters were determined during the biological analysis: standard body length (*SL*) to the end of the scale cover, total length (*TL*) to the posterior margin of the caudal fin, body weight (*W*), eviscerated body weight (*w*), and gonadal weight (*g*). The gonadosomatic index (*GSI*) was determined according to the following formula:  $GSI = g/w \times 100$ . The relationship between body weight (*W*, g) and body length (*TL*, cm) was approximated by the power equation (Sparre and Venema, 1998):  $W = aTL^b$ . The batch fecundity of the females with ovaries at maturity stage IV was determined by the weight method based on the calculation of the number of oocytes >600 μm in diameter in gonadal fragments 0.04–0.10 g in weight.

To analyze oocyte size composition, the gonadal fragments were placed into physiological solution. Images of sex cells were obtained under a Nikon Eclipse E-200 microscope and video camera. Oocytes (>50 μm) were measured with the ImageJ program.

Sexually mature males of several species of the suborder Scorpaenoidei have hypertrophied urinary bladders. As is supposed, the urinary bladder content is used during the spawning (Pavlov and Emel'yanova, 2007, 2010, 2013). The length (*K*) and width (*k*) of filled urinary bladder was measured by a digital caliper. The urinary bladder volume (*V*) was calculated based on the formula for a flattened spheroid:  $V = \pi/6 \times K^2 \times k$ .

For light microscopy, ovarian fragments were fixed in Bouin solution, and histological treatment was conducted according to a generally accepted method (Roskin and Levinson, 1957).

For scanning electron microscopy (SEM) and transmission electron microscopy (TEM), the fragments of ovaries and testes of sexually mature exemplars, as well as single ovulated oocytes obtained from a dissected female, were fixed in 2.5% glutaraldehyde mixed with 2% paraformaldehyde made in phosphate buffer, pH 7.4, with the addition of 2.5% NaCl and 1.5% sucrose. The samples were then postfixed in a 1% solution of OsO<sub>4</sub>. Subsequent mounting of the samples for SEM and TEM was carried out according to generally accepted methods (Weakley, 1972). The preparations for SEM were coated by gold and palladium alloy and investigated using a CamScan S-4 (Cambridge Instruments, United Kingdom) scanning electron microscope at accelerating voltage 20 kV. The ultrafine sections were investigated under a JEM-1011 (Jeol, Japan) transmission electron microscope at accelerating voltage 80 kV. The images were obtained with an ES-500W (Gatan, United States) digital camera and Digital Micrograph software.

The statistical analysis was conducted using the GraphPad Prism 5.03 program.

**Table 1.** Biological parameters of *Inimicus sinensis* with gonads at maturity stage IV

Sex (number of fishes)	Body length, mm		Weight, g			GSI, %
	<i>TL</i>	<i>SL</i>	<i>W</i>	<i>w</i>	<i>g</i>	
Females (22)	120–230	96–195	32–193	24–156	0.85–19.88	2.65–13.17
	184 (33)	157 (28)	111 (55)	95 (45)	7.23 (5.91)	7.03 (3.22)
Males (16)	130–183	110–158	32–117	31–107	0.06–0.32	0.08–0.41
	163 (15)	137 (10)	71 (20)	66 (18)	0.17 (0.08)	0.25 (0.09)

*TL*, total length; *SL*, standard length; *W*, body weight; *w*, eviscerated body weight; *g*, gonad weight; GSI, gonadosomatic index; above the line, range of the values; below the line, mean value and (in the parentheses) standard deviation.

## RESULTS

**Fish size and structure of internal organs.** The biological parameters of the fishes with gonads at maturity stage IV are given in Table 1. The body length (*TL*) of the females and males has the normal distribution of the variables, but the distribution of the values of female's body weight (*W*) and eviscerated body weight (*w*) is nonnormal (D'Agostino–Pearson test). Based on body length, the females are larger than the males, and the difference is significant (*t*-test:  $p = 0.028$ ). The body weight (*W* and *w*) does not differ in the females and males (Mann–Whitney test:  $p = 0.06$  and  $p = 0.11$ , respectively). The body length (cm)–body weight (g) relationship for the fishes of both sexes is approximated by the following equation:  $W = 0.0183 TL^{2.9556}$ ,  $R^2 = 0.927$ .

The ovaries of a sexually mature female are paired structures at two-thirds of their length, and their caudal parts are fused (Figs. 1a, 1b). In mature males, the testes are separated, and the spermiducts are fused just before the genital opening (Fig. 1c). The hypertrophied urinary bladder comprises a substantial part of the body cavity locating in its rostral, middle, and caudal parts. The spermiducts are attached to the urinary bladder surface. In the male 176 mm *TL* (GSI 0.30%), the length of the testes and spermiducts is 17.8 and 23.7 mm, the length and largest width of the urinary bladder is 22.0 and 9.0 mm, and the urinary bladder volume is 2.28 mL. In the females, the size of the urinary bladder is substantially lower. In the female of the same length (176 mm *TL*), the urinary bladder parameters are as follows: 12.0, 6.0 mm, and 0.45 mL. In the males 140–183 mm *TL* ( $n = 6$ ), the urinary bladder length is 11.4–23.6 (18.4 on average) mm, the largest urinary bladder width is 6.5–9.0 (7.8) mm, and the urinary bladder volume is 0.45–2.50 (1.53) mL.

**Size composition of oocytes and female's fecundity.** Based on oocyte diameter frequency distribution in sexually mature females, the cells of the smaller size prevail, and the cells of intermediate size (between previtellogenic oocytes and oocytes that completed vitellogenesis) are present. The cells that terminated vitellogenesis are most likely  $\leq 500$   $\mu\text{m}$  in diameter (Fig. 2). A hiatus is seen between these cells and

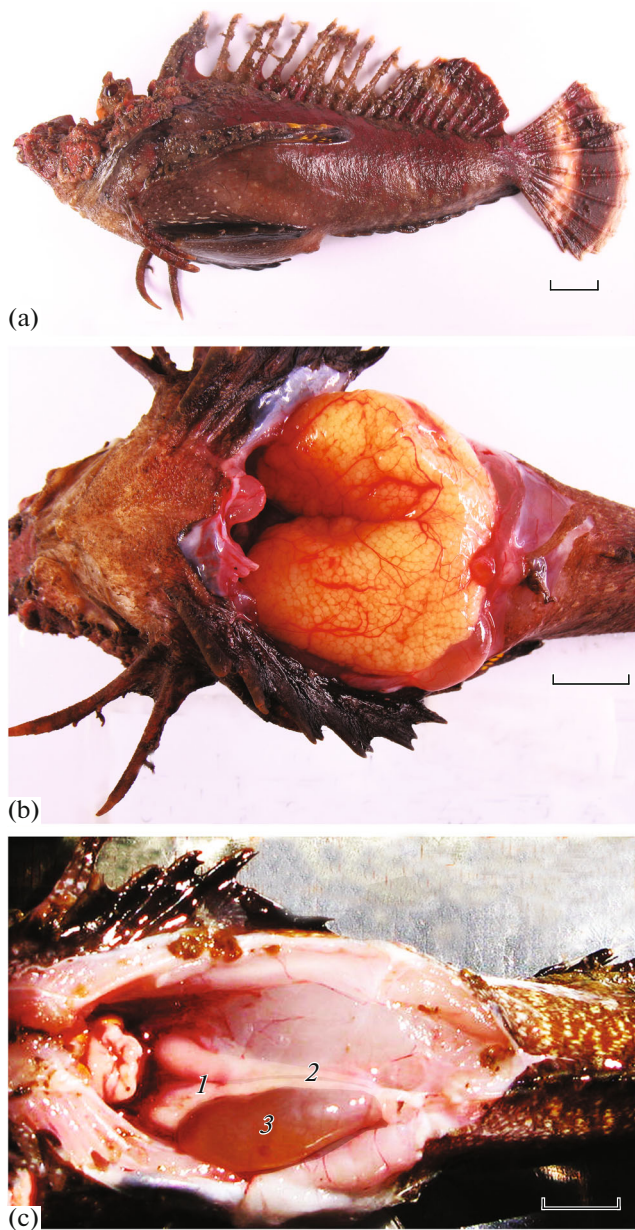
oocytes entering into maturation period. The oocytes of maturation period  $>600$ – $700$   $\mu\text{m}$  in diameter develop asynchronously showing different developmental phases. In the largest oocytes  $\sim 900$   $\mu\text{m}$  in diameter, the beginning of cytoplasm hydration is observed, and comparatively transparent narrow layer of peripheral cytoplasm is seen (based on in vivo observation).

The batch fecundity of the females (*F*) calculated based on the number of oocytes  $>600$   $\mu\text{m}$  in diameter is 4233–50080 (15380) oocytes ( $n = 15$ ). The dependence of this parameter on fish body length (*TL*, mm) is approximated by the following linear equation:  $F = 249.47 TL - 30539$ ;  $R^2 = 0.38$ .

**Cytological condition of oocytes.** The sex cells are located in well-developed ovigerous lamella. In the ovaries of single female at maturity stage II–III, the majority of oocytes are represented by previtellogenic oocytes of different size, and their maximum diameter is 150  $\mu\text{m}$ . Only a small part of cells enters into the period of vitellogenesis. These oocytes reach  $\sim 200$   $\mu\text{m}$  in diameter, and a layer of vacuoles (precursors of cortical alveoli) is seen at the periphery (Fig. 3). Small yolk granules appear in the peripheral cytoplasm.

In the ovaries at maturity stage IV, yolk granules of oval shapes 1–10  $\mu\text{m}$  in diameter are seen in the entire cytoplasm of oocytes that completed their growth. The layer of cortical alveoli reaches 9  $\mu\text{m}$  in width. Cortical alveoli are located in the peripheral cytoplasm in one or (more often) two and three rows (depending on their diameter), and they are situated close to each other. The size of cortical alveoli is substantially different; their maximum diameter is 8  $\mu\text{m}$ . Lipid droplets are absent.

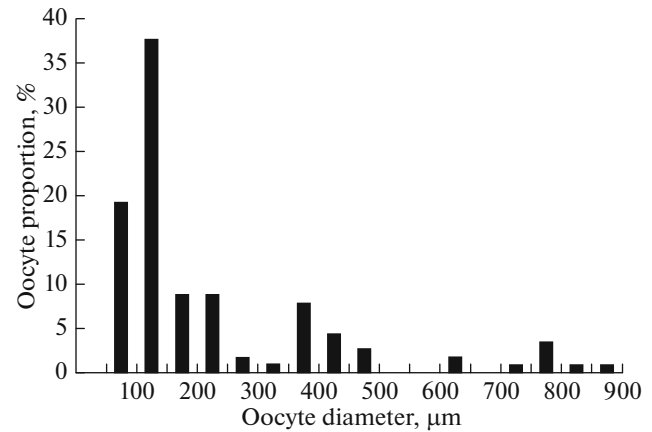
**Ultrastructure of oocyte envelope.** The oocyte surface is proportionally covered by oval pores that appeared after the contraction of micro- and macrovilli during the final maturation phases (Fig. 4a). The pore diameter ranges from 0.11 to 0.25  $\mu\text{m}$  ( $M \pm \sigma$ :  $0.19 \pm 0.13$   $\mu\text{m}$ ,  $n = 54$ ). The distance between the pores is 0.47–0.97 ( $0.75 \pm 0.03$ )  $\mu\text{m}$  ( $n = 64$ ). The oocyte surface is comparatively smooth and some-



**Fig. 1.** *Inimicus sinensis*: (a) female 121 mm *TL*, maturity stage IV, GSI 11.3%; (b) ovaries of the same exemplar; (c) male 176 mm *TL*, IV, 0.30%; 1, testes; 2, spermiducts; 3, urinary bladder. Scale bar: 1 cm.

times with particles (most likely, of alien nature) of different shape and size.

The zona radiata of oocytes that completed growth 9.25–11.37 (10.19)  $\mu\text{m}$  ( $n = 6$ ) in width consists of two layers with the substance of intermediate electron density (Fig. 4b). The widest internal layer has a homogenous structure with small spaces. Its width is 5.95–7.50 (6.65)  $\mu\text{m}$ . The external homogenous layer 2.45–5.10 (3.19)  $\mu\text{m}$  in width differs from the internal layer in slightly higher electron density. The outermost very narrow layer 0.31–0.37 (0.34)  $\mu\text{m}$  in width is most



**Fig. 2.** Oocyte diameter frequency distribution ( $n = 114$ ) in *Inimicus sinensis* female 171 mm *TL*, maturity stage IV, GSI 5.2%.

likely the chorion. This layer consists of narrow filaments oriented in different directions and restricted by the external electron dense band. The canals with micro- and macrovilli are seen in the envelope. Numerous oval mitochondria with light matrix, vesicles of agranular endoplasmic reticulum, and cortical alveoli are seen in the cytoplasmic layer of the oocyte (Fig. 4c).

**Spermatozoon ultrastructure.** The spermatozoon is characterized by an oval head, distinctly expressed midpiece, and flagellum (Fig. 5a). The size of the head, midpiece, and flagellum length are as follows:  $1.53 \times 1.63$ ,  $0.43 \times 1.19$ , and 21.4  $\mu\text{m}$ . Chromatin material of the head is densely packed and small spaces can sometimes be seen. The fossa in the base of the nucleus (with the centriolar complex) is  $\sim 1/3$ – $1/4$  of the head diameter. The fossa is located in the center of the nuclear base, less often, slightly eccentrically (Figs. 5b, 5c). The proximal centriole is directed orthogonally to the distal centriole (basal body) (Fig. 5d). The mitochondrial sections (four to seven) are seen in the midpiece. The mitochondria have oval shape and matrix of intermediate electron density. The axoneme of the flagellum has a classical structure, and it includes nine duplets of peripheral microtubules and two central microtubules:  $(9 \times 2) + 2$ . All microtubules are electron-transparent. The axoneme is surrounded by a cytoplasmic membrane of various widths in different parts of the flagellum (Fig. 5e).

## DISCUSSION

Almost all *I. sinensis* individuals collected in Nha Trang Bay in March and April had the gonads at maturity stage IV. Therefore, intensive reproduction during the spring can be supposed. In Japan, the spawning of *I. japonicus* in the coastal waters of Niigata Prefecture occurs from June to August with the peak in July (Watanabe, 2006). In the more southern region (off





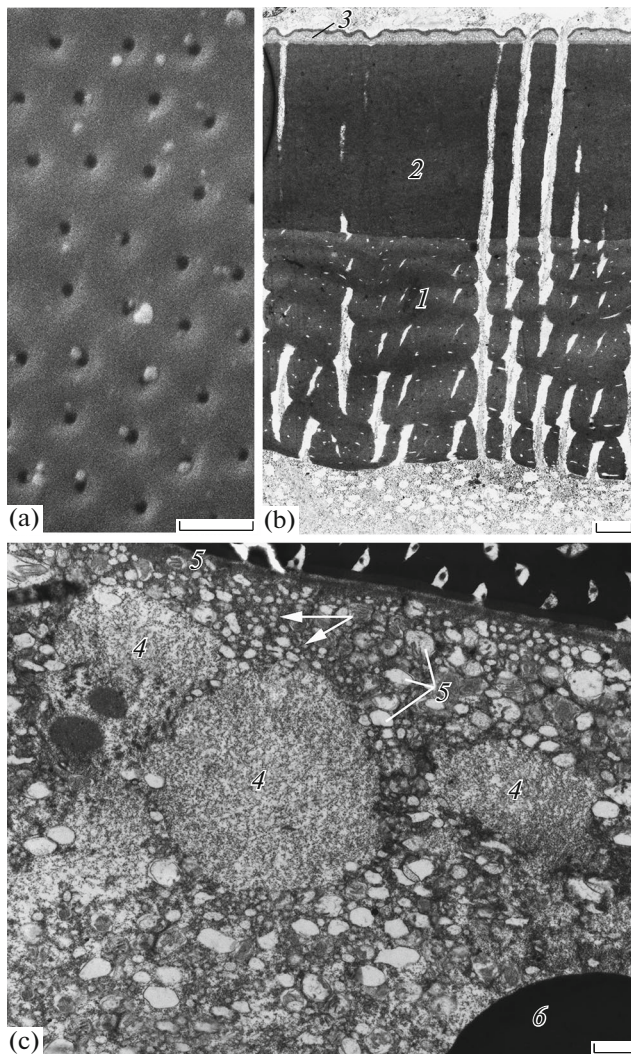
Fig. 3. Oocyte morphology in *Inimicus sinensis* female 145 mm *TL*, maturity stage II–III, GSI 2.0%. Scale bar: 200  $\mu$ m.

Shimabara Bay), the spawning of this species is observed from May to August with the peak in May and June (Nozaki et al., 2003). The smallest body length (*TL*) of sexually mature *I. sinensis* females with ovaries at maturity stage IV is 120 mm. The female 145 mm *TL* with the ovaries at maturity stage II–III, most likely, matured for the first time. Therefore, a variation of the female's size at onset of sexual maturation can be supposed. The smallest body length (*TL*) of *I. japonicus* females that participated in the spawning is 190 mm; and these fishes are at the age of 3 years (Watanabe, 2006).

Similar to many other representatives of the suborder Scorpaenoidei with external insemination, *I. sinensis* males possess hypertrophied urinary bladders. The first observation of sexual dimorphism by the size of the urinary bladder is made on the males of bighead sculpins from Lake Baikal (Scorpaeniformes: Cottoidae: Cottocomephoridae). A hypertrophied urinary bladder is registered before the spawning, during the spawning, and during the protection of demersal egg clutches (Chernyaev, 1977, 1979). In particular, in bighead sculpin *Batrachocottus baicalensis* female according to Chernyaev (1979. P. 1054), "the urinary bladder is of a normal size, but this organ in the male extends to the entire body cavity, and the urinary bladder should be pricked to make the drawing of the urogen-

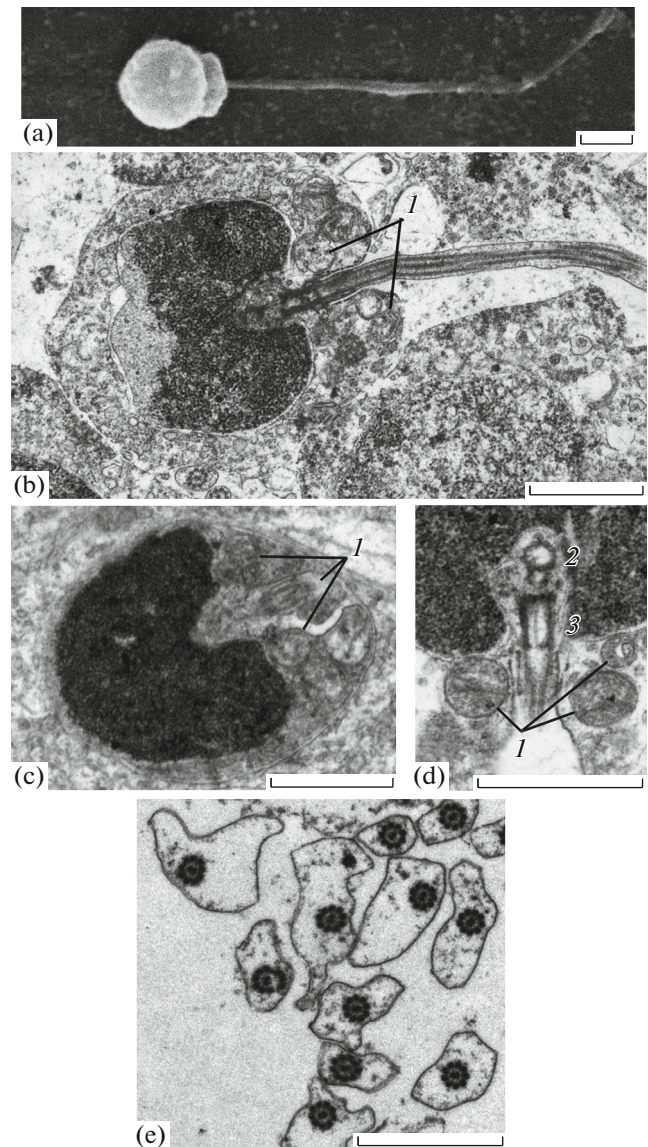
ital system." In black scorpionfish *Scorpaena porcus* (Scorpaenidae), the urinary bladder is also substantially larger in the male than in the female (Soin and Chepurnov, 1986). In subsequent studies, a hypertrophied urinary bladder is found in the males of other representatives of the family Scorpaenidae: *Scorpaenopsis possi*, *Sebastapistes cyanostigma* (Pavlov and Emel'yanova, 2007) and *Dendrochirus zebra* (Pavlov and Emel'yanova, 2010, 2019). A hypertrophied urinary bladder is also observed in *Synanceia horrida* (Synanceiidae) males (our unpublished data). The urinary bladder reaches the largest size in sexually mature individuals: a positive allometry of the growth of this organ in relation to male body length is registered in *D. zebra* (Pavlov and Emel'yanova, 2019). Spermatozoa are most often found in the urinary bladder content, and their concentration can be similar to that in the ejaculate of fishes with internal insemination. Following by activation in marine water, the progressive movements of spermatozoa from the urinary bladder are longer than those of spermatozoa obtained from the testes (Pavlov and Emel'yanova, 2007, 2010). The initial hypothesis on a possible direct connection between the urinary and genital systems in scorpaenoid males with hypertrophied urinary bladders (Pavlov and Emel'yanova, 2007, 2010, 2013) has not been supported. Despite the location of elongated





**Fig. 4.** Ultrastructure of oocyte envelope in *Inimicus sinensis*: (a) surface of ovulated oocyte; (b) envelope of oocyte filled with yolk with a part of peripheral cytoplasm; (c) fragment of peripheral cytoplasm of the oocyte filled with yolk; 1, internal layer of zona radiata; 2, external layer of zona radiata; 3, narrow outermost layer of oocyte envelope (chorion) located above the external layer of zona radiata; 4, cortical alveoli; 5, mitochondrion; 6, fragment of yolk granule; (→) vesicles of endoplasmic reticulum. Scale bar: 1  $\mu$ m.

spermiducts on the urinary bladder surface, canals connecting these organs have not been observed, and the genital pore is located at the top of the urogenital papilla close to the urinary pore. However, these pores are separated from each other (our unpublished data). A similar structure of excretory ducts is described in viviparous Bocaccio rockfish *Sebastes paucispinis* (= *Sebastes paucispinis*), and (in this species) the urinary bladder's size is similar in the female and male (Moser, 1967). Therefore, the features of spermatozoa penetration into the male's urinary bladder remain unknown. According to Chernyaev (1977, 1979), the



**Fig. 5.** Spermatozoon ultrastructure of *Inimicus sinensis*: (a) general view of the spermatozoon; (b) spermatid longitudinal section; (c) longitudinal section of spermatozoon head and midpiece; (d) a part of spermatozoon head with the centriolar complex; (e) transversal sections of the flagella; 1, mitochondrion, 2, proximal centriole; 3, distal centriole. Scale bar: 1  $\mu$ m.

excretion of hypertrophied urinary bladder of bighead sculpin males of Lake Baikal (including sperm) is important as attractants for the females and repellents for invertebrates. Based on our hypothesis (Pavlov and Emel'yanova, 2007, 2010, 2013), the urinary bladder is discharged during the insemination, and its spermatozoa can participate in fertilization of oocytes.

The spawning of *I. japonicus* is induced (under the effect of hormonal injections of gonadotropin-releasing hormone analogue and chorionic gonadotropin) in the culture conditions (Wang et al., 2013). However,

the pattern of oocyte deposition and insemination is not followed. Maximum GSI value of *I. sinensis* males is 0.4% vs. maximum GSI value of cultivated *I. japonicus* males  $\approx$  1.0% (Watanabe, 2012). A similar maximum GSI value (0.7%) is registered in *D. zebra* males (Pavlov and Emel'yanova, 2019). The spawning ritual of *D. zebra* includes a close contact between the male and female, and sperm is released inside of the clutch of oocytes embedded into gelatinous matrix (Fishelson, 1975; Moyer and Zaiser, 1981). The spawning of the representatives of the genus *Inimicus* also most likely occurs in pairs, the group of oocytes is released by a compact clutch immediately surrounded by sperm, and the clutch disintegrates into separated eggs following fertilization.

Based on size distribution of oocytes in the ovaries of sexually mature *I. sinensis* females, this species is characterized by continuous oogenesis and multiple spawning. Nevertheless, the GSI values (reaching 13.17%) are comparatively high. Maximum GSI values of *I. japonicus* females from natural and culture conditions are 23.28 and 26.97%, respectively (Watanabe, 2006). The *I. japonicus* individuals with the highest GSI values were most likely in a prespawning condition. Comparatively high GSI values of mature females of the genus *Inimicus* can be connected with large ovulated oocytes and comparatively high batch fecundity. The representatives of the family Scorpaenidae, *Sc. possi* and *S. cyanostigma*, with continuous oogenesis and multiple spawning are characterized by extremely low GSI values of the females during the spawning reaching 0.97 and 1.49%, respectively, on average (Pavlov and Emel'yanova, 2007). The average GSI value of *D. zebra* females is 4.45% (Shadrin and Emel'yanova, 2019), but the maximum value can exceed 8% (Pavlov and Emel'yanova, 2019). The seasonal periodicity of *I. sinensis* spawning is unknown, and the intervals between spawning events are most likely longer than in other scorpaenoid fishes of the tropical zone with smaller mature oocytes.

A brief description of *I. japonicus* oogenesis, including oocyte morphology in the end of vitellogenesis and at the final phases of maturation (with migrated nuclei), is given by Watanabe (2006). Unfortunately, the size of ovulated oocytes is not indicated. At the initial developmental stages, the average egg diameter of *I. japonicus* is 1.40 mm (Wang et al., 2013). The perivitelline space is extremely narrow (Wang et al., 2013, Fig. 2), and, therefore, the diameter of ovulated oocytes and egg diameter in the beginning of embryogenesis is almost the same. The diameter of the egg of *I. sinensis* with the embryo 12 h to hatching is 1.25 mm (Shadrin et al., 2003).

Despite a pelagic spawning mode in *I. sinensis*, the zona radiata width (10.2  $\mu$ m on average) is substantially larger than in other scorpaenoid fishes with the eggs developing in water mass, e.g., in *Sc. possi* (1.4  $\mu$ m), *S. cyanostigma* (0.7  $\mu$ m) (Pavlov and

Emel'yanova, 2007, 2010), and *D. zebra* (0.6–0.9  $\mu$ m) (Pavlov and Emel'yanova, 2019). A wide zona radiata in *I. sinensis* eggs is most likely connected with a protective function of comparatively large eggs developing in the water mass without gelatinous matrix. In *I. sinensis*, the zona radiata includes two layers, and it differs from the zona radiata of *Sc. possi* and *S. cyanostigma* composed of the single layer (Pavlov and Emel'yanova, 2007, 2010) and zona radiata of *D. zebra* composed of three layers (Pavlov and Emel'yanova, 2019). The outermost narrow layer of the *I. sinensis* oocyte's envelope (most likely, chorion), in addition to scorpaenoid fishes, is described in other marine fish species with pelagic eggs (Emel'yanova and Pavlov, 2012, 2014). Average pore diameter at the surface of oocyte envelope and average distance between the pores do not notably differ in scorpaenoid fishes with various size of ovulated oocytes: 0.19 and 0.75  $\mu$ m in *I. sinensis*, 0.17 and 1.04  $\mu$ m in *S. cyanostigma* (our unpublished data), 0.14 and 0.99  $\mu$ m in *D. zebra* (Pavlov and Emel'yanova, 2019).

Based on spermatozoon ultrastructure, *I. sinensis* is similar to other scorpaenoid fishes with external insemination. The *I. sinensis* spermatozoon head has a spherical shape, and its width is slightly larger than its length that is also described in other scorpaenoid fishes (Pavlov and Emel'yanova, 2018). The centriolar complex is characterized by the orthogonal position of the centrioles that is also described in *D. zebra* (Pavlov and Emel'yanova, 2018). It is important to note that a variable location of the centrioles in spermatozoa is observed in *S. horrida*: the orthogonal position or lateral displacement of the proximal centriole and its location at an acute angle to the distal centriole (Pavlov and Emel'yanova, 2018). This variation can be associated with a different degree of development of spermatozoa from spermatids. The similar changes in mutual localization of centrioles in the centriolar complex are registered in *Helicolenus dactylopterus* (Sebastidae) (Vila et al., 2010): the position of the proximal centriole in relation to the distal one becomes more orthogonal during the completion of spermatogenesis. *I. sinensis* differs from *S. horrida* in a deeper nuclear fossa (containing the centriolar complex in its cytoplasm).

In *I. sinensis*, the duplets of microtubules in the axoneme of the flagellum are electron-transparent as is observed in many other fish species. However, in several representatives of the families Clupeidae, Engraulidae, Alepocephalidae, Searcidae, and some other families, electron-dense fibrils are located in distinct microtubules, which is most likely connected with mobile properties of spermatozoa (Mattei, 1991). Based on flagellum length, *I. sinensis* does not differ from other scorpaenoid fishes, and it is similar to *D. zebra* having the shortest flagellum (Pavlov and Emel'yanova, 2018).

Based on ovarian structure, the representatives of the genus *Inimicus* do not differ from the majority of teleost fishes: oocytes develop in ovigerous lamellae attached to the dorsal side of the ovary, and ovulated oocytes are concentrated in the ovarian cavity in the ventral part of the gonad (Watanabe, 2006; this study). This gonadal structure is indicated as cystovarian type II-1 (Takano, 1989, cited by: Koya and Muñoz, 2007). Many other representatives of the suborder Scorpaenoidei with egg clutches embedded into gelatinous matrix are characterized by a unique ovarian structure. The ovigerous stroma surrounded by oocytes is located in the center of the ovary, it is attached to the ovarian wall only in the anterior part of the ovary, the internal ovarian wall and ovigerous tissues are lined with secretory cells producing gelatinous matrix, oocytes develop on peduncles, and oocytes are embedded into gelatinous matrix after ovulation (McMillan, 2007). This gonadal structure is designated as cystovarian type II-3 (Takano, 1989, cited by: Koya and Muñoz, 2007). The egg clutches of these fishes are represented by empty structures with the eggs located at the periphery; the clutches have bilobed shapes or they are spawned by two structures at the same time (Wourms, 1991; Pavlov and Emel'yanova, 2013). A similar production of gelatinous matrix is described in other teleost fish species, for example, in *Hexagrammos octogrammus* (Scorpaeniformes: Hexagrammidae) (Koya et al., 1993) and *Lophiomus setigerus* (Lophiiformes: Lophiidae) (Yoneda et al., 1998). However, these fishes possess ovaries of type II-1.

The following hypothesis is proposed to explain the variation of the scorpaenoid reproductive system. The generalized ovarian type (II-1) remained in the viviparous species of the genera *Sebastes* and *Sebastiscus*, and the type II-3 usual for other representatives of the suborder is more derived (Koya and Muñoz, 2007), which is in accordance with the phylogenetic scheme of Imamura (2004). The validity of this hypothesis is violated by the presence of ovarian type II-1 in at least the representatives of the genus *Inimicus*. Based on a recent phylogenetic scheme of the order Scorpaeniformes constructed with the use of a large number of morphological and molecular characters, the viviparous species of the genera *Sebastes* and *Sebastiscus* are the most derived (Smith et al., 2018), which is in accordance with traditional opinions on viviparity evolution of fishes (Wourms, 1991; Pavlov and Emel'yanova, 2010, 2013). Based on the phylogenetic scheme (Smith et al., 2018, Fig. 3), Synanceiidae and Scorpaenidae + Sebastidae (in traditional interpretation of these families (Smith and Wheeler, 2004)) are included into two large clades, and the second clade is interpreted as the family Scorpaenidae. Within the clade Synanceiidae, the genus *Inimicus* is characterized by ovaries of type II-1, but the representatives of the genus *Synanceia* possess demersal egg clutches embedded into gelatinous matrix (Fewings and Squire, 1999) and have ovaries of type II-3 (our

unpublished data). Within the clade Scorpaenidae, the majority of genera are characterized by ovaries of type II-3, but the genera *Helicolenus* (type II-3), *Sebastiscus*, and *Sebastes* (type II-1) form the single subclade. The species of the genus *Helicolenus* release fertilized eggs at early developmental stages followed by copulation and internal insemination: a spawning mode called zygoparity or embryoparity) (Wourms, 1991; Koya and Muñoz, 2007). Thus, based on the current phylogenetic scheme (Smith et al., 2018), parallel and independent evolutionary development (homoplasy) of the unique (for fishes) ovarian type II-3 is proposed. A possibility of this development is questionable, and none of the existing phylogenetic schemes gives a satisfactory explanation.

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#### COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interest.* The authors declare that they have no conflict of interest.

*Statement on the welfare of animals.* All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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