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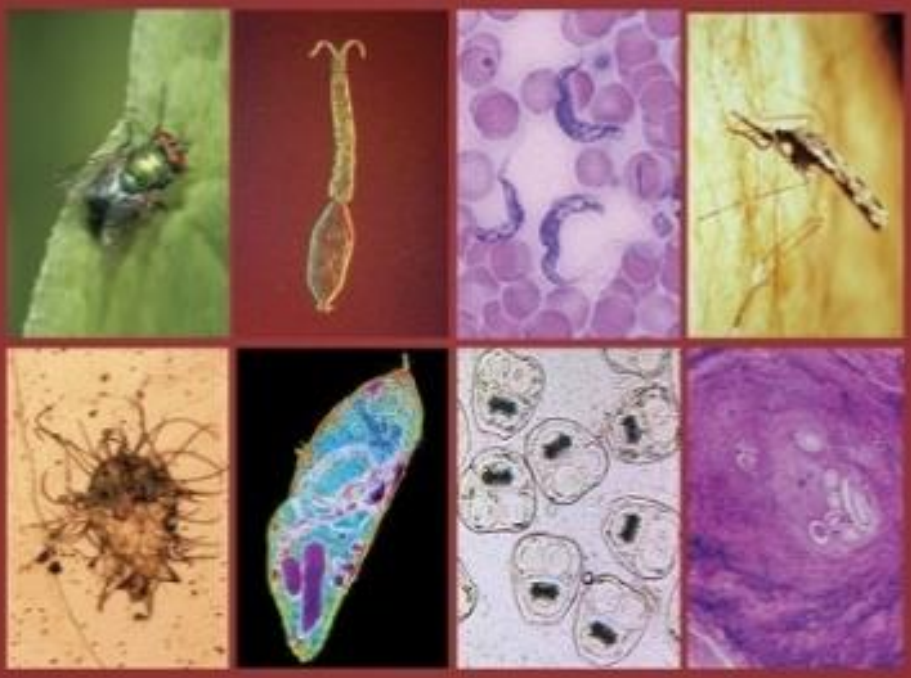

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# Light and Scanning Electron Microscopy of *Cainocreadium epinepheli* (Digenea: Opecoelidae) and *Prosorhynchus serrani* (Digenea: Bucephalidae) From Two Economically Important Serranid Fishes from the Red Sea in Egypt

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Opecoelidae, Bucephalidae, *Cainocreadium*, *Prosorhynchus*, fish.

**ABSTRACT**

The present study recorded the adult stages of two digenetic trematodes from the anterior part of the alimentary canal and pyloric caeca of two commercially important marine bony fishes belonging to the family Serranidae from the Red Sea in Egypt. They are opecoelid *Cainocreadium epinepheli* (Yamaguti, 1934) Durio and Manter, 1968 and Bucephalid *Prosorhynchus serrani* Durio and Manter, 1968 that infect areolate grouper, *Epinephelus areolatus* and yellow-edged lyretail, *Variola louti*, respectively. The incidence of infection is reported as 55.5% (5/9) for *Cainocreadium epinepheli* and 81.2 % (13/16) for *Prosorhynchus serrani*. The body of *Cainocreadium epinepheli* measured 2.003–3.667 (2.809) × 0.651–1.338 (0.986) (length × maximum width) while the body of *Prosorhynchus serrani* measured 1.664–2.210 (1.750) × 0.225–0.358 (0.287). *Cainocreadium epinepheli* is characterized by the presence of smooth tegument, median gonopore in the forebody, canalicular seminal receptacle, claviform cirrus sac, long excretory vesicle reaching forebody, pre-testicular intercaecal uterus, lobed ovary, non-filamentous eggs and has an extension of vitellaria into forebody. On the other hand, *Prosorhynchus serrani* devoids the oral and ventral suckers but has a single caecum, midbody-located mouth, conical rhynchus, pre-testicular ovary, and elongated seminal vesicle with curved proximal part of pars prostatica. The internal organs, body regions, and their ratios concerning total body length for both species were described, measured, and compared with some of the previously described species. Finally, this study postulates that it is the first time for studying surface topography using scanning electron microscopy of both species in Egypt.

## INTRODUCTION

Digenea contains more than 25,000 species and is considered the major subclass of class Trematoda within the phylum Platyhelminthes (Kostadinova and Pérez-del-Olmo, 2019). They mostly have an oral sucker, acetabulum, incomplete digestive system, and syncytial tegument that may be smooth or modified by spines, channels, or microvilli (Bogitsh *et al.*, 2019).

As adults, the digeneans inhabit all classes of vertebrates extending from fishes to humans and the majority of digenetic families infect class Osteichthyes (bony fishes), particularly marine fishes but they are less diverse in classes Agnatha (jawless fish) and Chondrichthyes (cartilaginous fish) (Roberts and Janovy, 2009; Kostadinova and Pérez-del-Olmo, 2019). In Egypt, many studies have been done on digenetic trematodes infecting fishes and terrestrial vertebrates (Nagaty, 1956; Nagaty and Abdel Aal, 1962; Saoud *et al.*, 1986; Hassanine, 2002; Mansour and Abd Elfatah, 2019; Mansour, 2019).

Genus *Cainocreadium* Nicoll, 1909 is classified in the family Opecoelidae, subfamily Hamacreadiinae (Pérez-Ponce de León and Hernández-Mena, 2019; Martin *et al.*, 2020). Opecoelidae Ozaki, 1925 contains over 90 genera, nearly 900 species, and inhabits the intestines of marine and freshwater fishes (Cribb, 2005). Opecoelids are generally characterized by smooth tegument, gonopore in the forebody, presence of oral and ventral suckers, I-shaped excretory bladder, and vitelline field occupying most of the body (Madhavi and Bray, 2018). *Hamacreadiinae* is erected by Martin *et al.* (2020*)* and inhabits piscivorous marine fishes. As listed by WoRMS (2023), Opecoelidae contains 12 subfamilies. Characters such as presence of well-developed cirrus sac, canalicular seminal receptacle, unequal fore- and hind body region, tubular excretory vesicle extending into forebody, non-filamented eggs, and intercaecal uterus between acetabulum and gonads can distinguish Hamacreadiinae from other opecoelid subfamilies (Cribb, 2005; Madhavi and Bray, 2018; Martin *et al*., 2020; Karar *et al*., 2020).

Discriminating features such as the presence of sessile ventral sucker, median gonopore, lobed ovary, non-diverticulated excretory vesicle and extension of the vitelline follicles into forebody can distinguish genus *Cainocreadium* from other genera within Hamacreadiinae (Durio and Manter, 1968a; Cribb, 2005; Andres *et al.*, 2014; Martin *et al.*, 2017; Madhavi and Bray, 2018). Based on the excretory vesicle extension, Bray and Cribb (1989) supposed the separation of *Cainocreadium* spp. into two groups; a group comprising *C. gulella, C. serrani, C. labracis* with excretory vesicle restricted to the hindbody while the other group comprising *C. lintoni, C. epinepheli and C. longisaccum* with excretory vesicle reached the forebody. Some *Cainocreadium* spp. had been reported from Red Sea fishes in Egypt such as *C. serrani* (nom. nov. for *Cainocreadoides serrani*) from *Serranus* sp. and *Lethrinus nebulosus* (Nagaty, 1956; Manter, 1963) and *C. pteroisi* (nom. nov. for *Hamacreadium pteroisi*) from the red lionfish, *Pterois volitans* (Nagaty and Abdel Aal, 1962). In addition, *C. epinepheli* has been reported from some fishes in the Arabian Gulf (Saoud *et al.*, 1986).

Genus *Prosorhynchus* Odhner, 1905 is classified within subfamily Prosorhynchinae, family Bucephalidae that can be easily distinguished by the absence of acetabulum and oral sucker, presence of anterior holdfast rhynchus, simple caecum without bifurcation, mouth located ventrally near midbody, gonopore located near posterior body region and terminal excretory pore (Overstreet and Curran, 2002; Madhavi and Bray, 2018). Characters such as the shape of rhynchus, seminal vesicle, pars prostatica, site of infection in the final host, and habitat of the fishes can discriminate the five subfamilies of family Bucephalidae (Overstreet and Curran, 2002; Nolan *et al.*, 2015). Prosorhynchinae inhabits the intestine of marine fishes (rarely freshwater) and possesses a spiny tegument, muscular or glandular infundibuliform rhynchus, elongated seminal vesicle, convoluted seminal duct and curved proximal part of pars prostatica (Madhavi and Bray, 2018). Genus *Prosorhynchus* can be differentiated from other Prosorhynchinae genera by

possessing a conical or pyriform rhynchus and pre-testicular ovary **(**Overstreet and Curran, 2002; Madhavi and Bray, 2018). More than 30 *Prosorhynchus* spp. had been recorded from serranid fishes (Bray and Justine, 2013; Truong *et al.*, 2016; Cutmore *et al.*, 2018). The present study postulates that it is the first time of studying the surface topography of *Cainocreadium* and *Prosorhynchus* species in Egypt in addition to their morphological redescription in detail.

## MATERIALS AND METHODS

### 1- Host Collection:

Nine areolate grouper, *Epinephelus areolatus* Forsskål, 1775, and sixteen yellow-edged lyretail, *Variola louti* Forsskål, 1775 were collected freshly from June 2019 to August 2020 from the fish market in the Suez area, Egypt. Fishes were transported immediately within ice cages to the Parasitology lab, Faculty of Science, Helwan University, Cairo, Egypt where they were dissected and examined. Fishes were identified according to Tesfamichael and Saeed (2016). After exiting the alimentary canal and its accessory organs for parasitic examination, the flesh of fish was used as a dietary food.

### 2- Alimentary Canal Dissection:

Fishes were dissected and the alimentary canal was removed, placed in a clean petri dish, and washed in physiological saline several times to remove debris or blood. The alimentary canal is cut into different parts in separated petri dishes to facilitate the finding of helminths and allow accurate recording of the site of infection.

### 3- Preparation for Light Microscopy:

The collected digeneans were washed in hot saline several times to remove any debris or mucous and facilitate body relaxation. Afterward, trematodes were fixed in formol acetic alcohol (85 ml ethanol absolute, 10 ml 40% formaldehyde, 5 ml acetic acid glacial) for 1-6 hours, washed in 70 % ethanol several times, and stained in acetocarmine for 5-10 hours depending on the thickness of the specimen. Stained specimens were color differentiated in weak acid-alcohol until a faint pink color was obtained, then dehydrated in an ascending ethanol series, cleared in xylene, and mounted in a correct orientation using Canada balsam on glass slides. Whole-mounted stained specimens were examined and photographed by using a Zeiss research photomicroscope (Carl Zeiss AG, Oberkochen, Germany).

### 4- Preparation for Scanning Electron Microscopy:

Digeneans were fixed in 3 % phosphate-buffered glutaraldehyde, washed in phosphate buffer, and dehydrated in an ascending series of ethanol with agitation using an automatic tissue processor (Leica Microsystems Pty Ltd, Macquarie Park, Austria). Subsequently, digenetic trematodes were dried using a $CO_2$ critical point drier (Model: Audosamdri-815; Tousimis, Rockville, MD, USA), coated by a gold sputter coater (SPI-Module). Finally, specimens were studied and photographed using scanning electron microscopy (Model: JSM-5500 LV; JEOL Ltd, Tokyo, Japan) at the Regional Centre of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

### 5- Helminths Diagnosis:

The collected digeneans were identified according to Bray and Cribb (1989), Overstreet and Curran (2002), Cribb (2005), and Madhavi and Bray (2018). All measurements are expressed, in millimeters (*mm*) *unless* otherwise mentioned, by the range (length × maximum width or width), and followed by the mean in parentheses.

## RESULTS AND DISCUSSION

### 1-Natural infection Rate:

In the present study, nine specimens of areolate grouper, *Epinephelus areolatus,* and sixteen specimens of yellow-edged lyretail, *Variola louti* were collected from the Red Sea, Egypt, and examined for the presence of digenetic trematodes. Only five fishes (55.5%) of *Epinephelus areolatus* and thirteen fishes (81.2 %) of *Variola louti* were found to be naturally infected with

adult opecoelid *Cainocreadium epinepheli* (Yamaguti, 1934) Durio and Manter, 1968a and Bucephalid *Prosorhynchus serrani* Durio and Manter, 1968b, respectively. The infections of both digeneans were found in the anterior part of the intestine within their hosts, especially the pyloric caeca. The current digenean worms were schematically drawn (Figs. A – C).

**1- Description of digenetic trematodes:**

**2.1. *Cainocreadium epinepheli* (Yamaguti, 1934) Durio and Manter, 1968a (Figs. A, 1-4, 11-21)**

**2.1.1. Light microscopy of *Cainocreadium epinepheli* (Figs. 1– 4):**

The description is based on 12 whole-mounted specimens. The body is elongated, pear-shaped, with a posterior 'teat'-like structure (Figs. 1, 4). The body length measures 2.003 – 3.667 (2.809). Maximum body width occurs in the hindbody at the anterior testicular level and measures 0.651 – 1.338 (0.986). The posterior end is broader than the anterior end. Length/maximum width (L/MW) measures 1.497 – 2.740 (2.099) while maximum width/length measures 0.422 – 0.678 (0.494).

The tegument is smooth. Oral sucker is subterminal, subspherical, and measures 0.169 – 0.282 (0.243) × 0.205 – 0.308 (0.258) (L×W) (Figs. 1, 2). Prepharynx is short and measures 0.023 – 0.063 (0.043) in length (Fig. 2). The pharynx is muscular and measures 0.114 – 0.225 (0.175) × 0.129 – 0.208 (0.176) (Figs. 1, 2). Oesophagus is long and measures 0.155 – 0.290 (0.245) × 0.087 – 0.156 (0.109) (Fig. 2). It bifurcates into two intestinal caeca that extend to the posterior end of the body (Fig. 1). Caeca end blindly and each caecum measures 0.124 – 0.133 (0.129) in width at cirrus sac level and 0.080 – 0.104 (0.092) at the testicular level (Fig. A). The ventral sucker is spherical, larger than the oral one, and measures 0.317 – 0.496 (0.421) × 0.318 – 0.498 (0.419) (Figs. 1, 11). The distance between the two suckers measures 0.523 – 1.051 (0.739) and represents 22 – 31% (27%) of the total body length. Oral sucker/acetabulum length measures 0.455 – 0.662 (0.577) while oral sucker/acetabulum width measures 0.546 – 0.651 (0.586). The cirrus sac is muscular, clavate, and measures 0.410 – 0.583 (0.497) × 0.112 – 0.172 (0.149). It represents 12 – 18% (16%) of the entire body length and never extends to the hindbody (Fig. A). The forebody measures 0.765 – 1.310 (0.988) and constitutes 33 – 39 % (35%) of total body length while the hindbody measures 1.044 – 1.972 (1.506) and constitutes 46 – 57% (52%) of total body length. The vitelline follicles are extensive and extend from the post-testicular level to the pharyngeal level (1 worm), intestinal bifurcation (6 worms), and oesophageal level (5 worms) (Figs. 1, 2, 4).

The ovary is lobulated with 4 – 5 lobes, sinistral, located anterolateral to the anterior testis, and entirely measures 0.116 – 0.254 (0.167) × 0.162 – 0.267 (0.224) (Figs. 1, 3). The canalicular seminal receptacle is oval, entirely measuring 0.235 – 0.301 (0.268) × 0.146 – 0.193 (0.169), and located posterior to the ovary (Fig. A). The uterus is intercaecal, pre-testicular, and extends anteriorly to the ventral sucker level (Fig. 1). The uterine eggs are oval, without filaments, and measure 0.063 – 0.080 (0.069) × 0.036 – 0.047 (0.041) (Fig. A). The two testes are oblique, spherical to oval shape, and smooth to slightly lobed (Figs. 1, 3). The pre-testicular distance measures 1.172 – 2.056 (1.682) and represents 55 – 64 % (60%) of the entire body length, while the post-testicular distance measures 0.287 – 0.697 (0.476) and represents 16 – 22% (18%) of total body length. The anterior testis measures 0.262 – 0.473 (0.356) × 0.264 – 0.452 (0.353), while the posterior testis measures 0.281 – 0.541 (0.389) × 0.277 – 0.484 (0.363). The gonopore is median and located posterior to the intestinal bifurcation (Fig. A). The excretory vesicle seen in unstained specimens passes between the two testes and extends into the forebody. The excretory pore is terminal (Fig. 4).

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**2.1.2. Scanning electron microscopy of *Cainocreadium epinepheli* (Figs. 11– 21):**

At the ultrastructure level, the ventral body surface is smooth without any spines or scales (Figs. 11 – 16). The mouth opening is sub-terminal (Figs. 11 – 13). The tegument surrounding the oral sucker is provided with aggregations of papillae-like structures that are irregular in shape (Fig. 13). The ventral sucker is protuberant, rounded with a thick rim, devoid of any spines and bearing two domed papillae on its left lower margin (Figs. 11, 14). The gonopore is medially located between oral and ventral suckers (Figs. 11, 12, 15). The tegument surrounding the gonopore is smooth, devoid of spines but provided with three domed papillae (Fig. 15). The papillae are smooth, rounded in shape, unequal in size, and located anterior to the gonopore. Some specimens were observed with non-spinous and partially everted cirrus extended from the gonopore (Fig. 16).

At the dorsal surface of the worm (Figs. 17 – 21), the tegument is smooth and provided with different distributed minute papillae. The lowest concentration of these papillae had been found at the tegument dorsal to the oral sucker (Fig. 18) and at the posterior third of the body (Fig. 20) while the highest concentration had been noticed in the area dorsal to gonopore, ventral sucker, and excretory pore (Figs. 17, 19, 21), respectively. These papillae are spherical and randomly distributed. Two-minute pores were observed at the tegument dorsal to the oral sucker (Fig. 18).

**2.2. *Prosorhynchus serrani* Durio and Manter, 1968b (Figs. B, C, 5 – 10, 22 – 33):**

**2.2.1. Light microscopy of *Prosorhynchus serrani* (Figs. 5 – 10):**

The description is based on 10 whole-mount specimens. The body is elongated, and slender, showing the widest width at seminal vesicle level (Fig. 5) and measures 1.664 – 2.210 (1.750) × 0.225 – 0.358 (0.287) (L × MW). The anterior end of the body is provided with a simple, conical rhynchus, which measures 0.158 – 0.207 (0.184) × 0.089 – 0.117 (0.101) (Figs. 5, 6, 22). The rhynchus represents 9 – 17 % (11%) of total body length. The mouth opening is located at the mid-body (Fig. B), and the pre-oral distance represents 49 – 53 (51%) concerning total body length. Caecum is single (Fig. B), ovoid, extending anteriorly, and measures 0.182 – 0.249 (0.215) × 0.051 – 0.130 (0.092) (Fig. 8). It occupies 10 – 13 % (11%) of body length.

The two testes are tandem and post-ovarian. The anterior testis ranges from round to subspherical and measures 0.104 – 0.130 (0.120) × 0.103 – 0.196 (0.144) (Figs. 5, 7). The posterior testis is located at the level of the anterior end of the cirrus sac and measures 0.074 – 0.148 (0.117) × **0.0**80 – 0.171 (0.116) (Figs. 5, 7). The pre-testicular distance measures 0.951 – 1.182 (1.035) and represents 43 – 61 % (56 %) of total body length while the post-testicular distance measures 0.334 – 0.638 (0.481) and represents 19 – 34 % (27 %) in relation to whole body length. The cirrus sac is straight, sinistral, and located at the posterior end of the body. It extends anteriorly reaching or overlapping the posterior testis (Figs. 5, 7). It measures 0.323 – 0.464 (0.389) × 0.068 – 0.151 (0.100) and occupies 18 – 26 % (21 %) of the body length. The cirrus sac contains a seminal vesicle joining with the male duct (Fig. C). The seminal vesicle is elongated, locates at the proximal end of the cirrus sac, measures 0.123 – 0.184 (0.152) × 0.038 – 0.067 (0.046), and represents 32 – 39% (35%) of the total length of the cirrus sac. The male duct consists of a pars prostatica and an ejaculatory duct (Fig. C). Pars prostatica measures 0.280 – 0.391 (0.321) × 0.047 – 0.058 (0.053) and is divided into two parts. The proximal part is narrow, curved proximally to form a convoluted seminal duct and joins with the seminal vesicle's posterior end (Figs. 7, 9). The distal part of the pars prostatica is long and wide (Figs. 9, C). The ejaculatory duct is short and opens inside the genital atrium.

The genital atrium contains the genital lobe (Fig. C).

The ovary is pre-testicular, spherical to ovoid, contiguous with the anterior testis, and measures 0.092 – 0.112 (0.101) × 0.075 – 0.107 (0.089) (Figs. 5, 7). The pre-ovarian distance measures 0.846 – 1.074 (0.965) and represents 42 – 56 % (51 %) of the total body length. The uterus extends from the posterior end of body to the anterior of vitelline follicles (Figs. 5, 7). The pre-uterine distance measures 0.351 – 0.698 (0.519) and represents 21 – 32 % (28 %) of body length (Fig. B). The vitellaria consist of two lateral fields of follicles extending anteriorly below rhynchus and posteriorly to the ovarian level (Figs. 5, 8). The number of follicles at the right side is 12 – 13 while at the left side is 14 – 16. The pre-vitelline distance measures 0.443 – 0.667 (0.543) and represents 25 – 31 % (29 %) of total body length, while the post-vitelline distance measures 0.812 – 0.910 (0.862) and constitutes 42 – 50 % (47 %). The uterine eggs are operculated, numerous, and measure 0.020 – 0.025 (0.023) × 0.015 – 0.018 (0.017) (Figs. 10, 33).

**2.2.2. Scanning electron microscopy of *Prosorhynchus serrani* (Figs. 22-33):**

At the ultrastructural level, the dorsal surface of rhynchus (Figs. 22-24), dorsal body surface (Fig. 25), and ventral surface of the rhynchus (Figs. 26-28), ventral body surface (Figs. 29- 32) and the egg (Fig. 33) were analyzed and photographed using scanning electron microscopy. The dorsal and ventral tegumental surfaces of the rhynchus were provided with nearly circular scales with different measurements. The anterior scales on the dorsal part of rhynchus measure 2.06 – 3.11 (2.55) × 2.09 – 3.01 (2.61) µm, larger than the posterior one (Fig. 23), and the distance between two successive scales on transverse raw measures 4.50 – 5.33 (4.92) µm. The scales at the posterior part of the rhynchus are similar in shape to the anterior ones and measure 1.29 – 2.16 (1.80) × 1.67 – 2.04 (1.86) µm, and some pores are seen between them (Fig. 24). The distance between two successive scales in a transverse row in this region measures 4.13 – 5.09 (4.64) µm. At the anterior half of the body on the dorsal surface, the tegument is provided with ellipsoidal scales, wider than longer measuring 1.14 – 1.52 (1.38) × 2.89 – 3.16 (3.02) µm (Fig. 25), and the distance between the two adjacent scales in the transverse row measures 5.80 – 6.34 (6.05) µm in this region.

The ventral body surface is covered entirely with scales (Figs. 26-32). The shape of scales on the ventral surface of the rhynchus is similar to those in the dorsal one (Figs. 23, 24, 27, 28). The scales below the rhynchus are ellipsoidal, wider than longer and measure 1.42 – 1.72 (1.55) × 2.42 – 3.06 (2.77) µm (Fig. 29), and the distance between two adjacent scales in the transverse row measures 2.87 – 3.43 (3.11) µm (Fig. 29). Both rims of these scales are provided with up to 13 fingers like projections (Fig. 30). The mouth opening locates near mid-body but slightly shifted to the left (Fig. 31) and measures 3.9 × 20 µm. The scales below the mouth measure 1.5 × 2.2 µm. The gonopore is slit-shaped and measures 15.3 µm in width (Fig. 32). The excretory pore is terminal, round, and measures 11 – 16 (14) × 12 – 12.3 (12) µm (Fig. 32). The scales around the excretory opening measure 1.1 × *2* µm and the distance between the gonopore and the excretory opening measures 96 – 128 (108) µm. The eggs are oval and provided with an operculum that measures 5.97 × 7.22 µm. The egg surface is rough and carries many irregular shape projections (Fig. 33).

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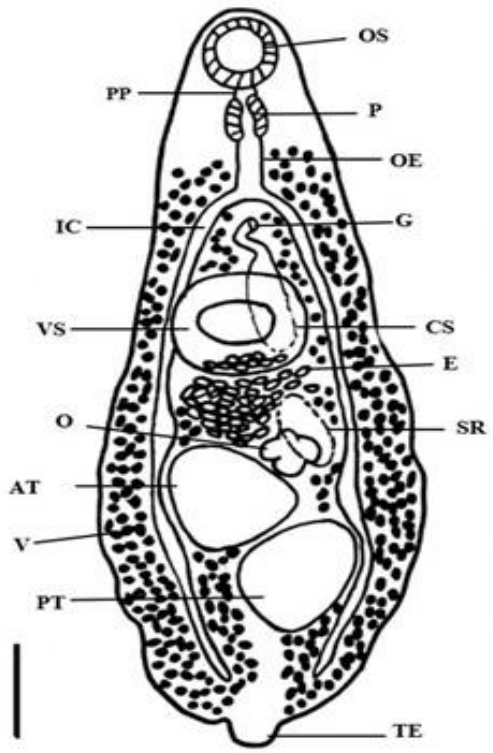



**Fig.** A: Diagram of the present *Cainocreadium epinepheli*. Ventral view. Abbreviations: AT, anterior testis; CS, cirrus sac; E, uterine egg; IC, intestinal caecum; G, gonopore; O, ovary; OE, oesophagus; OS, oral sucker; P, pharynx; PP, prepharynx; PT, posterior testis; SR, seminal receptacle; TE, teat-like structure; V, vitellaria; VS, ventral sucker. Scale bar = 0.3 mm.

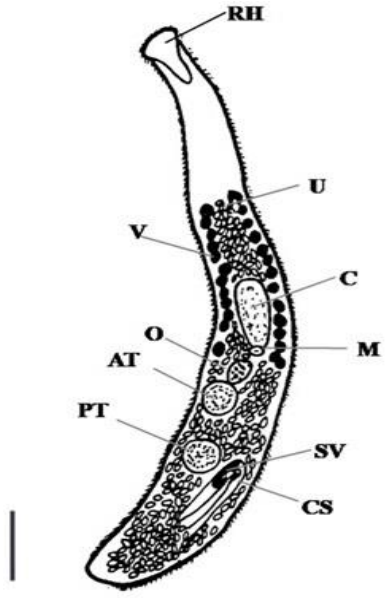



**Fig. B:** Diagram of whole ventral view of the present *Prosorhynchus serrani*. Abbreviations: AT, anterior testis; C, caecum; CS, cirrus sac; M, mouth; O, ovary; PT, posterior testis; RH, rhynchus; SV, seminal vesicle; U, uterus; V, vitellaria. Scale bar = 0.2 mm.

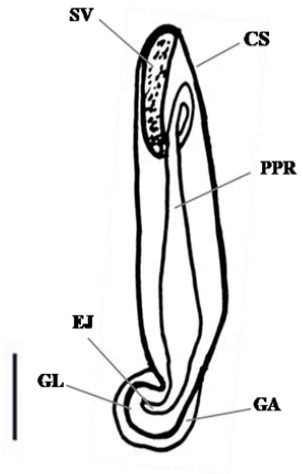
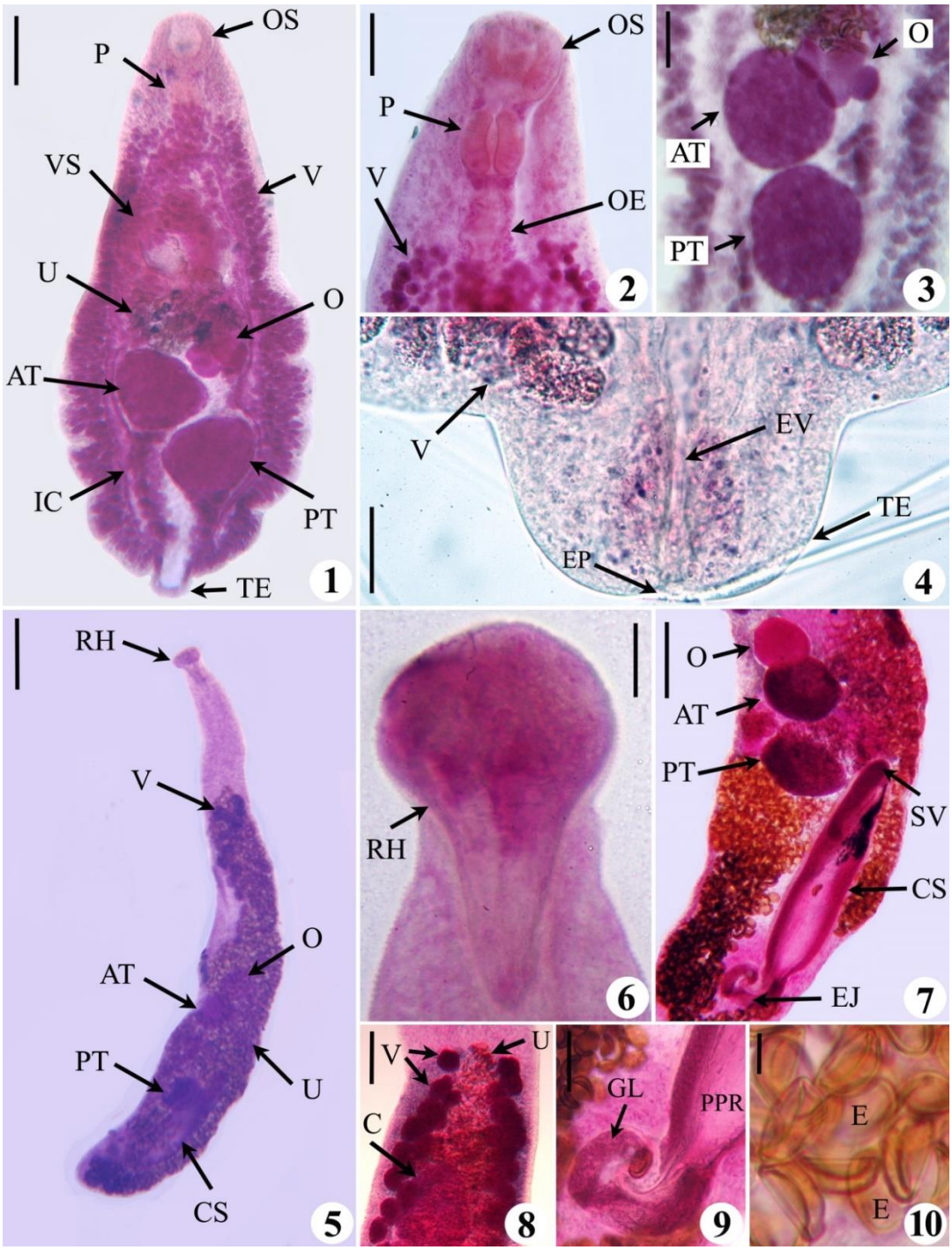



**Fig. C:** Diagram of Cirrus sac and genital atrium of the present *Prosorhynchus serrani*. Abbreviations: CS, cirrus sac; EJ, ejaculatory duct; GA, genital atrium; GL, genital lobe; PPR, pars prostatica; SV, seminal vesicle. Scale bar = 0.15 mm.




**Figs.** (1-10): Photomicrographs of *Cainocreadium epinepheli* (Figs. 1-4) and *Prosorhynchus serrani* (Figs. 5-10). Fig. 1: Whole worm of *Cainocreadium epinepheli*. Fig. 2: Anterior end of the worm from oral sucker to beginning of vitellaria. Fig. 3: Body region from the end of the uterus to posterior testis. Fig. 4: Posterior end of the body showing teat-like structure and excretory vesicle. Fig. 5: Whole worm of *Prosorhynchus serrani*. Fig. 6: Anterior end of body showing the rhynchus. Fig. 7: Body region from just before the ovary to the ejaculatory duct. Fig. 8: Body region showing anterior end of vitellaria, uterus, and caecum. Fig. 9: Posterior part of cirrus sac. Fig. 10: Uterine eggs. Abbreviations: AT, anterior testis; C, caecum; CS, cirrus sac; E, uterine egg; EJ, ejaculatory duct; EP, excretory pore; EV, excretory vesicle; GL, genital lobe; IC, intestinal caecum; O, ovary; OE, oesophagus; OS, oral sucker; P, pharynx; PPR, pars prostatica; PT, posterior testis; RH, rhynchus; SV, seminal vesicle; TE, teat-like structure; U, uterus; V, vitellaria; VS, ventral sucker. Scale bar (Fig. 1) = 0.3 mm, (Figs. 2, 3, 5) = 0.2 mm, (Figs. 4, 9) = 50 µm, (Fig. 6) =15 µm, (Fig.7) = 0.15 mm, (Fig. 8) = 0.1 mm, (Fig. 10) = 10 µm.

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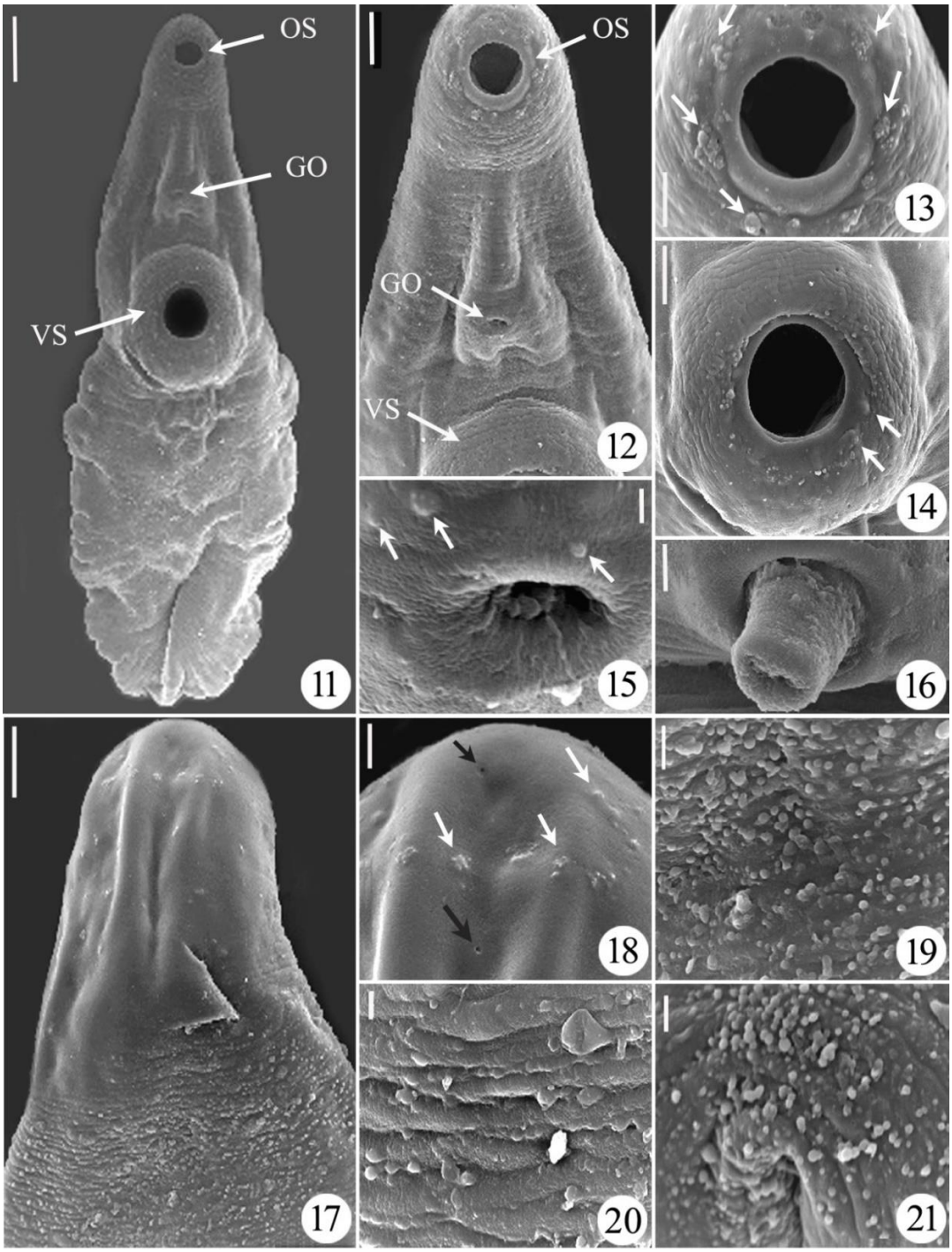
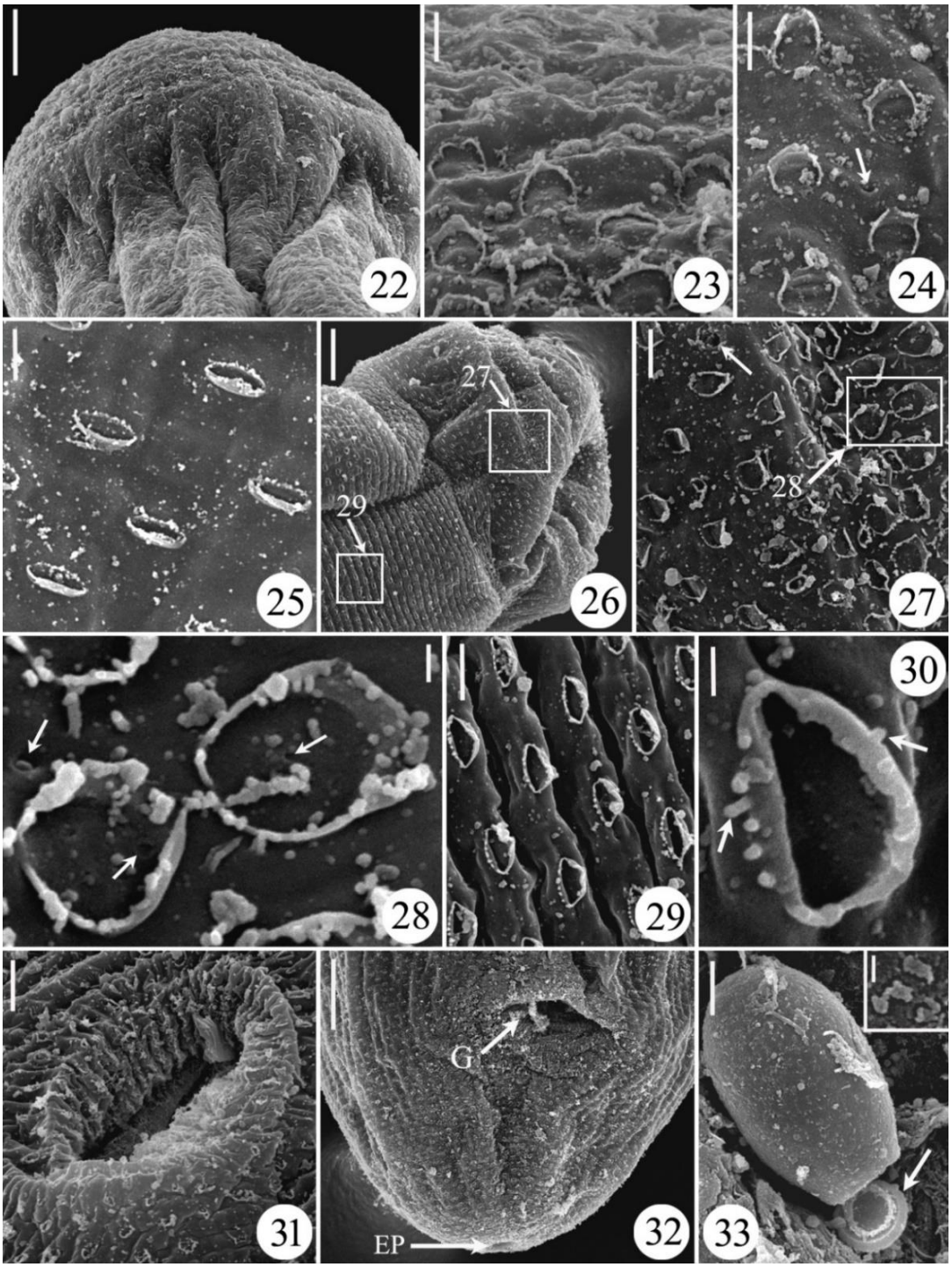



**Figs.** (11-21): Scanning electron micrographs of ventral (Figs. 11-16) and dorsal surfaces (Figs. 17-21) of the present *Cainocreadium epinepheli*. Fig. 11: Whole worm. Fig. 12: Anterior part of the worm from the oral sucker to the anterior rim of ventral sucker. Fig. 13: Oral sucker with clusters of papilla- like structures (arrows). Fig. 14: Ventral sucker with two papilla-like structures on its left lower margin (arrows). Fig. 15: Gonopore without everted cirrus. Note the presence of rounded shaped papilla- like structures (arrows). Fig.16: Gonopore with everted cirrus (arrow). Fig. 17: Dorsal anterior quarter of the worm showing different papillae density patterns along the body surface. Fig. 18: Anterior end of the worm dorsal to oral sucker with few papilla-like structures (white arrows) and minute pores (black arrows). Figs. (19-21): Different distribution pattern of papilla-like structures in body region dorsal to gonopore and ventral sucker region (Fig. 19), in posterior third of body (Fig. 20) and dorsal to excretory pore (Fig. 21). Abbreviations: G, gonopore; OS, oral sucker; VS, ventral sucker. Scale bar (Fig. 11) = 250 µm, (Figs. 12, 14, 17) = 100 µm, (Figs. 13, 19 and 21) = 50 µm, (Figs. 15, 20) = 10 µm, (Figs. 17, 19) = 25 µm.




**Figs.** (22-33): Scanning electron micrographs of the dorsal (Figs. 22-26), ventral (Figs. 27-33) surfaces of *Prosorhynchus serrani*, and the eggs (Fig. 33). Fig. 22: Dorsal view of the bulbous rhynchus. Fig. 23: Scales pattern at the anterior part of rhynchus. Fig. 24: Circular scales and pore (arrow) on the posterior part of rhynchus. Fig. 25: Slit-like scales at the anterior half of the body. Fig. 26: Ventral view of rhynchus. Two squares mark for the areas magnified in figures 27 and 29. Fig. 27: Scales from the middle area of rhynchus. Fig. 28: Two magnified neighboring scales from figure 27. Note the presence of pores (arrows). Fig. 29: Scales in the area just below rhynchus. Fig. 30: A magnified scale from figure 29 showing the presence of small projections from both scale rims (arrows). Fig. 31: Mouth opening. Fig. 32: Posterior end of the worm from gonopore to excretory pore. Fig. 33: Egg with opened operculum (arrow). The *inset* represents a part of the topography of the egg surface. Abbreviations: G, gonopore; EP, excretory pore. Scale bar (Figs. 22, 26, 32) = 20 µm, (Figs. 23, 24) = 2 µm, (Fig. 25) = 2.5 µm, (Figs. 27, 29) = 3 µm, (Figs. 28, 30, 33 inset) = 0.5 µm, (Figs. 31, 33) = 5 µm.

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**Table 1:** Measurements of the present *Cainocreadium epinepheli* and some recorded *Cainocreadium* species from fishes.

| Species | *C. lintoni* | *C. longisaccum* | *C. alanwilliamsi* | *C. labracis* | *C. dentecis* | *C. epinepheli* | *C. epinepheli* | *C. epinepheli* |
|---|---|---|---|---|---|---|---|---|
| **Hosts** | *Epinephelus striatus, Cephalopholis fulva* | *E. adscensionis* | *Pseudorhombus jenynsi* | *Dicentrarchus labrax* | *Dentex dentex* | *Lethrinus nebulosus, E. tauvina, E. chlorostigma, E. areolatus, E. summana* | *E. quoyanus, E. merra* | *E. areolatus* |
| **Locality** | Puerto Rico | Puerto Rico | Australia | North-western Mediterranean | North-western Mediterranean | Arabian Gulf | Australia | Red Sea |
| **References** | Siddiqi and Cable (1960) | Siddiqi and Cable (1960) | Bray (1990) | Jousson and Bartoli (2001) | Jousson and Bartoli (2001) | Saoud *et al.*, (1986) | Bray and Cribb (1989) | Present study |
| **Body (L×W)** | 3.502 – 5.822 × 0.734 – 1.034* | 2.050 – 2.870 × 0.490 – 0.812 | 1.640 – 2.600 × 0.370 – 0.610 | 3.400 – 7.055 × 1.020 – 2.125 * | 2.253 – 4.781 × 0.710 – 1.806 * | 2.28 – 7.3× 0.9 – 2.4 * | 2.600 – 3.928 × 0.602 – 1.008 | 2.003 – 3.667 × 0.651 – 1.338 |
| **Oral sucker (L×W)** | 0.180 – 0.273 × 0.247 – 0.287 | 0.154 – 0.196 (width only) | 0.170 – 0.220 × 0.155 – 0.210 | 0.314 – 0.666 × 0.336 – 0.744 | 0.189 – 0.341 × 0.213 – 0.352 | 0.200 – 0.570 × 0.200 – 0.640 | 0.198 – 0.266 × 0.218 – 0.314 | 0.169 – 0.282 × 0.205 – 0.308 |
| **Acetabulum (L×W)** | 0.380 – 0.474 (width only) | 0.231 – 0.366 × 0.196 – 0.273 | 0.235 – 0.330 × 0.225 – 0.310 | 0.437 – 1.009 × 0.544 – 1.275 | 0.213 – 0.533 × 0.304 – 0.533 | 0.310 – 1.070 × 0.340 – 1.020 | 0.326 – 0.458 × 0.338 – 0.461 | 0.317 – 0.496 × 0.318 – 0.498 |
| **Prepharynx (L)** | – | – | – | 0.016 – 0.149 | 0 – 0.091 | 0.020 – 0.080 | – | 0.023 – 0.063 |
| **Pharynx (L×W)** | 0.147 – 0.200 (width only) | 0.105 – 0.140 × 0.126 – 0.154 | 0.090 – 0.155 × 0.100 – 0.140 | 0.192 – 0.448 × 0.144 – 0.480 | 0.112 – 0.240 × 0.107 – 0.213 | 0.210 – 0.540 × 0.310 – 0.650 | 0.128 – 0.186 × 0.118 – 0.165 | 0.114 – 0.225 × 0.129 – 0.208 |
| **Oesophagus (L)** | – | 0.070 – 0.087 | 0.040 – 0.070 | 0.053 – 0.240 | 0.027– 0.267 | 0.110 – 0.850 | 0.099 – 0.134 | 0.155 – 0.290 |
| **Fore body %** | – | – | 20 – 30 | – | – | – | 32 – 36 | 33 – 39 |
| **Anterior testis (L×W)** | 0.343 – 0.454 × 0.327 – 0.427 | 0.175 – 0.238 (width only) | 0.195 – 0.280 × 0.135 – 0.195 | 0.373 – 0.640 × 0.346 – 0.906 | 0.320 – 0.746 × 0.267 – 0.650 | 0.240 – 1.070 × 0.280 – 0.850 | 0.251 – 0.326 × 0.229 – 0.298 | 0.262 – 0.473 × 0.264 – 0.452 |
| **Posterior testis (L×W)** | 0.343 – 0.454 × 0.327 – 0.427 | 0.175 – 0.238 (width only) | 0.185 – 0.365 × 0.125 – 0.190 | 0.405 – 0.746 × 0.267 – 0.906 | 0.373 – 0.800 × 0.277 – 0.692 | 0.280 – 1.080 × 0.280 – 0.920 | 0.285 – 0.394 × 0.152 – 0.275 | 0.281 – 0.541 × 0.277 – 0.484 |
| **Cirrus sac (L×W)** | – | – | 0.320 – 0.540 × 0.085 – 0.160 | 0.533 – 1.450 × 0.107 – 0.400 | 0.373 – 1.103 × 0.107 – 0.240 | 0. 700 – 1.280 × 0.210 – 0.280 | 0.470 – 0.774 × 0.154 – 0.218 | 0.410 – 0.583 × 0.112 – 0.172 |
| **Ovary (L×W)** | 0.280 – 0.287 × 0.347 – 0.367 | 0.091 – 0.161 × 0.098 – 0.231 | 0.145 – 0.210 × 0.160 – 0.230 | 0.235 – 0.586 × 0.213 – 0.666 | 0.176 – 0.480 × 0.181– 0.480 | 0.170 – 0.470 × 0.140 – 0.480 | 0.195 – 0.275 × 0.205 – 0.288 | 0.116 – 0.254 × 0.162 – 0.267 |
| **Ovarian lobes** | – | 3 – 5 | 4 | 3 – 5 | 3 | 3 – 6 | 3 – 4 | 4 – 5 |
| **Eggs (L×W)** | 0.063 – 0.072 × 0.037 – 0.042 | 0.054 – 0.069 × 0.033 – 0.048 | 0.076 – 0.092 × 0.041 – 0.052 | 0.065 – 0.087 × 0.033 – 0.048 | 0.059 – 0.074 × 0.029 – 0.043 | 0.042 – 0.089 × 0.017– 0.049 | 0.064 – 0.078 × 0.035 – 0.045 | 0.063 – 0.080 × 0.036 – 0.047 |

**Abbreviations:** L, length; W, width; * refers to maximum width.

**Table 2:** Comparison between hosts, distribution, and organ measurements of different *Prosorhynchus* spp. from some *epinepheline serranids.*

| Species | *P. maternus* | *P. conorjonesi* | *P. jexi* | *P. wrightae* | *P. tonkinensis* | *P. longisaccatus* | *P. serrani* | *P. serrani* | *P. serrani* |
|---|---|---|---|---|---|---|---|---|---|
| **Host** | *Epinephelus malabaricus* | *Cromileptes altivelis* | *E. quoyanus* | *Plectropomus leopardus* | *E. coioides* | *E. areolatus* | *Variola albimarginata* | *V. louti* | *V. louti* |
| **Locality** | New Caledonia | Australia | Australia | Australia | Vietnam | New Caledonia | New Caledonia | New Caledonia | Egypt |
| **References** | Bray and Justine, (2006) | Bott and Cribb, (2009) | Bott and Cribb, (2009) | Bott *et al.* (2013) | Truong *et al.* (2016) | Bray and Justine, (2013) | Bray and Justine, (2013) | Bray and Justine, (2013) | Present study |
| **Body (L×W)** | 2.052 –2.227 × 0.392 – 0.476 | 1.904 – 3.360 × 0.112 – 0 224 | 1.104 –1.424 × 0.208 – 0.352 | 0.800 – 1.088 × 0.128 – 0.224 | 0.830 – 1.247 × 0.367 – 0.519 | 0.639 – 1.203 × 0.185 –0.382 | 1.322 × 0.253 | 1.163 – 2.321 × 0.169 – 0.311 | 1.664 – 2.210 × 0.225 – 0.358 |
| **Rhynchus (L×W)** | 0.280 – 0. 360 × 0.189 – 0.243 | 0.177 – 0.305 × 0.112 – 0.161 | 0.167 – 0.212 × 0.128 – 0.151 | 0.051 – 0.077 × 0.059 – 0.083 | 0.240 – 0.363 × 0.180 – 0.279 | 0.146 – 0.262 × 0.135 – 0.300 | 0.177 × 0.141 | 0.147 – 0.247 × 0.102 – 0.139 | 0.158 – 0.207 × 0.089 – 0.117 |
| **Caecum (L×W)** | 0.202 – 0.231× 0.134 – 0.178 | 0.193 – 0.321 | 0.145 – 0.202 | 0.080 – 0.114 | 0.159 – 0.245 × 0.078 – 0.151 | 0.101 – 0.164 × 0.065 – 0.134 | 0.177 × 0.101 | 0 – 0.327 × 0.060 – 0.121 | 0.182 – 0.249 × 0.051 – 0.130 |
| **Pharynx (L×W)** | 0.058 – 0.101 × 0.069 – 0.100 | 0.058 –0.090 × 0.061 – 0.087 | 0.048 – 0.071 × 0.048 – 0.071 | 0.032 – 0.048 × 0.039 – 0.045 | 0.057 – 0.100 × 0.046 – 0.102 | 0.044 – 0.072 × 0.049 –0.078 | 0 | 0 – 0.078 × 0 – 0.076 | 0.046 – 0.049 × 0.049 – 0.054 |
| **Ovary (L×W)** | 0.127 – 0.153 × 0.098 – 0.131 | 0.061 – 0.116 × 0.061 – 0.119 | 0.058 – 0.090 × 0.058 – 0.109 | 0.085 – 0.096 × 0.088 – 0.090 | 0.096 – 0.137 × 0.084 – 0.131 | 0.054 – 0.112 × 0.034 – 0.124 | 0.107 × 0.081 | 0.077 – 0.134 × 0.063 – 0.128 | 0.092 – 0.112 × 0.075 – 0.107 |
| **Ant. testis (L ×W)** | 0.152 – 0.188 × 0.124 – 0.160 | 0.083 – 0.119 × 0.077 – 0.128 | 0.077 – 0.118 × 0.071 – 0.148 | 0.091 – 0.098 × 0.078 – 0.098 | 0.125 – 0.173 × 0.106 – 0.161 | 0.059 – 0.147 × 0.051 – 0.133 | 0.126 × 0.108 | 0.084 – 0.178 × 0.076 – 0.162 | 0.104 – 0.130 × 0.103 – 0.196 |
| **Post. testis (L×W)** | 0.152 – 0.191 × 0.117 – 0.148 | 0.083 – 0.122 × 0.074 – 0.128 | 0.074 – 0.128 × 0.077 – 0.173 | 0.088 – 0.096 × 0.074 – 0.081 | 0.115 – 0.179 × 0.128 – 0.182 | 0.058 – 0.141 × 0.048 – 0.149 | 0.138 ×0.109 | 0.072 – 0.168 × 0.070 – 0.138 | 0.074 – 0.148 × 0.080 – 0.171 |
| **Cirrus-sac (L×W)** | 0.468 – 0.541 × 0.091 – 0.116 | 0.376 – 0.565 | 0.321 – 0.482 | 0.212 – 0.262 | 0.351 – 0.457 × 0.101 – 0.159 | 0.206 – 0.439 × 0.084 – 0.178 | 0.338 × 0.080 | 0.222 – 0.359 × 0.077 – 0.135 | 0.323 – 0.464 × 0.068 – 0.151 |
| **S.V. (L×W)** | 0.198 – 0.275 × 0.061 – 0.088 | 0.138 – 0.189 × 0.048 – 0.071 | 0.087 – 0.209 × 0.039 – 0.119 | 0.049 – 0.077 × 0.026 – 0.049 | 0.107 – 0.206 × 0.032 – 0.054 | 0.083 – 0.150 × 0.027 – 0.054 | 0 | 0 – 0.387 × 0 – 0.087 | 0.123 – 0.184 × 0.038 – 0.067 |
| **PPR (L×W)** | 0.181 – 0.291 × 0.049 – 0.090 | 0.450 – 0.706 | 0.321 – 0.562 | 0.128 – 0.179 | 0.185 – 0.297 × 0.052 – 0.084 | 0.243 – 0.655 × 0.043 – 0.083 | 0.177 × 0.141 | 0.084 – 0.178 × 0.076 – 0.162 | 0.280 – 0.391× 0.047 – 0.058 |
| **Eggs (L×W)** | 27–28 × 14–22 | 31–32 × 16 | 32–33 × 16 | 20–24 × 12–13 | 32–36 x 19–22 | 26–36 × 17–21 | 0.024 × 0.013 | 0.024 – 0.033 × 0.015 – 0.022 | 0.020 – 0.025 × 0.015 – 0.018 |

Abbreviations: ant., anterior; L, length; PPR, Pars Prostatica; post., posterior; S.V.; Seminal vesicle; W., Width.

***1-Cainocreadium epinepheli* (Yamaguti 1934) Durio and Manter, 1968a:**

In his revision on the family Opecoelidae, Cribb (2005) reported eleven species within the genus *Cainocreadium*. Martin *et al.*, (2017) transferred three species that were originally described in the genera *Hamacreadium*, *Podocotyle* to that genus. The measurements of *C. epinepheli* recognized by Saoud *et al.* (1986) from the Arabian Gulf are larger than the same species recorded from Australia (Bray and Cribb, 1989) and the present species from the Red Sea in Egypt (Table 1). Previous studies and the present investigation recorded *C. epinepheli* from areolate grouper, *Epinephelus areolatus* beside other hosts (Saoud *et al.*, 1986; Bray and Cribb, 1989). The measurements and body ratios of the current *Cainocreadium*

*epinepheli* are compared with some previously recorded *Cainocreadium spp.* (Table 1).

The current *Cainocreadium epinepheli* is tapered anteriorly and broad posteriorly with a teat-like structure. Although *C. alanwilliamsi* has a posterior teat-like tail, it differs from the present species in that the excretory vesicle extends anteriorly reaching ventral sucker level or into the forebody. In addition, it has a shorter forebody, oesophagus, and slightly larger eggs (Bray, 1990) (Table 1). Furthermore, the body is tapered toward both ends in *C. lintoni* and *C. serrani*, however, it is tapered posteriorly with a particular narrow posterior end in *C. pteroisi* (Nagaty, 1956; Siddiqi and Cable, 1960; Nagaty and Abdel Aal, 1962). Moreover, the body shape is elongated with rounded ends in *C. musculometra* and *C. longisaccum* (Bravo-Hollis and Manter, 1957; Siddiqi and Cable, 1960). *C. epinepheli* under investigation has maximum body width in the hind body region at the anterior testicular level. On the contrary, maximum width occurs at the ventral sucker level in *C. lintoni* and *C. labracis* (Siddiqi and Cable, 1960; Jousson and Bartoli, 2001). Besides, the body is equally broad in *C. musculometra* (Bravo-Hollis and Manter, 1957).

The current *C. epinepheli* has extensive vitelline follicles from the hindbody to the forebody. Similar observations were reported in many *Cainocreadium* spp. (Nagaty, 1956; Siddiqi and Cable, 1960; Nagaty and Abdel Aal, 1962; Schroeder, 1970; Bray, 1990; Korniychuk and Gaevskaya, 2000; Jousson and Bartoli, 2001). On the other hand, *C. oscitans* and *C. musculometra* had the vitelline follicles restricted only to the hindbody (Manter, 1940; Bravo-Hollis and Manter, 1957). In agreement with the present study, Bray and Cribb (1989) reported a pre-testicular uterus that extends from the anterior testis to the ventral sucker. A similar observation was reported in *C. dentecis* (Jousson and Bartoli, 2001). However, the uterus was located pre-ovarian in *C. serrani*, *C. lintoni*, *C. longisaccum*, *C. pteroisi*, *C. alanwilliamsi*, and *C. labracis* (Nagaty, 1956; Siddiqi and Cable, 1960; Nagaty and Abdel Aal, 1962; Bray, 1990; Jousson and Bartoli, 2001). Furthermore, the uterus passes between the testes in *C. consuetum*, and locates pre-ovarian, in contact with the anterior testis in *C. musculometra* (Bravo-Hollis and Manter, 1957).

The excretory vesicle of the present *C. epinepheli* reaches the forebody and passes anteriorly to the ventral sucker. Similar observations were recorded for *C. epinepheli* (Bray and Cribb, 1989; Saoud *et al.*, 1986), *C. musculometra*, *C. lintoni*, and *C. longisaccum* (Bravo-Hollis and Manter, 1957; Siddiqi and Cable, 1960). On the other hand, the excretory vesicle reaches the level of the ovary in *C. flesi*, *C. labracis*, and *C. dentecis* (Korniychuk and Gaevskaya, 2000; Jousson and Bartoli, 2001). *C. lintoni* differs from the present species in having a longer body, larger ovary, and possessing lobed testes (Siddiqi and Cable, 1960). Moreover, *C. longisaccum* differs in having a shorter body, oesophagus, smaller oral and ventral sucker width, pharynx, and ovary along with a cirrus sac extending posteriorly to the ventral sucker (Siddiqi and Cable, 1960). In addition, *C. musculometra* differs in possessing a vitelline field restricted to the hindbody, shorter prepharynx and oesophagus, longer post-testicular space, and shorter eggs (Bravo-Hollis and Manter, 1957). As well as, *C. labracis* has longer forebody, hindbody, prepharynx, larger oral and ventral suckers, pharynx, cirrus sac, ovary, and shorter oesophagus. Moreover, *C. dentecis* has a shorter hindbody, larger testes, cirrus sac, ovary, and smaller eggs (Jousson and Bartoli, 2001). *C. serrani* differs from the species under investigation in possessing an ovary with 4 – 9 lobes. It also has a relatively narrower oral sucker, and a wider ventral sucker (Nagaty, 1956). Furthermore, *C. pteroisi* and *C. flesi* have a larger oral sucker, ventral sucker,

oesophagus, ovary, and eggs than the present species (Nagaty and Abdel Aal, 1962; Korniychuk and Gaevskaya, 2000).

Characters such as the presence of smooth tegument, median gonopore in the forebody, canalicular seminal receptacle, claviform cirrus sac, intercaecal uterus, lobed ovary, non-filamentous eggs in addition to the extension of vitellaria anteriorly into forebody contribute to classify the current species within genus *Cainocreadium* family Opecoelidae. Moreover, morphological features such as the presence of a teat-like tail in the posterior end of the body, long excretory vesicle that reaches the forebody, and pre-testicular uterus in addition to body region measurements contribute to assigning it to *Cainocreadium epinepheli* Durio and Manter, 1968a.

**2- *Prosorhynchus serrani* Durio and Manter, 1968b**

The body region ratio of *P. serrani* is compared from different *Variola* hosts and also with other *Prosorhynchus* spp. recorded from different epinepheline serranids (Table 2). Few scanning electron microscopic studies have been done on species within the family *Bucephalidae* (Shalaby and Hassanine, 1996; Santos and Gibson, 2002; Filippi *et al.*, 2010; Maghrabi and Gharabawi, 2014). The current *P. serrani* is mainly found in the intestine's anterior end, especially the pyloric caeca, and rarely inhabits the posterior part of the alimentary canal of *V. louti*. This is in agreement with Matthews (1973) who reported larger worms and rhynchus in the anterior part of the intestine while smaller worms and rhynchus are located in the posterior end of the intestine. Santos and Gibson (2002) described *P. aculeatus* with a shorter body length (0.880 – 1.320) mainly in the rectum while *P. crucibulum* with a larger body length (1.880 – 2.600) in the anterior and middle intestine of the European conger, *Conger conger.*

The body shape of present *P. serrani* is elongated showing the maximum width at the seminal vesicle level. Similarly, the body of *P. conorjonesi* is elongated but with uniform width (Bott and Cribb, 2009). In contrast, the body is subpyriform, tapers anteriorly, and rounded posteriorly in *P. mizellei* (Kruse, 1977). In addition, variations in body shape and maximum body width were recorded for many other *Prosorhynchus* species, for example, it is ovoid and tapered posteriorly in *P. lafii*, ovoid showing maximum width at vitelline follicles level in *P. robertsthomsoni* (Bott and Cribb, 2009), ovoid with maximum width at the seminal vesicle level in *P. wrightae* (Bott *et al.*, 2013), pyriform with maximum width at the two-thirds of the body in *P. tonkinensis* (Truong *et al.*, 2016), fusiform with maximum body width at caecum level in *P. jexi* and *P. brayi* (Bott and Cribb, 2009; Cutmore *et al.*, 2018).

The rhynchus of the present *P. serrani* is globular in shape and completely provided with nearly circular scales with different measurements. However, *the* rhynchus of *P. crucibulum* is provided with five indentations, central apical depression, and multi-pointed scales-like spines except in the median region of ventral indentation and apical depression while the rhynchus of *P. aculeatus* is circular and spiny except the neck and depressed central region (Santos and Gibson, 2002). The rhynchus of *P. longisaccatus* and *P. tonkinensis* is larger than the rhynchus of the present *P. serrani while P. wrightae* has a smaller rhynchus *(*Table 2). The presence of scales on the body surface supports the attachment of the parasite within the host (Matthews, 1973). In species under investigation, *the* largest tegumental scales are located on the anterior part of the rhynchus. On the contrary, *P. crucibulum* has the largest tegumental spines in the area between the rhynchus base and mouth level that measure (4.3 × 3.3 µm) and these spines are multi-pointed tips exposed toward the posterior end of the worm. Besides, *P. aculeatus has* the largest tegumental spines located just posterior to the rhynchus and measure (3.6 ×3.6 µm)

while smaller spines are located around the mouth (Santos and Gibson, 2002).

The mouth opening in the current *Prosorhynchus sp.* is located very near the mid-body. A similar observation was recorded in *P. serrani* (Durio and Manter, 1968b). However, the mouth is located between the third and last quarters of the body in *P. aculeatus* (Santos and Gibson, 2002). Besides, the mouth is located inside the anterior half of the body at the ovarian or anterior testis level in *P. maternus* with a shorter pre-mouth distance than the present *P. serrani* (Bray and Justine, 2006). In addition, the mouth is located at the anterior third of the body in *P. magnacirrus* and *P. jupe* (Shaukat *et al.*, 2008; Maghrabi and Gharabawi, 2014), the middle third of the body in *P. freitasi* **(**Bott *et al.*, 2013), and posterior half of the body in *P. tonkinensis* **(**Truong *et al*., 2016). The present *P. serrani* has a larger caecum length than *P. robertsthomsoni*, *P. wrightae*, and *P. heronensis* (Bott and Cribb, 2009; Bott *et al.*, 2013). Moreover, the single caecum extends anteriorly in the current *P. serrani*. As well, it extends anteriorly in *P. serrani* (Durio and Manter, 1968b). In addition, there are many species of *Prosorhynchus* with an anterior extension of caecum (Truong *et al.*, 2016; Cutmore *et al.*, 2018). On the contrary, the caecum extends posteriorly in *P. longicollis* and *P. manteri* (Nahhas *et al.*, 2006).

The two testes of *P. serrani* under investigation are tandem making this species distinguishable from *P. australis* as they are opposite to slightly diagonal (Etchegoin *et al.*, 2005). The testes are diagonal in *P. epinepheli* (Nahhas *et al.*, 2006), oblique in *P. freitasi* and *P. brayi* (Bott *et al.*, 2013; Cutmore *et al.*, 2018). In addition, the ratio of post-testicular distance with the body length in the current *P. serrani* is smaller than *P. maternus*, *P. jexi*, and *P. longisaccatus* (Bray and Justine, 2006, 2013; Bott and Cribb, 2009), but shows some resemblance to *P. conorjonesi*, *P. robertsthomsoni*, *P. milleri* and *P. tonkinensis* (Bray and Justine, 2013; Truong *et al.*, 2016). The present *P. serrani* possesses a cirrus sac reaching the level of posterior testis and making it distinct from *P. gonoderus*, *P. aguayoi*, *P. jupe*, *P. maternus*, and *P. platycephali* with cirrus sac separated from the posterior testis (Manter, 1940; Vigueras, 1955; Maghrabi and Gharabawi, 2014; Bray and Justine, 2006; Cutmore *et al.*, 2018). Moreover, the cirrus sac reaches the pre-equatorial body region in *P. magnacirrus* (Shaukat *et al.*, 2008).

The current *P. serrani* has an elongate seminal vesicle located at the proximal end of the cirrus sac while the cirrus sac contains a reniform seminal vesicle in *P. milleri (*Bott and Cribb, 2009). Moreover, it is U-shaped in *P. heronensis* (Bott *et al.*, 2013), and round-shaped in *P. stunkardi* (Siddiqi and Cable, 1960). In addition, *P. longus* has a subglobular seminal vesicle (Velasquez, 1959) and an ovoidal shape in *P. apertus* (McFarlane, 1936). The seminal vesicle is almost occupying one-third of the cirrus sac in the present *P. serrani while in P. mizellei*, it extends about two-thirds or anterior half of the cirrus sac length (Kruse, 1977), and the anterior half of the cirrus sac in *P. brayi* (Cutmore *et al.*, 2018). Furthermore, *P. longisaccatus* and *P. wrightae* have a smaller seminal vesicle than *P. serrani* (Bray and Justine, 2013; Bott *et al.*, 2013).

The ovary in the present *P. serrani* is located posterior to the pharynx and contiguous with the anterior testis. Also, in *P. serrani* (Durio and Manter, 1968b), the ovary is pre-testicular, while in *P. jupe*, *P.* pacificus, and *P. gonoderus* it locates at the same level as the pharynx (Manter, 1940; Maghrabi and Gharabawi, 2014**)**, and in *P. magnacirrus*, the ovary locates close to the anterior testis (Shaukat *et al.*, 2008) or at the level of the mouth in *P. maternus* (Bray and Justine, 2006). The ovary of *P. heronensis*, *P. wrightae*, and *P. freitasi* are smaller than the ovary of current *P. serrani* (Bott *et al.*, 2013; Bray and Justine, 2013). The present study recorded the uterus extended anterior to the ovary. However, many

*Prosorhynchus* spp. had the uterus restricted to the post-ovarian region (Bray and Justine, 2006).

*P. serrani under investigation has two lateral fields of* vitelline follicles that extend anteriorly below the rhynchus and posteriorly to the ovarian level. On the contrary, many *Prosorhynchus* spp. possess confluent vitelline follicles (Vigueras, 1955; Velasquez, 1959; Durio and Manter, 1968b; Kruse, 1977; Etchegoin *et al.*, 2005; Nahhas *et al.*, 2006; Bott and Cribb, 2009; Bott *et al.*, 2013; Cutmore *et al.*, 2018). The vitelline follicles extend from the pharyngeal region to the base of the rhynchus in *P. rotundus* (Manter, 1940). Besides, in *P. lafii*, the vitellaria are presented as two lateral clumpes at the caecum level, and in *P. conorjonesi*, they are separated into two fields; anterior and posterior to caecum and pharynx (Bott and Cribb, 2009). Additionally, the vitelline follicles are present in two groups; the anterior one is lateral to the ovary while the posterior one is intertesticular in *P. jupe* (Maghrabi and Gharabawi, 2014). In contrast, the anterior extension of follicles is located near the posterior margin of the rhynchus in *P. luzonicus* **(**Truong *et al.*, 2016).

The present species has non-filamentous eggs measuring 0.020 – 0.025 (0.023) × 0.015 – 0.018 (0.017). In possessing filamented eggs, *P. caudovatus* is separated from other *Prosorhynchus* species (Manter, 1940). Moreover, the eggs of *P. stunkardi* measure 0.016 – 0.018 × 0.011– 0.015 (Siddiqi and Cable, 1960). Characters such as the absence of oral and ventral suckers, presence of single caecum, mouth at midbody, conical rhynchus at the anterior end of body, pre-testicular ovary, and elongated seminal vesicle with curved proximal part of pars prostatica contribute to assign the present species within genus *Prosorhynchus* within family Bucephalidae. Besides, it shows a morphological and morphometric similarity with *P. serrani* described by Bray and Justine (2013).

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**Declarations:**

**Ethical Approval**: All procedures contributing to this work comply with the ethical standards of the relevant national guides to the care and use of laboratory animals and have been approved and authorized by the Institutional Animal Care and Use Committee (IACUC) at the Faculty of Science, Helwan University, Egypt with approval number HU-IACUC/Z/RM1508-32.

**Conflict of interests**: The authors declare no conflict of interest.

**Contributions:** I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission. Dr. Irene S. Gamil and Dr. Reda M. Mansour designed the research idea, objectives, and hypotheses. Dr. Amina Abd Elfatah was responsible for material preparation, host collection and light microscopy study. All authors contribute to scanning electron microscopy analysis in addition to writing and editing the article before the final form. Dr. Irene S. Gamil and Dr. Reda M. Mansour evaluated and approved the final article.

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