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MORPHOLOGICAL FEATURES OF THREE SPECIES OF *PHYLLODISTOMUM* (TREMATODA: GORGODERIDAE) FROM SOME MARINE FISHES IN THE SOUTHERN BLACK SEA

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Three species of the genus *Phyllodistomum* Braun, 1899 are identified infecting marine teleost fishes from Sinop coast (southern Black Sea, Turkey). Those are *Phyllodistomum acceptum* from *Parablennius sanguinolentus*; *Phyllodistomum crenilabri* from *Symphodus tinca* and *Symphodus ocellatus*; and *Phyllodistomum* sp. from *Gobius cruentatus*. Standard parasitological investigation methods were implemented, and morphological diagnostic features of these species were studied in detail under both light microscope and scanning electron microscope. The measurement data of all morphological diagnostics are provided; photomicrographs of each part of the parasites are presented. Infection prevalence and intensity values are given, as well as morphometric data for each parasite species. This research is the first on *Phyllodistomum* sp. presence in *Gobius cruentatus*. Moreover, this study is the first one, in which the tegumental surface of *P. acceptum* and *P. crenilabri* was examined by scanning electron microscopy.

Keywords: Gorgoderidae, *Phyllodistomum acceptum*, *Phyllodistomum crenilabri*, Blenniidae, Labridae, Gobiidae, Black Sea

Phyllodistomum spp. Braun, 1899 are digenetic trematodes of the family Gorgoderidae, subfamily Gorgoderinae; those are commonly called bladder flukes because of their preference for the urinary bladder and ureters (Goodchild, 1950). They have also been reported in the swim bladder and ovary. Usually, adult individual parasites prefer marine and freshwater fishes, but they occasionally infect amphibians and reptiles (Cribb, 1987). This cosmopolitan Trematoda genus contains about 120 species (Cribb et al., 2002). In the previous studies, 27 *Phyllodistomum* species were reported in 17 teleost fish families (Ho et al., 2014), but Cutmore & Cribb (2018) noted that species richness of this genus is likely to be far greater than presently known. As the researchers have explained, the reason is that the fish species infected with these parasite species are studied rarely or accidentally. Members of this genus do not have a well-defined host specificity pattern. Moreover, in terms of morphological features, *Phyllodistomum* species significantly differ from each other, but generally their large body appears like a leaf-shaped (Namuleno & Scholz, 1994). The genus is characterized by having a more-or-less foliate and broad hindbody, simple blind caeca, oblique two testes in the widest part of hindbody, and a slender excretory vesicle, I-shaped (Campbell, 2008). This Trematoda genus has complex life cycle,

and there is not enough research on it (Stunžėnas et al., 2017). These species have more than one intermediate host; they show a variety of life cycles with two or three hosts. Furthermore, one individual may serve as both first and second intermediate host (Goodchild, 1950).

Phyllodistomum species are distributed in marine water and freshwater worldwide. Despite the fact that these species infecting fishes in the Palaearctic realm have been studied for a very long time, data on *Phyllodistomum* existence and morphology in Turkish Black Sea coastal areas are rather limited.

In this paper, information is presented about morphology employing both light and scanning electron microscopic observations of three *Phyllodistomum* species in marine fish species (*Parablennius sanguinolentus*, *Symphodus tinca*, *Symphodus ocellatus*, and *Gobius cruentatus*) from Turkish Black Sea coasts. This study is the first detailed research on *P. acceptum* and *P. crenilabri* morphology, and it would serve as a basis for future investigation.

MATERIAL AND METHODS

Fishes were sampled by gill net from the southern Black Sea of Sinop in Turkey (42°01′55″N, 35°16′36″E) June 2016 to May 2017. Fish samples were transported to the laboratory and examined for the presence of *Phyllodistomum* trematodes. Throughout the research study, 34 fish species belonging to 26 different families were examined, and only 4 fish species from 3 families were found to be infected with *Phyllodistomum* spp. *Phyllodistomum* spp. specimens were recovered from fish urinary bladders. Individuals were set out in a Petri dish containing physiological saline and washed. Morphological diagnostic features of three *Phyllodistomum* species were studied in detail under light and scanning electron microscopes (hereinafter LM and SEM, respectively). Parasite specimens were studied when they were alive and later fixed and preserved in 70 % ethanol and Trump's fixative. Alive individuals were placed between slide and cover glass without pressure; some preparations were stained in acetocarmine and then examined under an Olympus BX51 microscope and photographed with DP-25 digital camera. For scanning electron microscopy, specimens preserved with Trump's fixative were placed in 1 % osmium tetroxide (OsO_4) in cacodylate buffer for 3 hours and then dehydrated in graded ethanol series. Species were dried in an E3100 critical point dryer (Quorum Technologies) using liquid carbon dioxide, then attached on stubs with double-sided adhesive tape, sputter-coated with gold/palladium, and examined under a Quanta FEG 250 SEM (FEI).

The infection parameters, prevalence (share, % of infected fish), mean intensity (mean number of parasites *per* infected fish), and abundance (mean number of parasites *per* examined fish), were calculated following Bush and co-authors (1997). The parasites were identified based on morphological criteria, according to (Opredelitel' parazitov pozvonochnykh, 1975) and (Gaevskaya, 2012).

RESULTS AND DISCUSSION

Three *Phyllodistomum* species were identified from fishes sampled from the coastal area off Sinop, Black Sea, Turkey. Those were *Phyllodistomum acceptum* Looss, 1901 from *Parablennius sanguinolentus*; *Phyllodistomum crenilabri* Dolgikh & Naidenova, 1968 from *Symphodus tinca* and *S. ocellatus*; and *Phyllodistomum* sp. from *Gobius cruentatus*. Hosts, site of infection, prevalence, and intensity of infections are presented, as well as morphological and morphometric features of each recorded digenean species.

***Phyllodistomum acceptum* Looss, 1901** (Figs 1 and 2).

Syn.: *Phyllodistomum (Catoptroides) acceptum* Looss, 1901.

Hosts: *Parablennius sanguinolentus* (Perciformes: Blenniidae).

Site in host: urinary bladder.

Prevalence: 40 %.

Mean intensity: 11.50.

Abundance: 4.60.

Infected/Examined fish number: 2/5.

Geographical distribution: Adriatic Sea (Pigulewsky, 1953); Balearic Sea (Campos & Carbonell, 1994); Aegean Sea (Papoutsoglou, 1976); and Black Sea (Korniychuk, 2001 ; Osmanov, 1940 ; Öztürk & Güven, 2020).

Description: measurements are of 10 gravid specimens. Body spatulate in shape; adult specimens' body slightly narrower at anterior end and broader and rounder at posterior end (Figs 1A, 2A, and 2B); length 3.94–4.70; maximum width 1.50–2.00 at level of anterior testis (Fig. 1A). Tegumental surface covered with knob-like protuberances (Figs 1D and 2E). Edges of body slightly serrated or straight. Oral sucker opening subterminally (Fig. 1C), rounded; length 0.32–0.38; width 0.29–0.34; bearing irregular developed papillae (Fig. 2C). Ventral sucker (Figs 1A and 2D) in second quarter of body, rounded; length 0.28–0.34; width 0.28–0.35; bearing six well-developed papillae. Oral sucker / ventral sucker length and width ratios: 1 : 0.91 to 1 : 0.97 and 1 : 0.89 to 1 : 1.12, respectively. Prepharynx and pharynx absent. Oesophagus length 0.26–0.37. Testes oblique, well separated, slightly lobed testes (Fig. 1A) located in the widest part of hindbody. Posterior testis is usually bigger than anterior one and covers large part of hindbody. Posterior testis greatest width 0.69–0.94; anterior testis greatest width 0.65–0.75. Genital pore (Fig. 2a) opening between bifurcation and ventral sucker. Ovary (Fig. 1A) globular, slightly lobed or slightly indented, located behind ventral sucker and at level of anterior testes; greatest width 0.40–0.44. Eggs (Fig. 1B) oval; length 0.32–0.70; width 0.20–0.40. Vitellarium (Fig. 1A) from 2 compact lobed glands; right gland (Fig. 1A) greatest width 0.35–0.47; left gland greatest width 0.35–0.40. Distance between vitelline glands 0.18–0.26. Excretory pore (Fig. 2b) median; dorso-subterminal posterior notch clearly invisible.

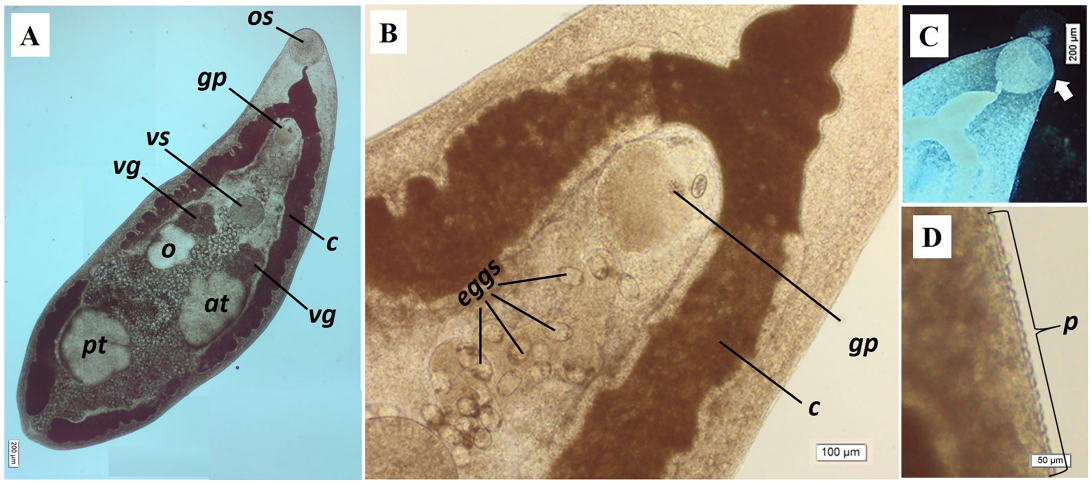

Fig. 1. Light micrographs of *Phyllodistomum acceptum*. A, body of mature worm, ventral view; B, forebody, ventral view showing eggs and genital pore; C, oral sucker; D, protuberances on body surface. Os denotes oral sucker; gp, genital pore; c, cecum; vs, ventral sucker; vg, vitelline glands; o, ovary; at, anterior testis; pt, posterior testis; p, protuberances

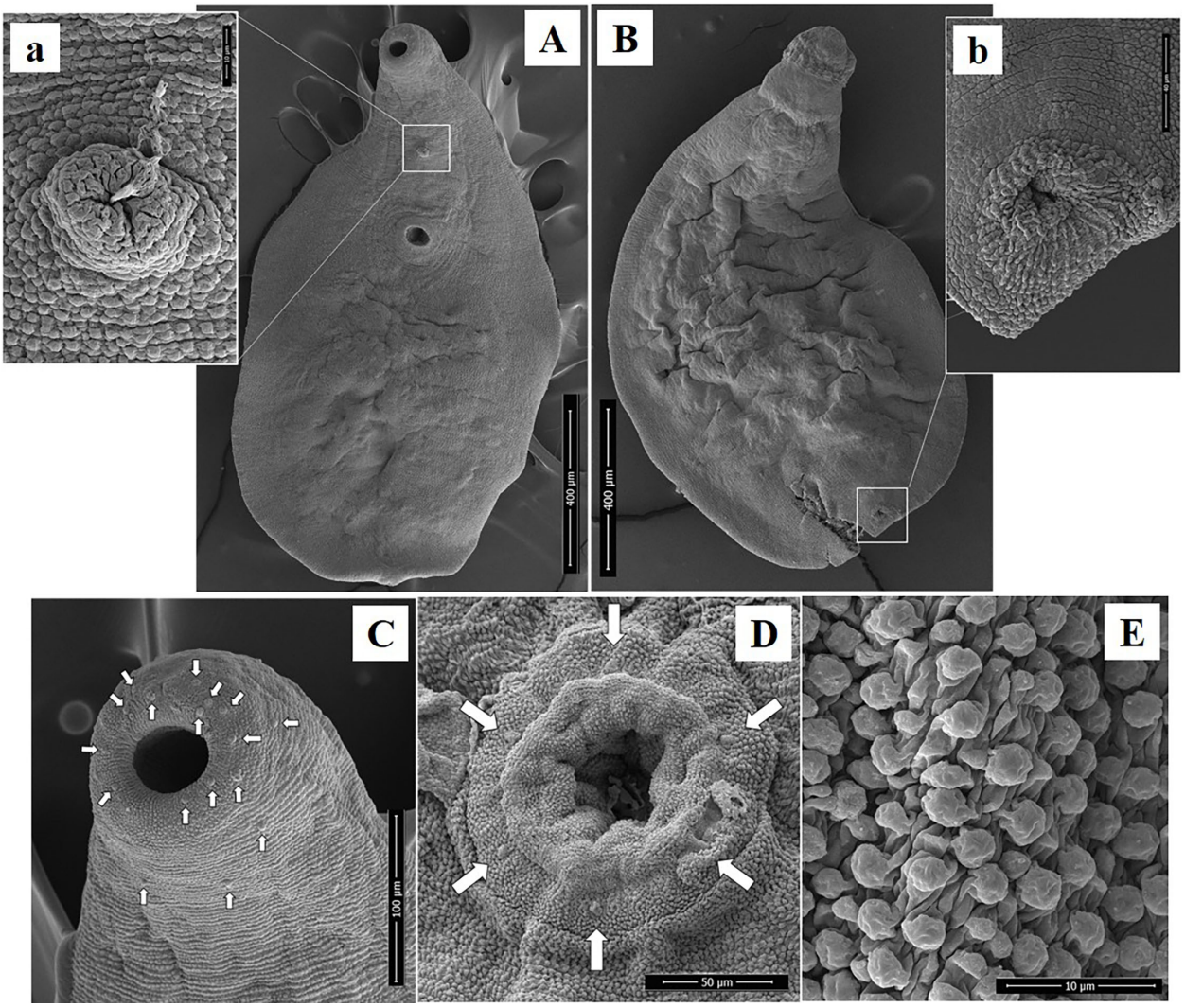

Fig. 2. Scanning electron micrographs of *Phyllodistomum acceptum* specimen. A, adult, ventral view; B, dorsal view; C, oral sucker showing irregular papillae; D, ventral sucker showing three pairs of papillae; E, protuberances on body surface; a, genital pore on anterior ventral surface with extruding sperm; b, subterminal excretory pore opening at posterior end of body, dorsal side

To date, Labridae (*Symphodus tinca*, *S. ocellatus*, and *S. cinereus*) are typical hosts for *Phyllodistomum acceptum* (Ho et al., 2014 ; Korniychuk, 2001, 2004 ; Nikolaeva & Solonchenko, 1970 ; Radujković & Šundić, 2014). *Serranus scriba*, *Mullus barbatus*, and *Parablennius tentacularis* were reported to be *P. acceptum* hosts in the Black Sea as well (Gaevskaya & Korniychuk, 2003 ; Kornyychuk et al., 2016). In this study, *P. acceptum* was detected in urinary bladder of *Parablennius sanguinolentus*. Prevalence and intensity values calculated to be 40 % and 1–22, respectively.

***Phyllodistomum crenilabri* Dolgikh & Naidenova, 1968** (Figs 3 and 4).

Hosts: *Symphodus tinca* and *Symphodus ocellatus* (Perciformes: Labridae).

Site in host: urinary bladder.

Prevalence: 44 % and 50 %, respectively.

Mean intensity: 10.75 and 2.00, respectively.

Abundance: 4.78 and 1.00, respectively.

Infected/Examined fish number: 12/27 and 1/2, respectively.

Geographical distribution: Black Sea (Dolgikh & Naidenova, 1968 ; Nikolaeva & Solonchenko, 1970 ; Öztürk & Güven, 2021).

Description: measurements are of 5 gravid specimens. Body of mature specimens elongated in shape (Figs 3A, 3B, and 4A); length 2.40–3.37; maximum width 0.73–1.08 at level of anterior testis. Tegument aspinose (Fig. 3E). Oral sucker (Figs 3A, 3C, and 4B) opening subterminally, oval; length 0.22–0.30; width 0.21–0.26; papillae not observed. Ventral sucker (Figs 3A and 4C) rounded; length 0.20; width 0.25. Oral sucker / ventral sucker length and width ratios: 1 : 0.73 to 1 : 0.83 and 1 : 0.95 to 1 : 0.96, respectively. Prepharynx and pharynx absent. Oesophagus length 0.18–0.27. Testes (Fig. 3A and 3B) oval, oblique, well separated, in the widest part of the hindbody. Posterior testis is usually bigger than anterior one and covers large part of hindbody; posterior testis 0.25–0.51 × 0.20–0.32; anterior testis 0.22–0.43 × 0.15–0.32. Genital pore (Figs 3A and 4B) opening between bifurcation and ventral sucker, 0.3 from anterior end. Ovary (Fig. 3B) oval or slightly lobed, in left side of body opposite to anterior testes; greatest width 0.15–0.20. Vitellarium from 2 elliptical lobed glands; each lobe irregularly indented (Fig. 3B). Eggs small and elongate (Fig. 3D). Excretory pore (Fig. 4D) median, terminal.

Dolgikh & Naidenova (1968) described *P. crenilabri* in *Symphodus tinca* from the Black Sea and stated the following: in this species, “the body surface is covered with spines”. It might happen since they were wrong and misinterpreted the papillae covering the body surface as spines. In fact, based on our LM and SEM examinations, we did not observe spines on the surface of any specimens studied. Moreover, in our LM observations, we detected that the body surface of the specimens was covered with tegumental protuberances and that there were shallow transverse tegumental ridges. However, in our SEM observations, shallow transverse tegumental ridges and papillae were not clearly apparent on the body surface.

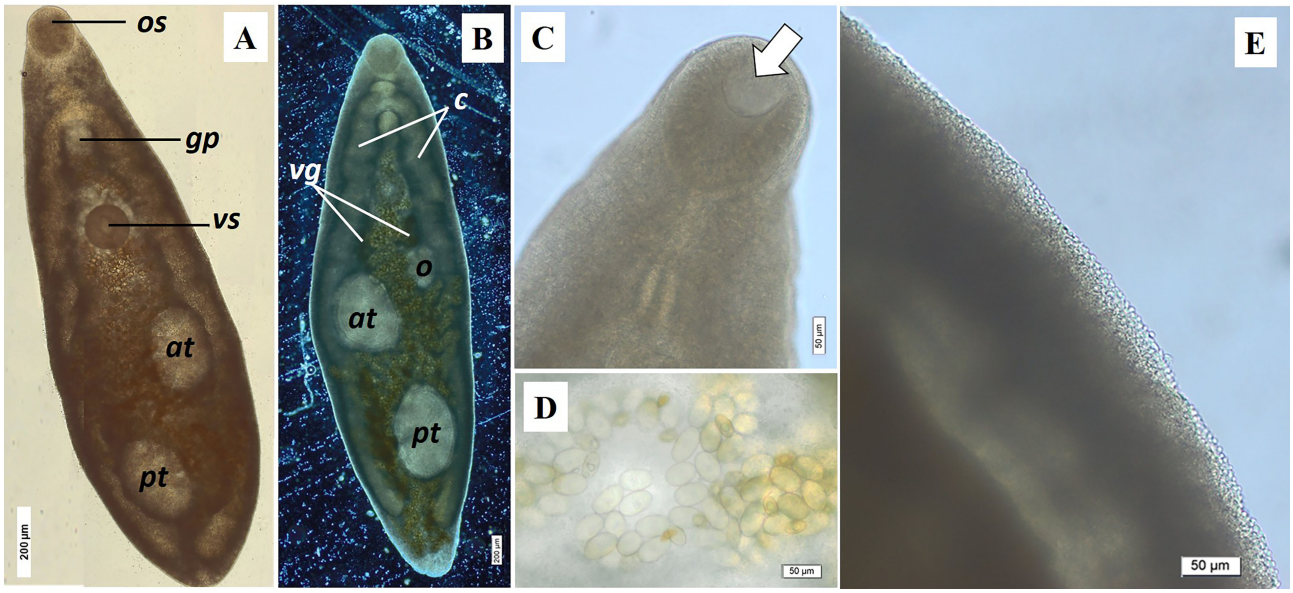

Fig. 3. Light micrographs of *Phyllodistomum crenilabri*. A, body of mature worm, ventral view; B, dorsal view (phase contrast micrograph); C, oral sucker; D, eggs; E, protuberances on body surface. Os denotes oral sucker; gp, genital pore; c, cecum; vs, ventral sucker; vg, vitelline glands; o, ovary; at, anterior testis; pt, posterior testis

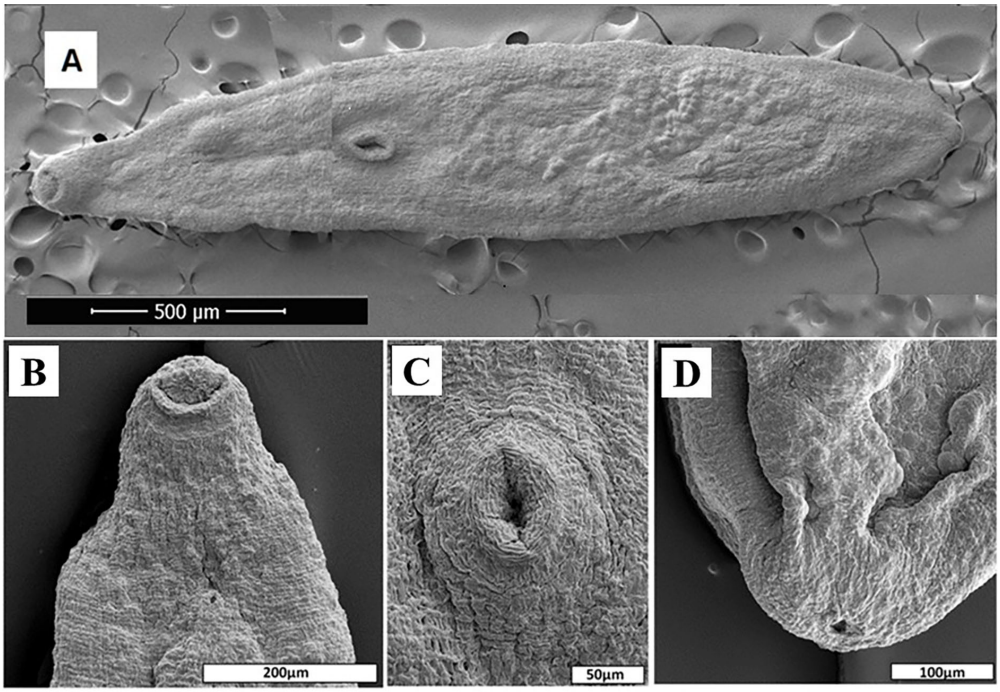

Fig. 4. Scanning electron micrographs of *Phyllodistomum crenilabri* specimen. A, adult, ventral view; B, oral sucker and genital pore; C, ventral sucker; D, terminal excretory pore

Phyllodistomum crenilabri was detected in urinary bladders of *S. tinca* and *S. ocellatus*. Prevalence and intensity values calculated to be 50 % and 1–50 for *S. tinca* and 50 % and 11 for *S. ocellatus*, respectively. This Trematoda species was reported in *S. tinca*, *S. ocellatus*, and *S. cinereus* in Black Sea waters from (Korniychuk, 2001). When the studies compared, infection values detected are higher in this research.

***Phyllodistomum* sp.** (Fig. 5).

Hosts: *Gobius cruentatus* (Gobiidae).

Site in host: urinary bladder.

Prevalence: 20 %.

Mean intensity: 18.00.

Abundance: 3.60.

Infected/Examined fish number: 1/5.

Description: measurements are of 3 gravid specimens. Body elongated in shape; length 1.8–2.1; maximum width 0.8–1.0; greatest width at level of posterior part of anterior testes (Fig. 5A and 5B). Tegument smooth, aspinose. Oral sucker (Fig. 5A and 5C) terminal, with lip; length 0.22; width 0.20. Pharynx absent; oesophagus short; length 0.5–1.0; intestinal cecum (Fig. 5B) simple, broad, undulant, terminating near posterior end of testes or little beyond. Ventral sucker (Fig. 5A and 5C) 0.31–0.35 × 0.30–0.35. Oral sucker / ventral sucker length ratio: 1 : 1.5. Testes (Fig. 5A) asymmetrical or oblique, tandem in position, deeply lobed. Right testis 0.25–0.40 × 0.20–0.25, with 4–6 loculi; left testis 0.40–0.42 × 0.20–0.25, with 4–6 loculi. Seminal vesicle free in parenchyma. Genital pore opening (Fig. 5A and 5C) between bifurcation and ventral sucker, 0.4 from anterior end. Ovary (Fig. 5A) in right side of body, slightly indented, close to vitellarium; 0.15–0.17 × 0.10. Vitellarium (Fig. 5A) from two glands, irregularly lobed or entire; right gland 0.06–0.07 × 1.0–1.25; left gland 0.75 × 0.10. Eggs (Fig. 5D) 0.026–0.030 × 0.018–0.020. Excretory pore median, notch from the ventral side.

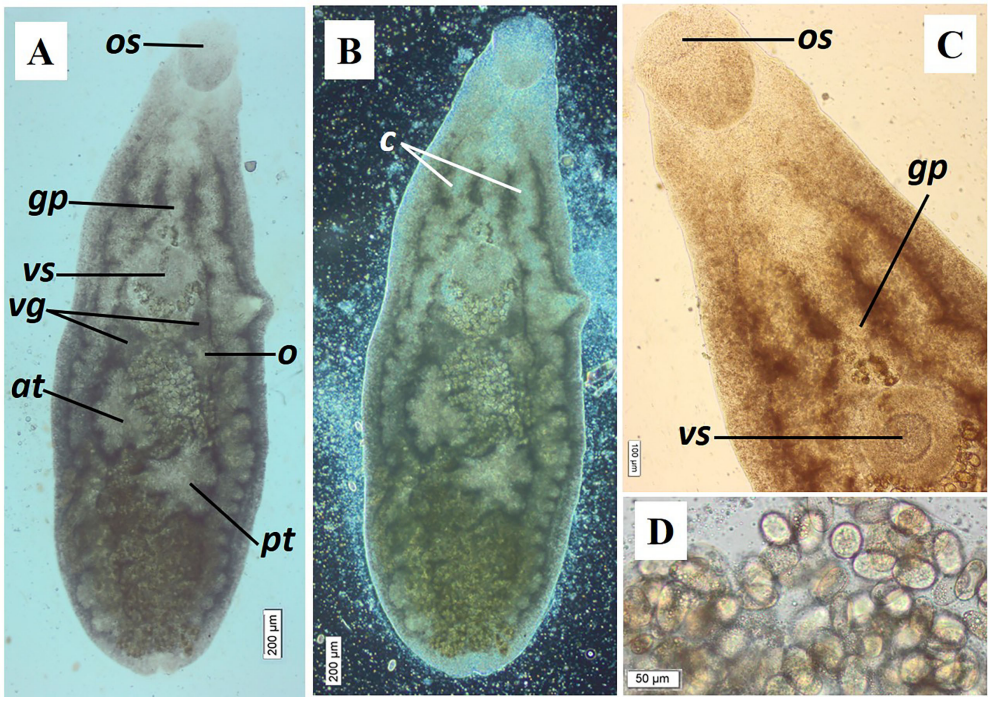

Fig. 5. Light micrographs of *Phyllodistomum* sp. A, body of mature worm, ventral view; B, phase contrast micrograph of mature worm (ventral view); C, forebody, ventral view showing oral sucker, genital pore, and ventral sucker; D, eggs. Os denotes oral sucker; gp, genital pore; c, cecum; vs, ventral sucker; vg, vitelline glands; o, ovary; at, anterior testis; pt, posterior testis

Phyllodistomum sp. was found in urinary bladder of only one of the fishes examined. No *Phyllodistomum* species have been registered in *G. cruentatus* so far, and this is its first report. The morphology of the species was quite different from that of the previously reported *Phyllodistomum* species in the Black Sea. Considering that *G. cruentatus* is a gobiid of Mediterranean origin, the parasite has a high potential to become a new species. Unfortunately, suitable photographic images were not obtained from *Phyllodistomum* sp. specimens examined under SEM.

Although more detailed investigations are needed on these species, this is the first study on the micromorphology and tegumental surface topography of *P. acceptum* and *P. crenilabri* containing useful data for their identification.

Conclusion. Three species – *Phyllodistomum acceptum*, *P. crenilabri*, and *Phyllodistomum* sp. – were detected in urinary bladder of four fish species, namely *Symphodus tinca*, *Symphodus ocellatus*, *Parablennius sanguinolentus*, and *Gobius cruentatus*. In the present study, we provided the first comprehensive data on both light and ultrastructural observations of *Phyllodistomum acceptum* and *P. crenilabri* in the Turkish Black Sea coasts. While tegumental papillae were observed in detail in *P. acceptum* samples in scanning electron microscopy examinations, we could not get appropriate results in *P. crenilabri* samples. This may be related to the fact that sputter coating, wavelength, and fixation time suitable for SEM examinations differ depending on *Phyllodistomum* species. All the illustrations and morphometric data presented contribute to our current knowledge and will also provide a base for further studies.

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МОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ТРЁХ ВИДОВ *PHYLLODISTOMUM* (TREMATODA: GORGODERIDAE) ОТ НЕКОТОРЫХ МОРСКИХ РЫБ В ЮЖНОЙ ЧАСТИ ЧЁРНОГО МОРЯ

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Определены три вида трематод рода *Phyllodistomum* Braun, 1899, поражающие морских костистых рыб у побережья Синопа (южная часть Чёрного моря, Турция): *Phyllodistomum acceptum* из *Parablennius sanguinolentus*; *Phyllodistomum crenilabri* из *Symphodus tinca* и *Symphodus ocellatus*; *Phyllodistomum* sp. из *Gobius cruentatus*. Применены стандартные методы паразитологического исследования; морфологические диагностические особенности этих видов детально изучены с использованием светового и сканирующего электронного микроскопов. Предоставлены морфометрические и морфологические данные, а также микрофотографии этих паразитов. Приведены показатели заражённости трематодами рыб-хозяев. Паразитирование *Phyllodistomum* sp. у бычков *Gobius cruentatus* отмечено впервые. Кроме того, тегумент *P. acceptum* и *P. crenilabri* впервые исследован с помощью сканирующей электронной микроскопии.

Ключевые слова: Gorgoderidae, *Phyllodistomum acceptum*, *Phyllodistomum crenilabri*, Blenniidae, Labridae, Gobiidae, Чёрное море