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The polyclad fauna (Platyhelminthes, Rhabditophora) of the Sinop Peninsula (Black Sea, Turkey) with a description of a new species of *Cryptocelis* Lang, 1884

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Abstract

Between October 2013 and July 2014, 40 polyclad specimens were collected during systematic sampling of mussel beds along the coast of the Sinop Peninsula (Western Black Sea). Six species were identified, including a new *Cryptocelis* species. *Cryptocelis sinopae* sp. nov. is characterized by possessing a prostatic vesicle lined with a ridged fold in its anterior end and the presence of two secretory glandular folds in the distal dorsal wall of the male atrium. Additionally, new records of *Echinoplana celerrima* Haswell, 1907 and *Leptoplana mediterranea* (Bock, 1913) are reported for Turkey and the Black Sea, as well as *Leptoplana tremellaris* (Müller OF, 1774) and *Prosthiostomum siphunculus* Delle Chiaje, 1822 for the coast of the Black Sea of Turkey. A short description of the reproductive behaviors of *Pleioplana okusi* Bulnes, Kalkan and Karahan, 2009 and *Cryptocelis sinopae* sp. nov. are provided.

Keywords: Marine flatworms; mussel bed; secretory glandular fold; taxonomy; diversity; reproductive behavior.
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Introduction

The first records of the polyclad fauna of the Black Sea go back to the turn of the twentieth century. Czerniavsky (1880) and Jacubowa (1909) registered six species from the Black Sea and described four new ones off the coasts of Georgia and the Crimean Peninsula. Additionally, Murina *et al.* (1995) remarked on the predatory behavior of *Stylochus tauricus* Jakubowa, 1909 feeding on the barnacle *Amphibalanus improvisus* (Darwin, 1854) in the Laspy and Sevastopol Bays. Although still scarce, the knowledge of polyclad biodiversity in Turkey has grown in the last ten years. Five new species have been described (Bulnes *et al.* 2009, Bulnes 2010) and several new records have been registered from the Aegean and Marmara Seas (Gözcelioğlu, 2011; Çinar, 2014; Teker *et al.* 2017). The Black Sea coast of Turkey remained unexplored with a single report of three known species (Çinar, 2014).

We report the presence of six species of polyclads associated with the mussel beds on the coast of the Black Sea in Turkey with the description of a new *Cryptocelis* species. The genus *Cryptocelis* includes 10 valid species (Tyler *et al.* 2006–2016): *Cryptocelis alba* (Schmidtlein, 1880); *C. amakusaensis* Kato, 1936; *C. compacta* Lang, 1884; *C. glandulata* Jacubowa, 1909; *C. ijimae* Bock, 1923; *C. insularis* Hyman, 1953 (a); *C. lilianae* Marcus and Marcus, 1968; *C. littoralis* Kato, 1937; *C. occidentalis* Hyman, 1953 (b) and *C. orientalis* Kato, 1939. Lang created the genus *Cryptocelis* based on the description of *Leptoplana alba*, a species collected in the Mediterranean Sea (Schmidtlein 1880; Lang 1880, 1884). Since then, there have been two different interpretations of the male copulatory apparatus of this genus. Bock (1923) interprets the male system as an extremely elongated prostatic vesicle, producing different secretions along its course without any copulatory organ. However, Lang (1880) describes an intrabulbar prostatic vesicle that opens to an unarmed secretory cirrus and to the male atrium, which are completely housed in a muscular bulb. We will describe *Cryptocelis sinopae* sp. nov. after Lang' s review of *C. alba* and follow up with a more detailed discussion in the following section. Finally, we will note two different reproductive strategies observed in these species, contributing to the knowledge of the distribution, ecology, and reproduction of marine flatworms.

Materials and Methods

Samples were collected on mussel beds at five stations along the Sinop Peninsula (Fig. 1, Table 1) by SCUBA diving using a quadrant (20x20 cm) of 4–5 m depth within the framework of the TÜBİTAK Projects (113Y312 and 2209/A) coordinated by Dr. Güley Kurt. At each station, three replicates were collected and sorted with a 0.5 mm mesh sieve. The retained material was fixed in a 4% seawater-formaldehyde solution and transported in plastic containers. In the laboratory, samples were rinsed with fresh water, separated under a stereomicroscope, and preserved in 70% ethanol. The polyclads were inspected under stereo- and compound microscopes, registering important taxonomic data of their external morphology and documenting the information with photos using a Nikon CoolPix 4500 camera.

For further taxonomical studies, nine fixed exemplars were prepared for histological examination, dehydrated in an ascending series of ethanol, embedded in paraffin, serially sectioned at 7 µm, and stained with eosin and toluidine blue. Some of the exemplars were processed whole, and others were prepared only in the sections containing the copulatory structures. The holotype and paratype have been deposited at the Museum of Ege University Faculty of Fisheries (ESFM), and other materials have been deposited at the Department of Biology, Faculty of Arts and Sciences at Sinop University.

Table 1. Coordinates, depths and localities of sampling stations.

Stations	Coordinates	Region	Depth (m)
M1	42.02200º N 35.15127º E	Liman-İskele	5
M2	42.02174º N 35.21512º E	Gazi Kayası	4
M3	42.03913º N 35.19309º E	Asma Kaya	5
M4	42.09350º N 34.98272º E	İnceburun-Başoz	4
M5	42.03777º N 35.16377º E	Üzümlü Dere	5

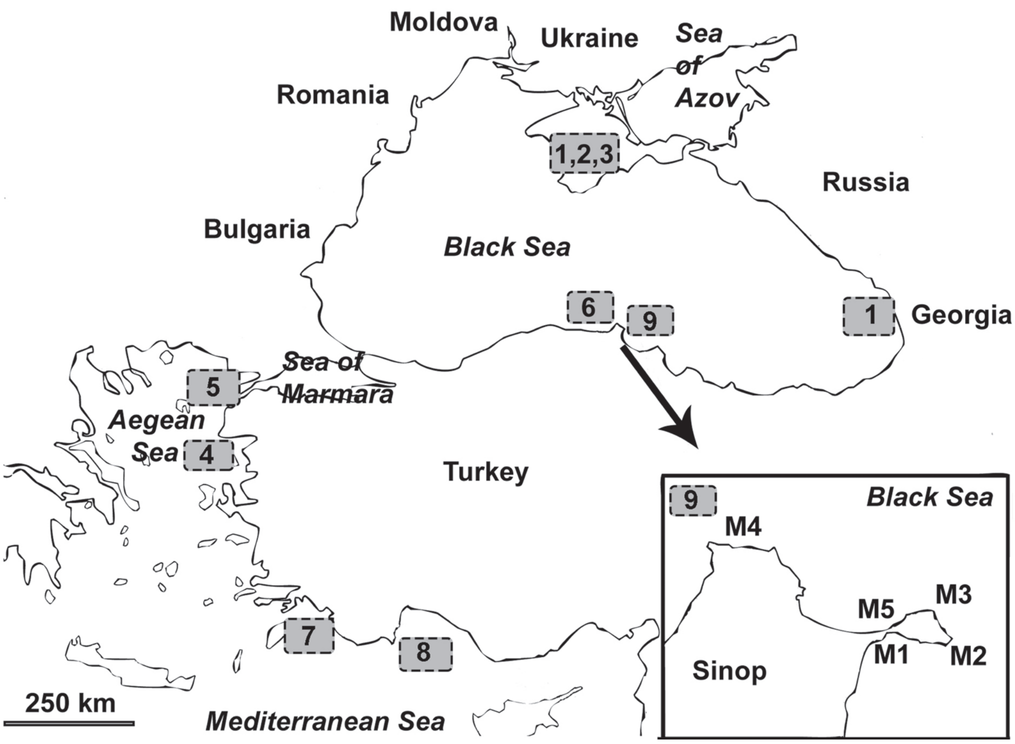

Fig. 1: Map of the coast of Turkey and the Black Sea showing the sampling site of the biodiversity studies carried out in the area, chronologically ordered. 1, Czerniavsky (1880); 2, Jacubowa (1909); 3, Murina *et al.* (1995); 4, Bulnes *et al.* (2009); 5, Bulnes (2010); 6, Çinar (2014); 7, Gözcelioğlu (2011); 8, Teker *et al.* (2017); 9, this study; M1, Liman-İskele; M2, Gazi Kayası; M3, Asma Kaya; M4, İnceburun-Başoz; M5, Üzümlü Dere.

Results

Taxonomic Account
Order Polycladida Lang, 1884
Suborder Acotylea Lang, 1884
Superfamily Discoceloidea Dittman, Cuadrado, Aguado, Noreña & Egger, 2019
Family Cryptocelidae Laidlaw, 1903
***Genus Cryptocelis* Lang, 1884**
***Cryptocelis sinopae* sp. nov.**
(Figs. 2–4, 8A)

Material examined. Holotype: ESFM-PLAT/2014-2, sagittal sections of the copulatory apparatus (20 slides), 12.05.2014, station M1, 5 m deep, Sinop, Black Sea, coll. G. Kurt. Paratype: ESFM-PLAT/2014-3, one specimen (18 slides), sagittal sections of the median region containing copulatory apparatus, 12.05.2014, station M1, 5 m deep, Sinop, Black Sea, coll. G. Kurt.

Description of the holotype. Body oval-shaped and compact, 15 mm long and 6 mm wide at the broadest part of the body. The anterior margin rounded, posteriorly somewhat pointed, laterally ruffled. Dorsal surface ground color yellowish to brownish with an unpigmented marginal band. The dorsal color pattern consists of minute brown spots distributed mostly behind the brain region. The spots are more densely distributed in two dorsal bands parallel to the main intestine and reproductive organs. Lateral to the central bands, the spots are concentrated in small clusters, resembling rounded blotches, up to the unpigmented margin (Fig. 2A). Ventrally whitish. Without tentacles. Tentacular eyes present, cerebral eyes in two clusters, with frontal eyes and marginal eyes along the entire body margin. The latter more concentrated in the anterior fourth of the body, arranged in rows but scattered on the rest of the body margin (Figs. 2F, 8A).

At the level of the mid-longitudinal body axis, the dorsal body wall consists of an epidermal layer of cylindrical ciliated cells with basal nuclei, short and numerous spindle-shaped rhabdites, and eosinophilic and basophilic granules six times higher (29 µm) than the compact and well-developed basement membrane (4.5 µm). Inwards, there is a circular muscle layer as high as the basement membrane, followed further inwards by a longitudinal muscle layer three times higher than the basement membrane (14 µm) and an innermost well-developed circular muscle layer, almost three times higher than the longitudinal muscle layer (35 µm), blending with apparent parenchymal transversal muscle bundles (Fig. 2C). The ventral body wall consists of an epidermis of cylindrical cells densely ciliated, with scattered rhabdites and acidophilic granules, six times higher (28 µm) than the compact and well-developed basement membrane (5 µm). Inwards, there is a circular muscle layer slightly higher (5.4 µm) than the basement membrane, followed inwards by a longitudinal muscle layer twice as high as the basement membrane (10.2 µm) a well-developed circular muscle layer (26 µm) and an innermost longitudinal muscle layer (12 µm). Parenchymal transversal muscle bundles apparent (Fig. 2D).

Necks of parenchymal eosinophilic and basophilic glands piercing the muscular wall apparent. The marginal body wall has a different arrangement. The columnar epithelium is not glandular, the cilia are longer, and nuclei are no longer basal, with scattered, small rhabdites. Basement membrane delicate and muscular body wall reduced to several longitudinal muscle fibers. The necks of two types of subepithelial glandular cells open in this area: the type with fine granular eosinophilic secretion, also distributed throughout the epidermis of the whole body, and a singular type with coarser secretion (glandular corpuscles after Bock, 1923, p. 20) (Fig. 2F). The body wall around the genital area is characterized by a lower columnar epithelium, shorter cilia, nuclei central, without rhabdites, and not glandular. The muscular body wall is disorganized, and the muscular layer coalesces with the muscular layers of the bulb housing the reproductive organs (Fig. 2E).

Digestive system. The mouth opens near the center of the body, and the pharyngeal pocket is located in the second third of the body, connected to the intestine at almost the same level as the mouth.

Reproductive system. Testis arranged ventrally between intestinal branches. Male copulatory apparatus immediately behind the pharyngeal pocket directed backward and enclosed in a well-developed muscular bulb. The outermost fibers of the muscular bulb intermingle with the longitudinal muscle layer of the body wall. The vasa deferentia also anchored to the body wall. The paired vasa deferentia filled with sperm, first run anteriad before turning to the middle body line and joining into a median vas deferens (Fig. 3A). The latter runs anteriad up to the level of the prostatic vesicle. After leaving the vas deferens, the ejaculatory duct develops a strong circular muscle layer between the ciliated inner epithelium and the external longitudinal muscle layer. The ejaculatory duct turns dorsal and, after piercing the muscular bulb from ventral, projects into the prostatic vesicle (Fig. 3B).

Anterior to the prostatic vesicle, there is a strong layer of transversal muscles, anchored dorsally and ventrally to the body's muscular wall. Prostatic vesicle rounded; its muscular wall consists of a few muscular bundles mixed with the muscular bulb. The inner glandular lining is deeply ridged and secretory, filling the lumen of the vesicle anteriorly, while distally the ridges become shorter and the lumen of the vesicle becomes apparent. Distally, the inner epithelium becomes cuboidal and ciliated before opening to the ejaculatory duct of the cirrus. Without extra vesicular glands. Cirrus long, unarmed, set coiled and somehow twisted in the muscular bulb. The cirrus muscular wall consists of a compact layer of circular muscle fibers intermingling with glandular cells, which run along the entire tortuous path up to the male atrium (Fig. 3C). The cirrus is enclosed in a muscular bulb. This bulb consists of an inner, looser muscular matrix and an outermost layer of circular muscles, both devoid of glandular secretions. The muscular fibers coating the muscular bulb blend dorsally and ventrally with the body's muscular layers, anchoring the male copulatory system to the body wall. Ejaculatory duct coated with a cuboidal,

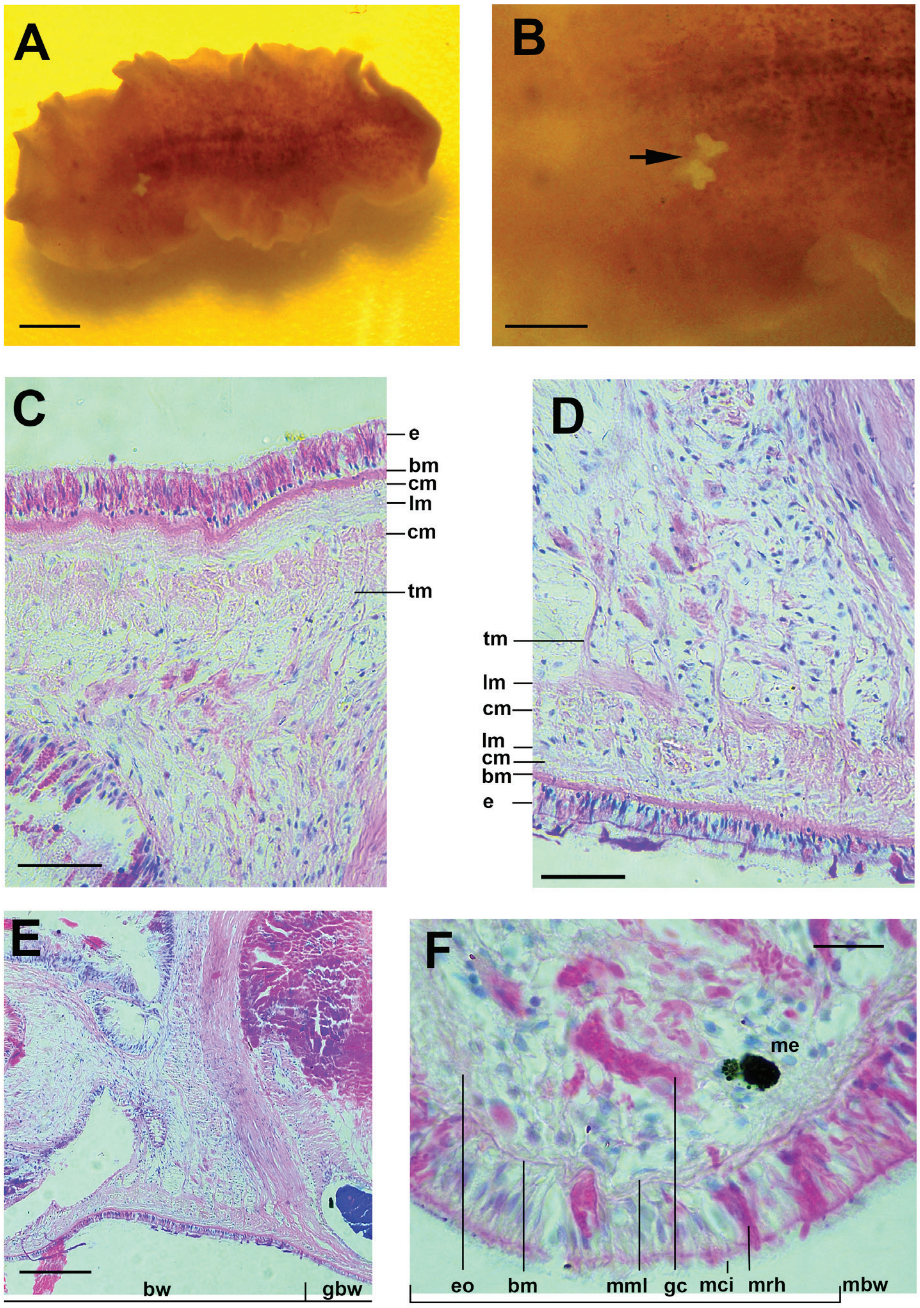

Fig. 2: *Cryptocelis sinopae* sp. nov. A, dorsal view of fixed specimen; B, detail of the spermatophores (arrow); C, sagittal section of the dorsal body wall; D, sagittal section of the ventral body wall; E, sagittal section of the ventral body wall at genital openings' area; F, sagittal section of the marginal body wall. Abbreviations to the figures: bm, basement membrane; bw, body wall; cm, circular muscle layer; e, eyes; eo, eosinophilic glands; gbw, body wall around the genital area; gc, marginal glandular cell (glandular corpuscles [Bock, 1923]); lm, longitudinal muscle layer; mbw, marginal body wall; mci, marginal cilia; me, marginal eye; mml, marginal muscle layer; mrh, marginal rhabdites; tm, transversal muscle fibres. Scale bars: A: 2 mm, B: 1 mm; C: 50 µm; D: 50 µm; E: 200 µm; F: 20 µm.

ciliated, folded epithelium (Figs. 3C-D), alternating with secretory patches. In the last third of its length, the ejaculatory duct widens. At this point, the folds of the duct´s wall become deeper. Shortly before its opening, the ejaculatory duct turns into a narrow tube (Fig. 3C). The cirrus opens to the male atrium in the center of shallow conical penis papillae. The male atrium is narrow, covered with a tall and heavily ciliated epithelium, except dorsally where it widens laterally, forming two pouches in the roof of the atrium. An anterodorsal intrabulbar musculo-glandular conical fold projects into each atrium´s pouch (Figs. 3E, E´, and F). The glandular folds consist of scatter muscular fibers, immersed in a mass of glandular cells, covered with a squamous epithelium without cilia. The male atrium opens to the exterior through a narrow canal via the male pore (Figs. 3C, 5).

Ovaries dorsal, immersed in the parenchyma. The uteri run slightly ventral to the ovaries, filled with eggs (Fig. 4D). The oviducts enter separated from the ventral side to a distended portion of the vagina interna (Fig. 4A). From this point, the female canal continues anteriorly. At the level of the male gonopore, the female canal makes

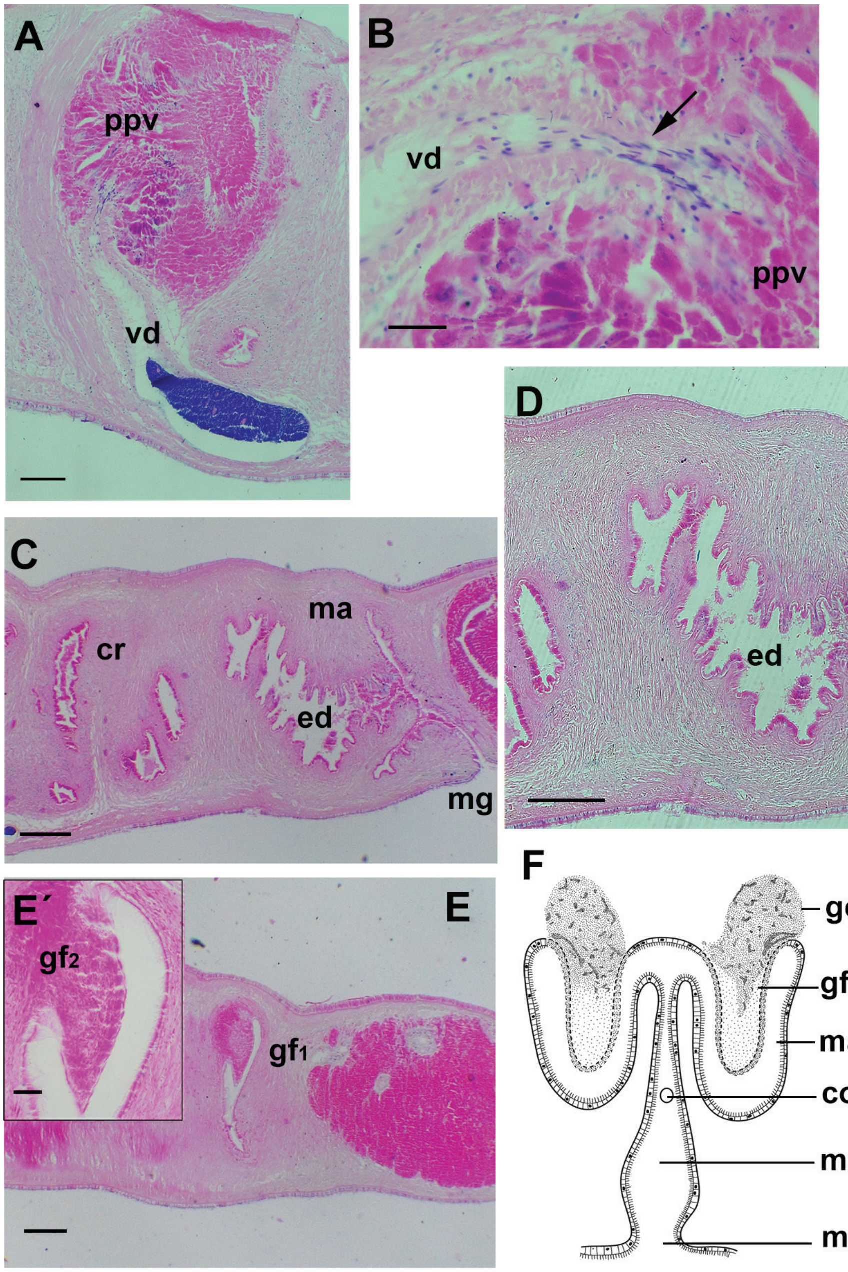

Fig. 3: *Cryptocelis sinopae* sp. nov. A, sagittal section of the male copulatory organ (vas deferens and prostatic vesicle); B, sagittal section of the ejaculatory duct, the arrow points the projection of the ejaculatory duct into the proximal prostatic vesicle; C, sagittal section of the male copulatory organ (cirrus and male atrium); D, sagittal section of the male copulatory apparatus (cirrus); E and E´, sagittal sections of the musculo-glandular folds; F, transversal diagram of the male atrium. Abbreviations to the figures: co, cirrus opening; cr, cirrus; ed, ejaculatory duct; gce, glandular cells; gf, musculo-glandular fold; gf_1, right musculo-glandular fold; gf_2, left musculo-glandular fold; ma, male atrium; mg, male gonopore; ppv, proximal prostatic vesicle; vd, vas deferens. Scale bars: A: 100 µm; B: 30 µm; C: 200 µm; D: 200 µm; E: 200 µm; E´: 30 µm.

a long posteroventral turn and then runs posteriorly up to the level of the female gonopore. Distally the vagina turns ventrally, opening to the ample female atrium and gonopore. The female gonopore is located 3 mm behind the male pore. From the vagina interna up to the female atrium, the female canal develops deep folds in its wall (Figs 4A-C). Oviducts slender, with cuboidal inner epithelium with short cilia. The vagina interna lined with high columnar epithelium and long cilia. The rest of the female canal and female atrium is covered by a columnar ciliated glandular epithelium. The female canal is immersed in a dense mass of cement glands (Figs 4A–C). Posteriorly, the cement glands mass extends not far from the level of the female gonopore and is limited by an external muscular layer. The outermost fibers of this layer blend dorsally and ventrally with the muscular body wall.

Whitish, spherical, ruffled structures were recognized on the dorsal surface of one specimen. They were identified as spermatophores (Fig. 2B). Histological sections also revealed sperm cells in several areas of the body's

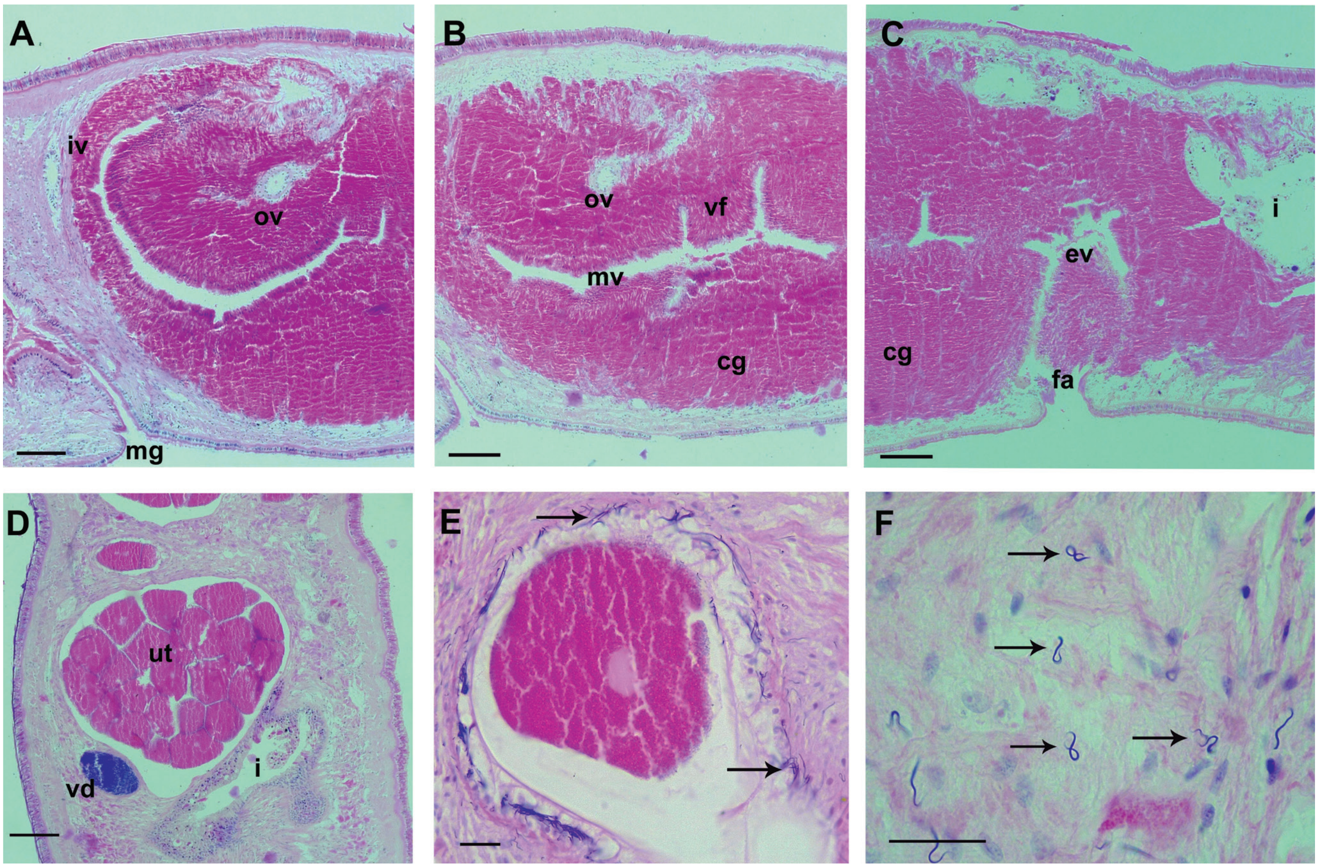

Fig. 4: *Cryptocelis sinopae* sp. nov. A-C, sagittal section of the female reproductive system; D, sagittal section of the uterus and vas deferens; E, sagittal section of an oocyte; F, sperm cells immersed in body parenchyma. The black arrows point sperm cells. Abbreviations to the figures: cg, cement glands; ev, external vagina; fa, female atrium; i, intestine; iv, internal vagina; mg, male gonopore; mv, median vagina; ov, oviduct; vd, vas deferens; vf, vaginal fold. Scale bars: A: 100 µm; B: 100 µm; C: 100 µm; D: 100 µm; E: 30 µm; F: 15 µm .

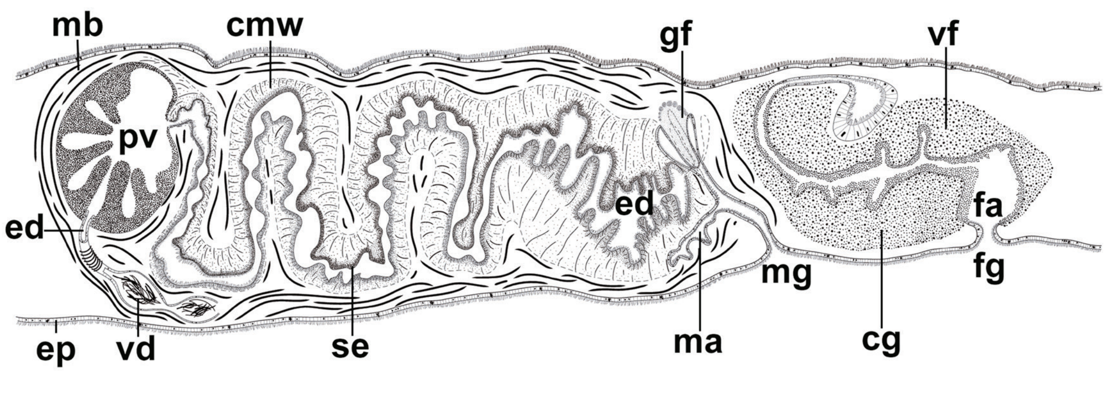

Fig. 5: *Cryptocelis sinopae* sp. nov. Sagittal reconstruction of reproductive system. Abbreviations to the figures: cg, cement gland; cmw, cirrus muscular wall; ed, ejaculatory duct; ep, epidermis; fa, female atrium; fg, female gonopore: gf, musculo-glandular fold; ma, male atrium; mb, muscular bulb; mg, male gonopore; pv, prostatic vesicle; se, secretory epithelium; vd, vas deferens; vf, vaginal fold. Scale bar: 1000 µm.

parenchyma (Fig. 4F) and around the uterine ducts (Fig. 4E), probably as a consequence of the indirect transfer of sperm (dermal impregnation).

Etymology. The species name is a specific term alluding to the type locality's ancient name, Sinop, which is taken from the name of the goddess Sinope from the Greek mythology.

Remarks. The oval body, without tentacles, with cerebral, nuchal, and marginal eyespots, male genital apparatus enclosed in a massive muscular bulb, lack of a seminal vesicle, with an intrabulbar prostatic vesicle with ridged glandular inner lining, and unarmed cirrus surrounded by

Table 2. Comparison between the valid *Cryptocelis* species and *C. sinopae.* (d), this information was obtained from the drawings in the original publications.

	alba	*amakusaensis*	*compacta*	*glandulata*	*ijimae*	*insularis*	*lilianae*	*littoralis*	*occidentalis*	*orientalis*	*sinopae*
Color	White, transparent	Uniformly light brown	White with hints of brown	Uniform grayish-yellow	Milky white	Indeter-minable	Colourless	Uniform brown	Indeterminable, transparent	Uniformly brown	Brownish, with spots and bands
Tentacular eyes	Yes	Yes	Yes	Yes	Yes	4 eyes each	Yes	Yes	Yes	Yes	Yes
Cerebral eyes	2 clusters	2 clusters	Yes	2 clusters	Yes	2 clusters	Yes	2 clusters	Yes	Yes	Yes
Frontal eyes	Yes (d)	Yes (d)	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes
Marginal eyes	Whole margin	Whole margin	Whole margin	Whole margin	Whole margin	¼ anterior margin	Whole margin	Whole margin	Yes	Whole margin	Whole margin
Body form	Elongate oval (d)	Elongate oval	Elongate oval	Elongate oval	Elongate, broadly rounded	Elongate oval	Oval	Oval	Elongate oval	Broadly rounded	Oval
Mouth	Mid body	Slightly posterior	-	Mid body	Mid body	-	Mid body	Mid body	Mid body	Mid body	Mid body
Pharynx	-	1/3 body length	-	1/3 body length	1/4 body length	1/5 body length (d)	1/3 body length	1/3 body length	1/3 body length	1/3 body length	1/3 body length
Spermiducal bulbs	No	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No
Vas deferens	1	2	1	1	2	1	2	2	1	2	1
Prostatic vesicle	Rounded	Concavo convex	Rounded, curved	Rounded	Elongate	Elongate	Concavo convex	Ovoid	Ovoid	Ovoid	Rounded
P.V. inner lining	Folded	Folded cylindrical cells	Folded antero ventrally	Not folded	Folded cylindrical cells	Folded, two regions	Folded	Folded	Folded, two regions	Folded	Anteriorly folded

Continued

Table 2 continued

	alba	*amakusaensis*	*compacta*	*glandulata*	*ijimae*	*insularis*	*lilianae*	*littoralis*	*occidentalis*	*orientalis*	*sinopae*
Extra vesicular glands	Yes	No	No	No	No	No	No	No	No	No	Yes
Extra bulbar glands	Yes (d)	Yes	No	No	Yes	No	No	Yes	No	Yes	No
Secretory parenchyma	No	No	-	No	No	-	Yes	No	No	No	No
Cirrus epithelium	Not secretory, flat	Secretory, folded	-	Folded	Secretory, not folded	Not secretory	Secretory, not folded	Secretory, folded	Secretory, not folded	Secretory, folded	Secretory, folded
Additional secretory structures	No	No	-	Annex glandular organ	No	No	Pouch of the ejaculatory duct	No	No	No	Musculo glandular folds
Vagina epithelium	-	Cylindrical	-	-	Cylindrical ciliated	Cuboidal	-	-	-	-	Cylindrical, folded
Vaginal duct enlargement	Distal	Distal	Distal	Distal (d)	Anterior, and distal (d)	Anterior, and distal	-	Medio-distal	-	Whole vagina (d)	Anterior, medial, distal.
Cement glands	Only around vagina	Slightly behind female gonopore (d)	Only around vagina	Up to distal body margin	Only around vagina	Only around vagina	Only around vagina	Only around vagina	Only around vagina	Only around vagina	Slightly behind female gonopore
Post genital vesicles	No	No	No	No	Yes	No	No	No	No	No	No

an intrabulbar secretory tissue, without Lang´s vesicle, agree with the characteristics of the species gathered in the genus *Cryptocelis*, Lang 1884. The presence of the paired lateral musculo-glandular folds in the roof of the male atrium represents an apomorphic character, which distinguishes *Cryptocelis sinopae* sp. nov. from its congeners (Table 2).

The presence of additional secretory structures associated with the male copulatory organs was described for *Cryptocelis glandulata* Jacubowa, 1909 and *Cryptocelis lilianae* Marcus and Marcus, 1968. The annex glandular organ of *C. glandulata* is oblong in shape with a slightly pointed anterior tip, opening to the distal ejaculatory duct, and filled with fine-grained basophilic star-shaped secretions organized in somewhat longitudinal rows. The muscular wall consists of a thin layer of longitudinal muscular fibers, underlined by a few small oval-shaped glands opening to the organ's lumen (Jacubowa, 1909, p. 16). Although Jacubowa was not able to find the connection to the ejaculatory duct, she believed the annex glandular organ opened to the distal part of the ejaculatory duct and hypothesized the secretions were related due to the formation of spermatophores. Marcus & Marcus (1968) observed that the distal portion of the ejaculatory duct of *Cryptocelis lilianae* forms a conspicuous dorsal diverticulum. They describe this diverticulum as an inner muscular wall and an outer glandular epithelium with sunken glands and nuclei partly immersed in the muscular layer (Marcus & Marcus 1968, p. 14). The lack of a well-defined muscular wall lined with a glandular epithelium led us to consider that these accessory secretory structures associated with the male systems of *Cryptocelis sinopae*, *C. glandulata*, and *C. lilianae* are not adenodactyls like that of *Adenodactyloplana uruguayensis* Bulnes, Faubel & Ponce de Leon, 2003, which also belongs to the family Cryptocelididae. Additionally, the paired musculo-glandular conical folds of *C. sinopae*, open to the male atrium, while the accessory secretory structures of *C. glandulata* and *C. lilianae* are connected to the ejaculatory duct.

Lang (1884) created the genus *Cryptocelis* based on the description of *Leptoplana alba*. In his description, he stated that the copulatory system of *Cryptocelis* resembles the copulatory organ of the genus *Planocera* (p. 248-249): an intrabulbar prostatic vesicle that opens to a cirrus and the male atrium. But while *Planocera* cirri are armed, meaning the inner lining of the ejaculatory duct secretes scleroproteins in the form of hooks and teeth, in *Cryptocelis* the ejaculatory duct is lined with an epithelium secreting viscous substances, probably acid mucins, since they are dyed red in basophilic stains. The extreme development of the muscular wall and the predominance of secretory epithelia lining the male system led Bock to consider that the muscular bulb housing the male copulatory organ of *Cryptocelis ijimae* was a prostatic vesicle, even when he discovered the secretions of the proximal bulbous component were different from those of the elongated distal component (Bock, 1923, p. 26). In the following years, the authors followed Bock's interpretation of the male copulatory organ, adding unnecessary complexity to the microanatomy of the genus (Faubel, 1983; Hyman, 1953a; 1953b; Kato, 1936; 1937; 1939; Marcus & Marcus, 1968).

The level of detail in descriptions of the *Cryptocelis* species varies, but most describe a long and tortuous, even a spiralized, secretory ejaculatory duct. Although a ciliated ejaculatory duct was described only for *C. sinopae*, we consider this may also be the case for other *Cryptocelis* species and that the proximal section of the ejaculatory duct contributes to the production of spermatophores. The prostatic secretions protect the sperm mass, and while this mass moves along the ejaculatory duct with the help of the metachronic movement of the cilia, the secretions of the ejaculatory duct are added, shaping the characteristic external striation (Lang, 1884, p. 248-249). The spermatophores are stored in the distal part of the ejaculatory duct, which is probably the only portion of the duct that protrudes during mating. The partial projection of the distal ejaculatory duct from the male atrium could explain the difference in the *Cryptocelis* species between having a blunt penis papilla (*C. occidentalis* Hyman, 1953), and single, or multiple penis sheaths (*C. glandulata*, *C. compacta* Lang, 1884). Regarding the prostatic vesicle, except for *C. glandulata*, all other *Cryptocelis* species display a regularly folded glandular lining filled with eosinophilic secretions. *Cryptocelis insularis* Hyman, 1953, also possesses the deeply folded inner lining with a proximal eosinophilous and a distal basophilic region. *Cryptocelis sinopae* is characterized by a rounded prostatic vesicle with a ridged glandular lining, only anteriorly showing some similarities to the prostatic vesicle of *C. compacta*, which has an inner glandular lining that is developed more antero-ventrally.

Bock (1923) describes the inner lining of the anterior portion of the prostatic vesicle of *C. ijimae* as an epithelium with a fine and inconspicuous basal membrane underlined by subepithelial gland cells, but in *C. sinopae* the glandular epithelium appears pseudostratified and ciliated. The reproductive system of the *Cryptocelis* species exhibits a striking number of glandular secretions. The glandular cells may be extravesicular, intrabulbar (*C. sinopae*), extrabulbar, (*C. alba*), or both (*C. ijimae*). The detailed description of the cirrus and the ejaculatory duct's inner lining is not available for all species, but in the examined species, the glandular cells are located beneath the ciliated epithelium. The species of the *Cryptocelis* genus lack a true seminal vesicle, and in most of them, the vasa deferentia develop spermiducal bulbs. From that point, the vasa deferentia turn into slender ducts, which may join in a common vas deferens (*C. glandulata*, *C. compacta*, *C. insularis*), or continue to be paired (*C. lilianae*, *C. amakusensis*, *C. ijimae*, *C. littoralis*, and *C. orientalis*) before piercing the muscular bulb of the copulatory organ and opening to the prostatic vesicle. Like *Cryptocelis occidentalis*, in *Cryptocelis sinopae*, the vasa deferentia join into a common vas deferens that pierces the muscular bulb and projects into the prostatic vesicle, but in *C. sinopae* there are no spermiducal bulbs, and the common vas deferens is characterized by a well-developed circular muscle layer.

The female system seems more or less homogeneous

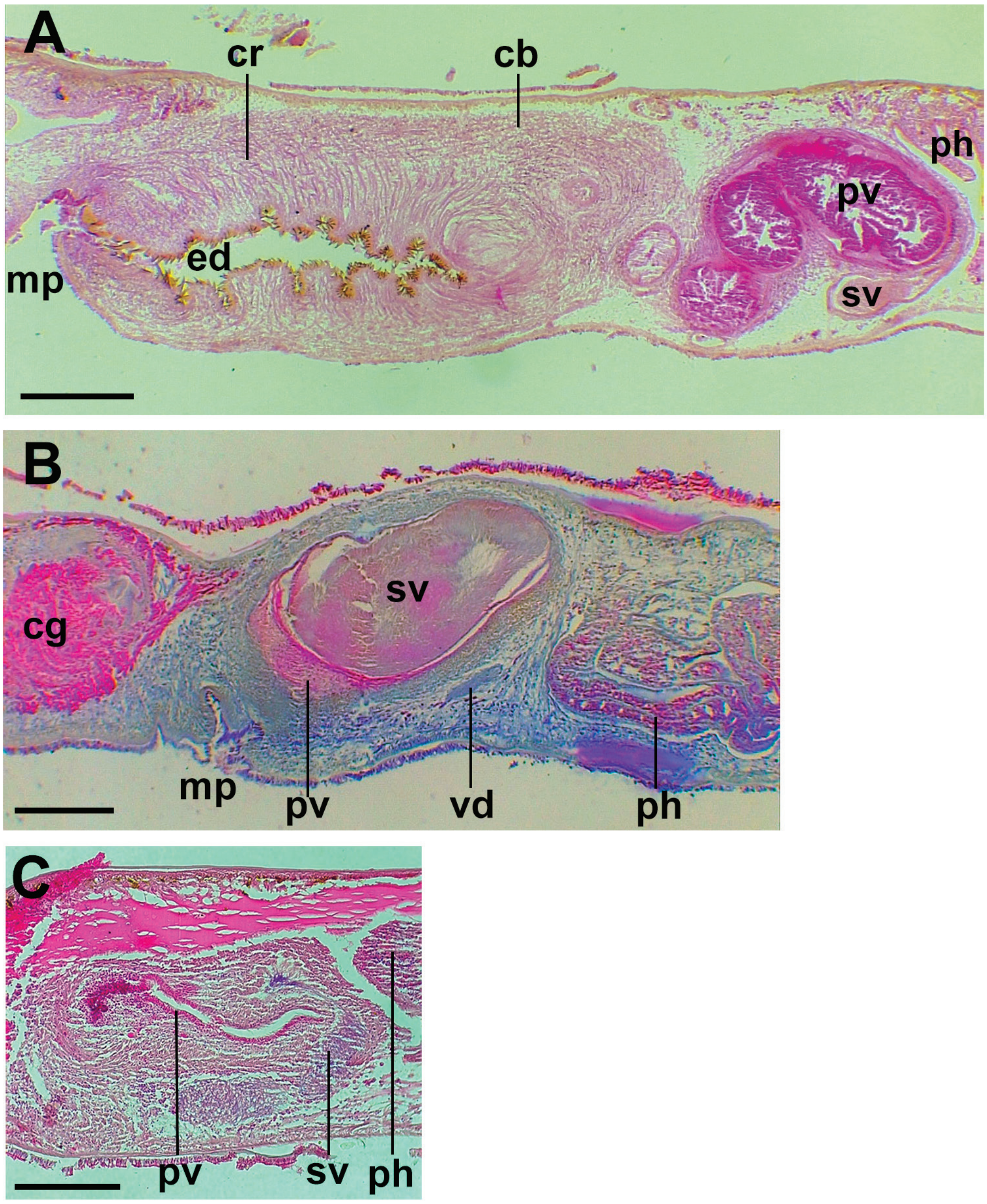

Fig. 6: Sagittal sections of the reproductive system of A, *Echinoplana celerrima*; B, *Leptoplana mediterranea*; C, *Leptoplana tremellaris*. Abbreviations to the figures: cb, cirrus bulb; cg, cement glands; cr, cirrus; ed, ejaculatory duct; mp, male gonopore; ph, pharynx; pv, prostatic vesicle; sv, seminal vesicle; vd, vas deferens. Scale bars: A: 500 µm; B: 200 µm; C: 500 µm.

in all species of the genus *Cryptocelis*. The oviducts open from the mid-lateral to the common female canal at the level of the female gonopore. This anterior part of the female canal is lined with ciliated non-glandular epithelium. Distally, the inner epithelium is ciliated and glandular. The canal runs dorsal and turns frontal, then turns backward, and finally ventral. The canal opens to the female atrium and gonopore. Along the vaginal canal, there are some distended areas, which vary among the different species. The female system is surrounded by a dense mass of glandular cells immersed in the parenchyma. Except for *Cryptocelis glandulata*, where the cement glands reach the distal margin of the animal, in all other *Cryptocelis* species, the glandular mass does not distally surpass the level of the female gonopore. The extreme development of the muscular fibers observed in *Cryptocelis sinopae* can also be observed in the female system, where the glandular mass seems limited by bundles of longitudinal muscle fibers.

There is little information available regarding the body wall of the *Cryptocelis* species. *Cryptocelis amakusensis* possesses a thick epidermis of 60 µm with a thin and delicate basement membrane, followed inwards by a circular, then a longitudinal, then an innermost diagonal muscular coat. The diagonal coat is divided into two layers. The ventral body wall is higher than the dorsal one since there is also a circular muscular layer between the diagonal layers. In *Cryptocelis sinopae*, the epidermis is lower; the basement membrane is well developed, and the dorsal and ventral body walls are different. While the dorsal epidermis is followed inwards by a circular, a longitudinal, and another circular muscle layer, ventrally there is an additional innermost longitudinal layer.

The dorsal and ventral epidermis of *Cryptocelis sin-*

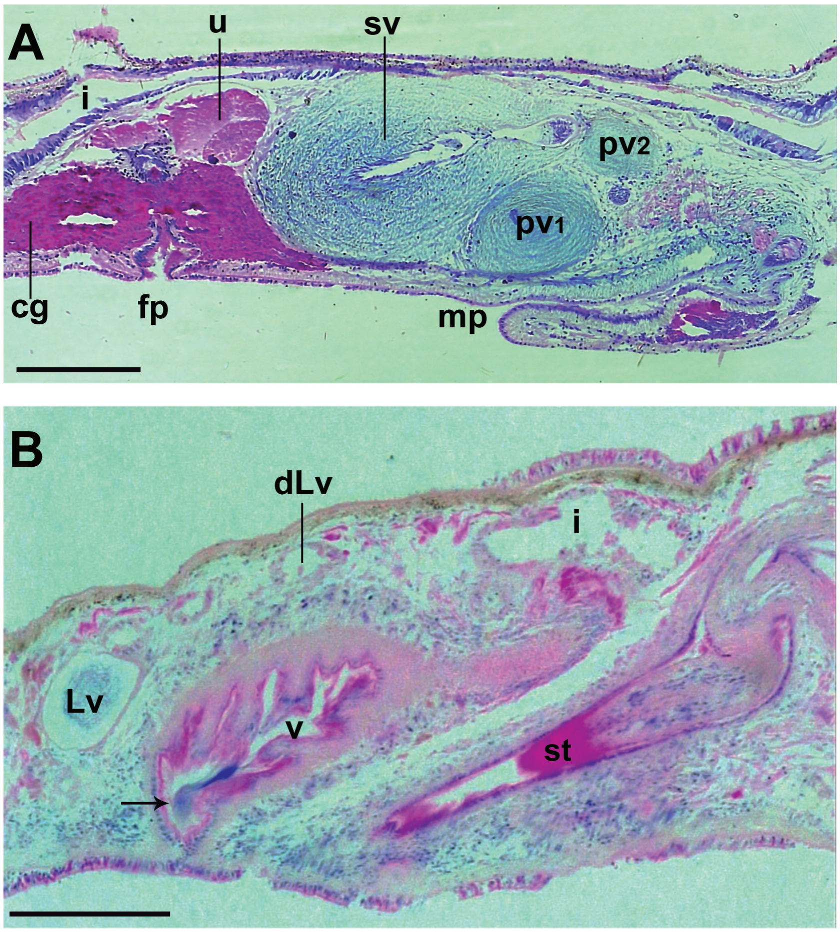

Fig. 7: A, *Prosthiostomum siphunculus*; B, *Pleioplana okusi*. The arrow points the sperm mass. Abbreviations to the figures: cg, cement glands; dLv, Lang's vesicle duct; fp, female gonopore; i, intestine; Lv, Lang´s vesicle; mp, male gonopore; pv1, prostatic vesicle 1; pv2, prostatic vesicle 2; st, stylet; sv, seminal vesicle; u, uterus; v, vagina. Scale bars: A: 200 µm; B: 200 µm.

opae have similar thicknesses. The only recorded differences agree with the observations made by Bock (1923) for *Cryptocelis ijimae*, where the dorsal and ventral epidermis of the body differs from the marginal and the ventral epidermis of the genital area.

The presence of masses of spermatozoa in the parenchyma of *Cryptocelis sinopae* agrees with the observation made by Lang (1884) for *Cryptocelis alba*, and the production of spermatophores and the hypodermic insemination may be a usual mating behavior of the *Cryptocelis* species.

Superfamily Leptoplanoidea Faubel, 1984
Family Gnesiocerotidae Marcus & Marcus, 1966
***Genus Echinoplana* Haswell, 1907**
***Echinoplana celerrima* Haswell, 1907**
(Fig. 6A and 8B)

Material examined. Nine mature specimens, 21.01.2014, station M2, 4 m deep. Three mature specimens, 22.01.2014, station M3, 5 m deep. One specimen, 12.05.2014, 5 m deep, station M1. One specimen sagittally sectioned (16 slides).

Description. Body broadly oval. Preserved specimens between 8–25 mm long and 3–7 width. Dorsal surface background color brownish, ventrally colorless, and transparent. Tentacles wanting. Two elongated clusters of cerebral eyes, tentacular eyes basal, apparent only in a few specimens (Fig. 8B).

Digestive system. A ruffled pharynx lies in the mid-third of the body and possesses 8 to 12 pairs of lateral folds. Mouth opening slightly posterior to the middle of pharyngeal pocket.

Reproductive system. Male gonopore behind the distal margin of the pharynx. Cirrus sac consisting of thick layers of musculature armed with sclerotized teeth. Prostatic vesicle interpolated, lined with glandular epithelium thrown into radial folds. Seminal vesicle four times smaller than prostatic vesicle and located ventrally to the latter (Fig. 6A).

Female gonopore 2.4 mm behind the male one. Between them, there is a corrugated surface consisting of glandular epithelium. The vagina is oriented anteriorly, Lang's vesicle and ductus vaginalis not developed.

Remarks. The color pattern, the organization of the male system, and the presence of a corrugated ventral area between the gonopores (genital sucker) agree with the descriptions of *Echinoplana celerrima* from the Indo Pacific and Mediterranean waters (Haswell, 1907; Galleni, 1979; Prudhoe, 1982; Holleman, 2007; Gammoudi

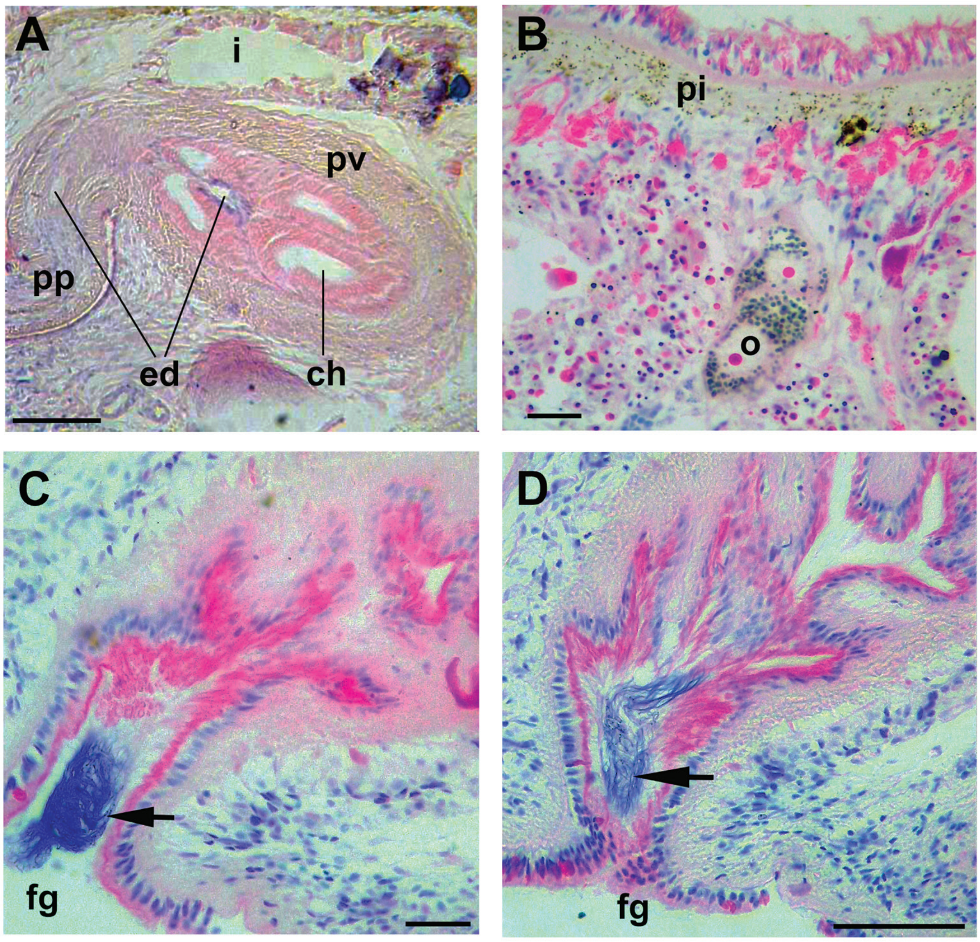

Fig. 8: *Pleioplana okusi*. A, sagittal section of the prostatic vesicle; B, sagittal section of the ovary; C-D, sagittal section of the vagina and female atrium. The arrows point the sperm mass. Abbreviations to the figures: ch, tubular chamber of the prostatic vesicle; ed, ejaculatory duct; fg, female gonopore; o, ovary; pi, epidermal pigment; pp, penis papilla; pv, prostatic vesicle. Scale bars: A: 100 µm; B: 50 µm; C: 30 µm; D: 50 µm.

et al. 2009); nevertheless, some variability was detected. The specimens from the Black Sea lack the median dark band and, in some of the revised material, the tentacular eyes were wanting although present in all other described specimens. Regarding the reproductive system, the Lang´s vesicle varies from rudimentary (Prudhoe, 1982) to well developed, and with a ductus vaginalis (Gammoudi *et al.* 2009). The rudimentary Lang´s vesicle in our sectioned specimen could suggest it was not fully mature.

Family Leptoplanidae Stimpson, 1857
***Genus Leptoplana* Ehrenberg, 1831**
***Leptoplana mediterranea* (Bock, 1913)**
(Figs. 6B and 8C)

Material examined. Ten specimens, 23.10.2013, M5, 5 m deep. Three specimens, 02.07.2014, station M5, 5 m deep. Two specimens were sagittally sectioned (7 slides and 6 slides).

Description. Body broadest anteriorly, tapering distally. Preserved specimens between 10-22 mm long and 4-8 mm wide. Tentacles lacking. Genital pit present. Cerebral eyes clusters distributed over the brain region, extending distally up to the area of the tentacular eyes. The tentacular eyes bigger than the cerebral ones (Fig. 8C).

Digestive system. Pharynx located in the posterior second third of the body. The deeply ruffled pharynx may develop up to 16 pairs of folds. Mouth opening in the second half of the body in the posterior third of the pharyngeal pocket.

Reproductive system. Testes are disposed in ventral parenchyma between digestive ramifications, whereas ovaries are arranged dorsally. Vasa deferentia extend anteriorly up to the level of the last third of pharynx, then turn posteriorly and join in a common vas deferens before entering a developed oval seminal vesicle (Fig. 6B). The interpolated prostatic vesicle slender, elongated, arranged ventrally to the seminal vesicle. The prostatic vesicle extends anteriorly up to the level of the union of the common vas deferens to the seminal vesicle. The anterior section of the prostatic vesicle, or diverticulum (proximal chamber after Marquina *et al.* 2014, p. 117), is arranged ventrally alongside the seminal vesicle. The ventral margin of the seminal vesicle is divided from the dorsal limit of the prostatic diverticulum by a common layer of cuboidal epithelial cells. The ejaculatory duct link the distal portion of the seminal vesicle to the distal section of the prostatic vesicle, near the connection to

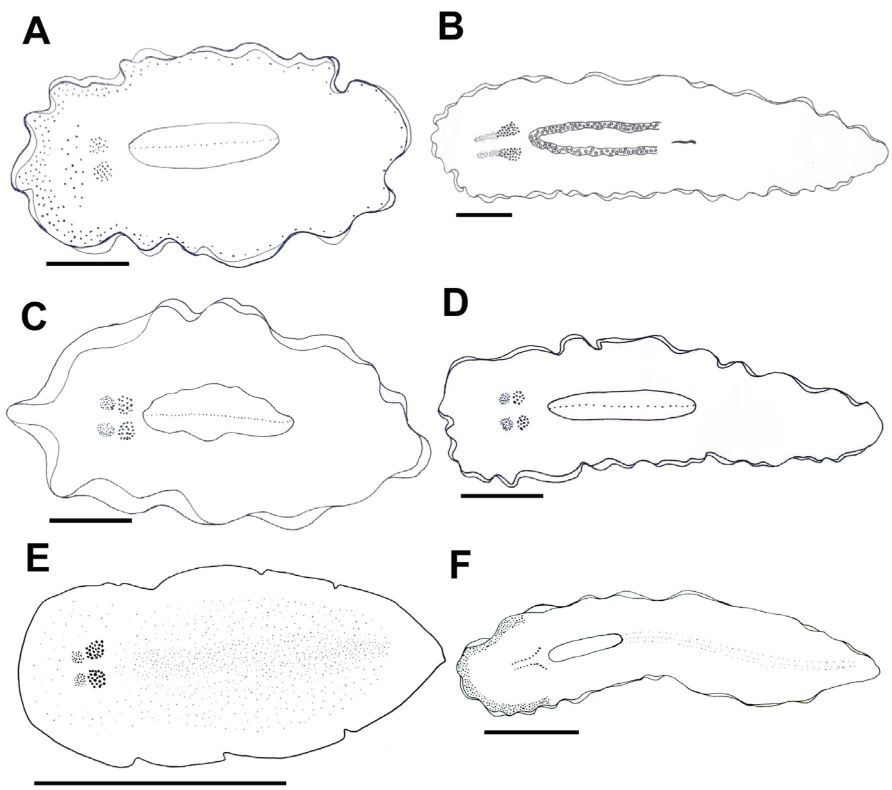

Fig. 9: Diagrams of the external morphology of: A, *Cryptocelis sinopae*; B, *Echinoplana celerrima*; C, *Leptoplana mediterranea*; D, *Leptoplana tremellaris*; E, *Pleioplana okusi*; F, *Prosthiostomum siphunculus.* Scale bars: A-F, 3 mm.

the short unarmed cirrus. With a distinct male atrium and male gonopore. Genital sucker present.

The female gonopore is located 2 mm behind male gonopore. The external vagina extends vertically, curves anteriorly, immersed in a dense mass of cement glands. The internal vagina receives ventrally the paired uterine ducts.

Remarks. The observed characters agree with the specimens described from Tunisia (Gammoudi *et al.*, 2012) and Spain (Marquina *et al.*, 2014). This is the first record from the oriental basin of the Mediterranean Sea.

***Leptoplana tremellaris* (Müller OF, 1773)**
(Figs. 6C and 8D)

Material examined. Five specimens, 22.10.2013, station M1, 5 m deep. Two specimens, 12.05.2014, station M1, 5 m deep. One specimen sagittally sectioned (7 slides).

Description. Body broadly oval, tapering posteriorly. Preserved specimens between 10–22 mm long and 4–10 mm width. Colour pattern lost during fixation. Tentacles lacking, with cerebral and tentacular eyes (Fig. 8D). Genital pit present.

Digestive system. Ruffled pharynx arranged in the second third of body; mouth opens in posterior half of pharyngeal pocket. Intestinal ramifications anastomosing.

Reproductive system. Testes are located mainly in ventral parenchyma between digestive ramifications. The paired vasa deferentia run anteriorly, turning then posteriorly joining in a common vas deferens communicating with a pear-shaped seminal vesicle. The interpolated prostatic vesicle tubular, with a well-developed muscular wall. The prostatic diverticulum is arranged ventral to the seminal vesicle. Between the ventral wall of the seminal vesicle and the dorsal wall of the prostatic vesicle´s diverticulum, there is a layer of parenchymatic tissue. Unarmed cirrus present. Male and female gonopores are well separated, arranged in the last third of body. Genital sucker present.

Female gonopore 1 mm behind the genital sucker. The external vagina runs antero-dorsally, surrounded by numerous cement glands, then curves posteriorly where the paired oviduct opens separately. The distal uteri often filled with eggs, extending anteriorly, joining at level of the post cerebral region. Ovaries are located mainly in dorsal parenchyma.

Remarks. Our specimen of *Leptoplana tremellaris* agrees with the original description of O.F. Müller (1774).

Leptoplana tremellaris and *L. mediterranea* are externally very similar. Only a histological study of the internal anatomy and the embryonic data provide the specific level of evidence to support the identification of these two species. Specifically, the relation of the seminal vesicle to the prostatic diverticulum (Gammoudi *et al.*, 2012). In *L. tremellaris*, there is a layer of parenchymatic tissue between the ventral muscular wall of the seminal vesicle and the dorsal muscular wall of the prostatic diverticulum. However, *L. mediterranea* displays no trace of muscular fibers between the seminal vesicle and the prostatic diverticulum. The ventral margin of the seminal vesicle is separated from the dorsal limit of the prostatic diverticulum by a common layer of cuboidal epithelial cells (Gammoudi *et al.* 2012).

Family Pleioplanidae Faubel, 1983
Genus *Pleioplana* Faubel, 1983
***Pleioplana okusi* Bulnes, Kalkan & Karhan, 2009**
(Fig. 7B, 8 and 9E)

Material examined. One mature specimen, 22.10.2013, station M4, 4 m deep. One specimen, 02.07.2014, station M4, 4 m deep. Both specimens sagittally sectioned (20 and 19 slides).

Description. Body elongated oval 5 mm long, 2 mm wide. Tentacles wanting. Dorsal background color brownish, with a pattern of black spots less densely arranged towards the margin (Fig. 8E). Ventrally colorless. Tentacular eyes present. Cerebral eyes in two clusters, deeply embedded in the parenchyma (Fig. 8E).

Digestive system. Ruffled pharynx located in the second third of body. Mouth opening at mid-body, in the middle of the pharyngeal pocket.

Reproductive system. Male copulatory complex directed backward. The vasa deferentia pierce separately the elongated oval seminal vesicle. The latter oriented horizontally and located near the ventral body wall. Ejaculatory duct projecting into the interpolated oval-shaped prostatic vesicle. Its inner lining folds organized as tubular chambers (Fig. 7A) filling completely the lumen of the prostatic vesicle (Atomata-type, Faubel 1983). Ejaculatory duct distally armed with a sclerotized stylet, housed in a ciliated narrow male atrium (Fig. 6C) opening to the exterior by a small male genital pore.

Female gonopore immediately behind the male one. The narrow external vagina runs dorsally to form the median vagina lined by a deeply folded epithelium. After receiving separately the paired oviducts, continues distally and opens to a spherical Lang's vesicle. Ovaries dorsal (Fig. 7B).

Masses of sperm were observed in the vagina, Lang's vesicle, and protruding from the female atrium (Figs. 6C, 7C and D).

Remarks. The color pattern, eye arrangement, form, and position of the pharynx, as well as the microanatomy of the reproductive system, agree with the description of the specimen of *Pleioplana okusi* from the type locality in the Bosporus Strait (Bulnes *et al*. 2009). The only difference we detected was the absence of any trace of tentacles in the specimen from the Black Sea, while the authors described the presence of tentacular knobs in the specimen from Altinkum (Bosporus Strait).

The presence of spermatozoa in the vagina and protruding from the female gonopore of a specimen of *Pleioplana okusi* suggests that direct copulation may be part of the mating behavior of this species. Galleni & Gremigni (1989) have already proposed that the presence of a Lang's vesicle may be associated with true copulation during mating, and this observation in *P. okusi* may provide further evidence that supports this hypothesis.

Suborder Cotylea Lang, 1884
Superfamily Prosthiostomoidea Bahia, Padula, & Schrödl, 2017
Family Prosthiostomidae Lang, 1884
Genus *Prosthiostomum* Quatrefages, 1845
***Prosthiostomum siphunculus* (Delle Chiaje, 1822)**
(Figs. 7A and 9F)

Material examined. Three mature specimens, 22.10.2013, station M5, 5 m deep. Three mature specimens, 22.10.2013, station M3, 5 m deep. Two mature specimens, 21.01.2014, station M2, 4 m deep. Two specimens, 02.07.2014, station M1, 5 m deep. One specimen, station M4, 4 m deep. The area of the reproductive structures was separated and sagittally sectioned in two specimens (3 slides and 4 slides).

Description. Body elongated with rounded anterior and pointed posterior margins. The cerebral eyes in two clusters. The marginal eyes encircle the anterior body margin not exceeding the level of cerebral eyes (Fig. 8F). Preserved specimens ranging 8–13 mm long and 2–3 mm wide

Digestive system. Tubular plicate pharynx housed in a pharyngeal pocket extending from behind the cerebral organ to the level of reproductive apparatus. Main intestine extending distally up to the level where the body starts tapering posteriorly.

Reproductive system. The paired vasa deferentia lead to the oval-shaped seminal vesicle, which opens to the ejaculatory duct. The latter also receives the sinuous prostatic ducts from two spherical prostatic vesicles (Fig. 6B). The horizontally oriented male antrium houses a penis armed with a tubular stylet. Sucker behind the female gonopore.

Female apparatus characterized by a short external vagina followed by a wide cement pouch (Fig. 6B), hence immersed in a dense mass of cement glands. The vagina continues dorsally, and before reaching the median intestine, turns posteriorly, where the internal vagina connects to the paired oviducts. Lang's vesicle lacking.

Conclusions

The description of a new species of *Cryptocelis* with a unique accessory glandular organ associated with the male copulatory organ has provided the evidence to support a long-time-abandoned interpretation of the reproductive system of the species gathered in this genus. The interpretation of the male system as an extremely elongated prostatic vesicle, producing different secretions along its course without any copulatory organ and completely immersed in a muscular bulb seems inaccurate since the same authors reported the presence of sperm cells in the parenchymal tissue near the female system. Is dermal impregnation possible without the presence of a protrusible copulatory male organ? The characterization of the male copulatory organ of the *Cryptocelis* species as possessing an intrabulbar prostatic vesicle opening to an unarmed secretory cirrus and the male atrium, completely housed in a muscular bulb, agree with our observations, including the presence of spermatophores and sperm cells in the parenchymal tissue.

The remaining collected species are known from the Mediterranean and the European Atlantic coasts, thus widening the geographical range of these species.

The presence of spermatophores on the dorsal surface and sperm cells in the parenchyma of *Cryptocelis sinopae* sp. nov. (Figs. 4E and F), as well as the presence of sperm masses and prostatic secretions in the female canal of *Pleioplana okusi* (Figs. 6C, 7C, and D), provide new evidence supporting the existence of two different mating strategies between polyclads. This introduces the opportunity to study both direct copulation and indirect fertilization via dermal impregnation in the same geographical area under the same ambient conditions. Our data support the deduction of Galleni & Gremigni (1989), who established a relationship between different insemination mechanisms depending on the presence or the absence of a Lang's vesicle. The authors suggest that the presence of a Lang's vesicle is related to direct (true) copulation behavior, while the absence of this vesicle is usually related to indirect copulation mechanisms.

Pleioplana okusi possesses a Lang's vesicle and engages in true copulation, whereas *Cryptocelis sinopae* lacks a Lang's vesicle and assembles spermatophores, which are structures usually associated with dermal impregnation or indirect copulation.

The complementary information provided by the histological studies, as well as the direct observations of living exemplars, support our opinion that detailed morpho-histological studies provide important clues that unravel the relatively unknown behavior of polyclads, such as their reproductive biology.

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