



The genus *Leptoplana* (Leptoplanidae, Polycladida) in the Mediterranean basin. Redescription of the species *Leptoplana mediterranea* (Bock, 1913) comb. nov.

MEHREZ GAMMOUDI^{1,2}, BERNHARD EGGER^{3,4}, SAÏDA TEKAYA¹ & CAROLINA NOREÑA^{2,5}

1: Unité de Recherche “ Biologie de la Reproduction et du Développement animal Département de Biologie, Faculté des Sciences de Tunis”, 2092 Campus Universitaire El Manar Tunis, Tunisia.

2: Museo Nacional de Ciencias Naturales (CSIC), c/ Jose Gutierrez Abascal 2, 28006 Madrid, Spain.

3: Department of Ultrastructure and Evolutionary Research, Institute of Zoology, University of Innsbruck, Technikerstr. 25, 6020 Innsbruck, Austria.

4: Department of Genetics, Evolution and Environment, University College London, Darwin Building, Gower Street, London WC1E 6BT, United Kingdom

5: Corresponding author

E-mail:

mehrezgammoudi@yahoo.fr

bernhard.egger@uibk.ac.at

saida.tekaya@fst.rnu.tn

norena@mncn.csic.es

Abstract

A revision of the genus *Leptoplana* Ehrenberg, 1831 in the Mediterranean basin is undertaken. This revision deals with the distribution and validity of the species of *Leptoplana* known for the area. The Mediterranean sub-species polyclad, *Leptoplana tremellaris* forma *mediterranea* Bock, 1913 is elevated to the specific level. *Leptoplana mediterranea* comb. nov. is redescribed from the Lake of Tunis, Tunisia. This flatworm is distinguished from *Leptoplana tremellaris* mainly by having a prostatic vesicle provided with a long diverticulum attached ventrally to the seminal vesicle, a genital pit closer to the male pore than to the female one and a twelve-eyed hatching juvenile instead of the four-eyed juvenile of *L. tremellaris*. The direct development in *L. mediterranea* is described at 15 °C.

Key words: *Leptoplana tremellaris*, planarian, turbellarian, embryonic development, juvenile, Tunisia

Introduction

The genus *Leptoplana* is one of the most controversial genera within the suborder Acotylea. A reason for this controversy can be found in the fact that the anatomy of the copulatory organs (female and male) of the type species, *L. tremellaris*, differs clearly from the rest of the species that belongs to or has belonged to the genus (Hyman 1939). Since its establishment in 1831 by Ehrenberg, more than 120 polyclads have been described as *Leptoplana*, but after the revisions of different authors, mainly Faubel (1983, 1984) and Prudhoe (1985), the genus is actually constituted by only 3 valid species:

L. tremellaris (Müller OF, 1773) Oersted, 1843: North Sea, Mediterranean Sea

L. vesiculata Hyman, 1939: only known from Mount Doughty, Puget Sound, Washington (North Pacific Ocean)

L. trapezoglana (Schmarda, 1859) Diesing, 1862: only known from Weligama Bay, Sri Lanka (Indian Ocean)

The genus was created by Ehrenberg (1831) for the species *Leptoplana hyalina* found in the coasts of the Red Sea near El-Tor. Unfortunately, the description is limited to external morphological features and is deemed unrecognizable (Lang 1884, Prudhoe 1989). Nonetheless, Lang in 1884 accepted the genus described by Ehrenberg in 1831, considered that the species *L. hyalina* is very similar to *Planaria tremellaris* (Müller OF, 1773) and

synonymised both species. Since then, *L. tremellaris* is considered the first species assigned to the genus *Leptoplana*, as well as the type species (Prudhoe 1989).

Leptoplana tremellaris is one of the most frequently registered polyclad species in the Mediterranean (Grube 1840 (Palermo); Lang 1884 (Naples); Lo Bianco 1888, 1899 (Naples); Pruvot 1897 (Gulf of Lyon); Micoletzky 1910 (Trieste); Palombi 1928 (Port Said), 1936 (Naples); Steinböck 1933 (Adriatic Sea); Arndt 1943 (Naples); Galleni & Gremigni 1989 (Italian coasts); Novell 2001 (Catalonia); Gammoudi et al 2009 (Tunisia) as well as in the North Atlantic (see Prudhoe 1985), but its internal morphology has rarely been described.

Although the type locality of *Leptoplana tremellaris* is in the Norwegian part of the North Sea (Müller 1773, described as *Fasciola tremellaris*), the first description of the internal anatomy and configuration of the male copulatory apparatus was made by Lang (1884), based on material from the coasts of the Gulf of Naples.

Bock (1913), in his study of Polycladida, also offers a full description of the internal anatomy of specimens of *L. tremellaris* from the North Sea (Denmark: Aarhus, Norway: Dröbak; Sweden: Gullmarfjord). Based on his observations, Bock (1913) questioned the synonymy of *L. tremellaris* with *L. hyalina* due to the lack of information on the internal anatomy and the distance given between the type localities of the two species, the North Sea and the Red Sea, respectively. Moreover, comparing the Mediterranean individuals of *L. tremellaris* described by Lang (1884) with the specimens of the North Sea, Bock concluded that the two species differ enough from one another to characterize the specimens of the Gulf of Naples as *Leptoplana tremellaris* forma *mediterranea* and the population of the North Sea as *L. tremellaris* sensu stricto.

Owing to the fact that the only way to recognize a *Leptoplana* species are histologically sectioned series (Lang 1884) and that most of the Mediterranean species have been described through external features only, there are serious doubts about the validity of the determinations and even about the presence of *Leptoplana tremellaris* in the Mediterranean basin.

In the present study we revise the distribution and validity of the species of *Leptoplana* known for the Mediterranean Sea and elevate *Leptoplana tremellaris* forma *mediterranea* to the specific level, as *Leptoplana mediterranea* (Bock, 1913) comb. nov., based on material from the Tunisian coasts. We describe the embryonic development of *L. mediterranea*, compare it with the development of *L. tremellaris*, and we use the different type of juveniles as a diagnostic character.

Material and Methods

Specimens were collected from rocky habitats from the east shores of the lake of Tunis, near Port La Goulette and carefully transferred into a plastic bottle filled with sea water. In the lab, animals were measured and photographed in vivo using an Axio-Cam MRC5 and a Leica DFC490 camera. For histological preparation, animals were fixed in Bouin Hollande fluid. In order to maintain the flat shape of the specimens, the fixation procedure was carried out as follows: live animals (including copulating ones) were put on a cover slide. When they were completely flat, the cover slide with the animal was placed on one edge vertically inside a petri dish and was dropped upside down in a small volume of the fixative contained in the petri dish. The fixed animals were embedded in paraffin and subsequently serially sectioned at 7 µm and stained with eosin and toluidine blue. The reconstruction of the copulatory apparatus was derived from serial sagittal sections. The scheme of classification follows Faubel (1983).

The mode of insemination was determined by fixing animals during or just after copulation, and studying the histological sections for the presence or absence of sperm on the epidermis, in the parenchyma or in the vagina.

For developmental studies, flatworms were kept in a culture room at a temperature of 15 °C and 22 °C in plastic containers covered by a plastic transparent layer. Adults and juveniles were offered algae, calf liver and crustaceans, which they did not ingest.

The plastic layer with deposited eggs was cut out for light microscopical observations of the embryonic development with a Zeiss Axiovert 135 and a Zeiss AxioPlan microscope, and always returned to its fish tanks. Embryonic stages were also recorded as movies using a Lumix camera.

Results

Systematic studies

Superfamily Leptoplanoidea Faubel, 1984

Syn.: Schematommatidea Bock, 1913

Family Leptoplanidae Stimpson, 1857

Genus *Leptoplana* Ehrenberg, 1831

Diagnosis (after Faubel 1983, emended): Leptoplanidae without tentacles with a genital pit between male and female gonopore. Male copulatory apparatus with seminal vesicle and penis developed as eversible unarmed cirrus. Prostatic vesicle with ventral diverticulum at its proximal end. Female apparatus usually with rudimentary Lang's vesicle.

Leptoplana mediterranea (Bock, 1913) comb. nov.

Material examined: A total of 74 specimens were collected. Ten of them were sagittally sectioned, the rest of the specimens served for study of reproduction and embryonic development.

Material deposited (Voucher): One specimen as serial sections (18 slides). Collected on the 4th August, 2009 and deposited in the Museo Nacional de Ciencias Naturales, Madrid. MNCN 4.01/57.

Type locality: Lake of Tunis (36° 49' 06.61" N; 10° 17' 23.37" E). Specimens were collected from under rocks in the Lake of Tunis, Tunisia, a natural lagoon between the city of Tunis and the Gulf of Tunis.

Etymology: Name given by Bock (1913) to the forma "mediterranea" of *Leptoplana tremellaris* and for the geographical area of distribution.

External features: *Leptoplana mediterranea* showed two distinct colour morphotypes, found in the same locality (Figs. 1A–B).

The first morphotype (40 specimens), with a translucent dorsal surface, with a white marginal rim. The digestive branches apparent, which ramify from the main intestine towards the periphery. The digestive branches appear to anastomose only in the pharyngeal region (Fig. 1A).

The second morphotype (34 specimens) with beige dorsal surface and with brown speckles. Pigmentation occurs and is more concentrated in the pharyngeal median region. Digestive ramifications were not apparent (Fig. 1B).

Both morphotypes with an elongate oval body form. The largest specimen was 33 mm long and 20 mm wide, while the smallest one was 25 mm long and 16 mm wide. The body is rounded anteriorly and tapering posteriorly. The maximal width is situated at the level of the anterior third of the flattened body. Tentacles are absent. The cerebral organ is situated near or under the eyes. The eyes are arranged in two groups on either side of the median line. The cerebral eyes are smaller and arranged deeper, the tentacular eyes are arranged posteriorly to the cerebral eyes and lying more superficially (Fig. 1A–C).

The surface is covered with a ciliated cellular epidermis; the basal membrane is two times thicker dorsally than ventrally. The rod-like rhabdites are located between epidermal cells, and are more numerous dorsally than ventrally. The body wall musculature is more developed ventrally than dorsally. The mouth is situated ventrally behind the middle of the body and opens in the posterior third of the pharyngeal pocket. The plicate ruffled pharynx is located in the first two thirds of the body and has up to 15 pairs of heavy folds. The opening of the intestine into the pharynx is directly above the mouth.

Reproductive system: In live animals, the sperm ducts extend anteriorly on both sides of the pharynx and then turn posteriorly (M-shaped) (Fig. 1D) and join in a common expanded sperm duct which curves dorsally to enter the seminal vesicle. The latter is identified easily in vivo: dorsally, as a whitish mass just behind the pharynx and ventrally as a white oval vesicle seen clearly by transparency. The uteri extend from behind the seminal vesicle up to behind the brain, where they converge (Fig. 3F).

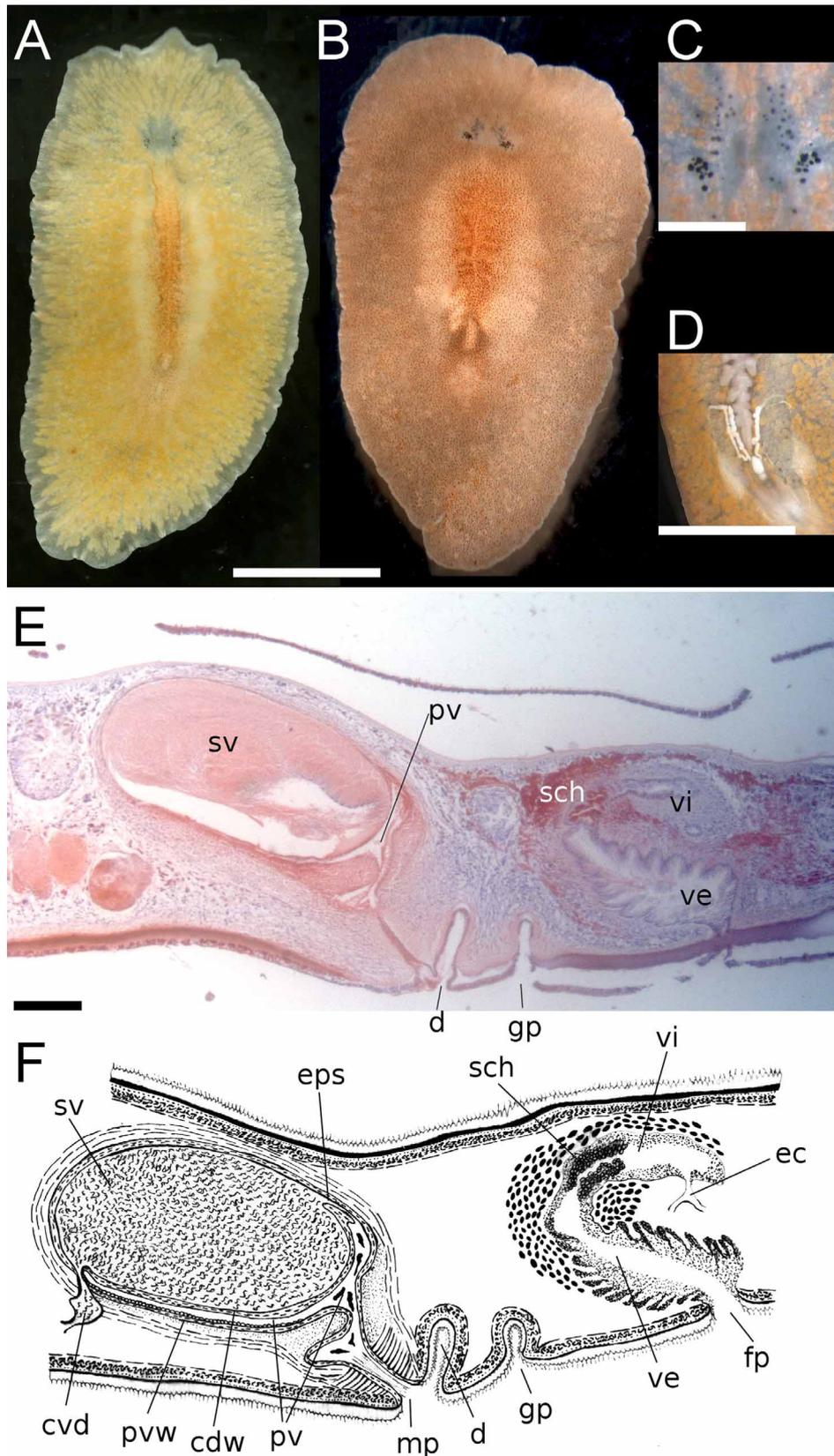


FIGURE 1. Anatomy of *L. mediterranea*. (A) Morphotype 1, dorsal view. (B) Morphotype 2, dorsal view. (C) Eyes. (D) Ventral view of copulatory apparatus. (E) Sagittal section of copulatory apparatus. (F) Diagrammatic representation of copulatory apparatus. cvd: common vas deferens, cdw: common dividing wall, ec: entrance of common uterus, eps: entrance of prostatic vesicle in seminal vesicle, d: diverticulum, fp: female pore, gp: genital pit, mp: male pore, pv: prostatic vesicle, pvw: prostatic vesicle wall, sch: shell chamber, sv: seminal vesicle, ve: vagina externa, vi: vagina interna. Scale bars: 1A–B, 1D: 1 cm; 1C: 1 mm; 1E–F: 0.1 mm

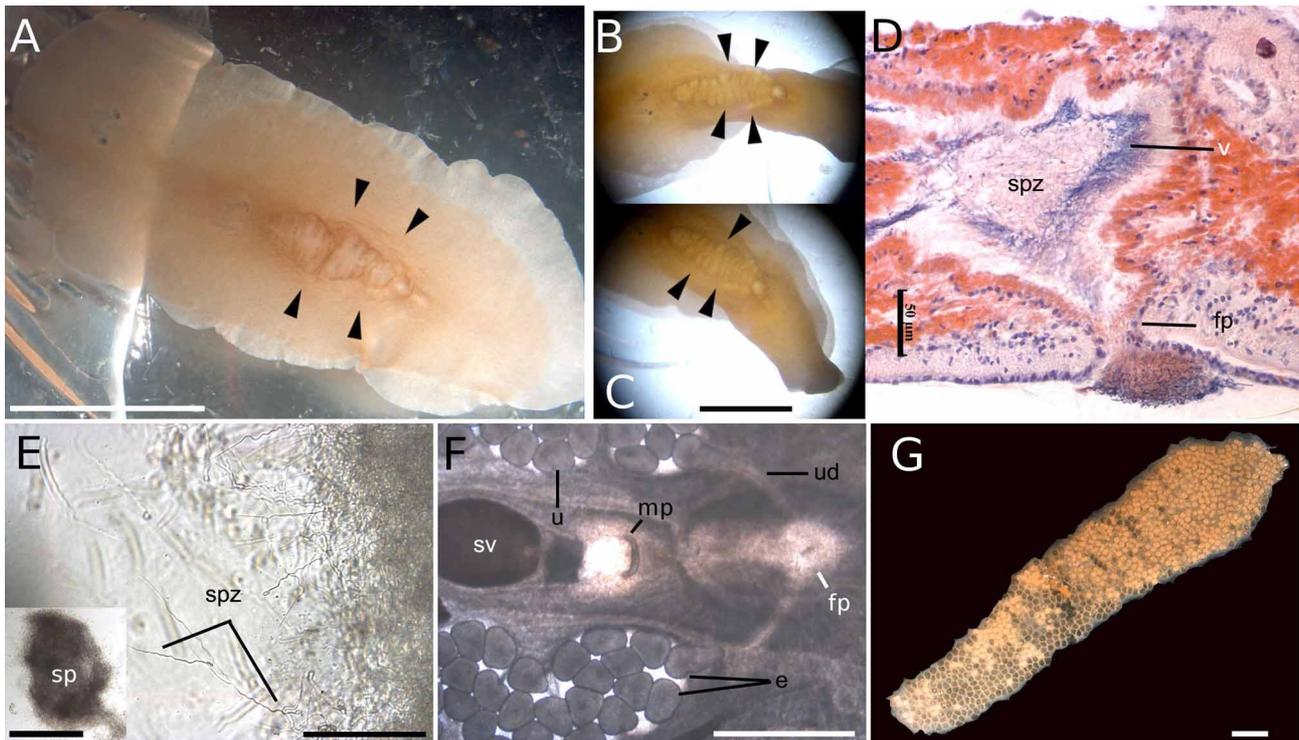


FIGURE 2. Reproduction in *L. mediterranea*. (A–C) Copulation, single pictures from a movie. Arrowheads signify wave contraction movements. (D) Sperm in the vagina shows direct copulation. (E) Spermatophore and spermatozoa. (F) Ventral side showing eggs passing from the uteri to the uterine ducts connected with the vagina. (G) Ribbon-shaped egg plate. Anterior of the animals is left in (A–D, F). e: eggs, fp: female pore, mp: male pore, sp: spermatophores, spz: spermatozoa, sv: seminal vesicle, u: uterus, ud: uterine duct, v: vagina. Scale bars: 2A–C: 1 cm; 2D: 0.05 mm; 2E: 0.02 mm; 2E–F: 1 mm.

Two genital pores are evident in the ventral face, between them and closer to the female pore is the genital pit. The genital pit is a depression of the ventral wall of epidermal nature, also known as genital sucker (Rawlinson et al 2008), but not comparable with the true sucker of the Cotylea due to a lack of characteristic muscle-layers and the position between the genital openings instead of posterior to them (Figs. 1E–F; 3F).

Internally, the male apparatus is situated inside a muscular bulb.

The *vasa deferentia* arise in the ventral parenchyma and open together into a common *vas deferens* which communicates with a well developed (about 400 µm thick and 600 µm long) oval shaped seminal vesicle (Figs. 1D–F; 2A). In some specimens, the seminal vesicle occupies more than two thirds of the whole thickness and it is usually filled with spermatozoa.

The interpolated prostatic vesicle is divided into three sections: proximal, median and distal. The proximal section is an elongated diverticulum which extends along the ventral side of the seminal vesicle to the entrance of the common vas deferens (Figs. 1E–F). Besides the contact zone, both vesicles are separated only by an epithelial common wall, whose ventral side is of a cubic nature (Figs 1F; 2E). There is no musculature between the prostatic and the seminal vesicle. The median section of the prostatic vesicle is orientated dorsally and opens into the dorso-distal region of the seminal vesicle via a narrow opening. The lumen of the distal section of the prostatic vesicle forms a small chamber which opens to the proximal part of the ejaculatory duct, the unarmed penis cirrus. The latter leads to the male atrium that shows a dorso-posteriorly oriented diverticulum. This diverticulum is of various sizes and can reach half of the animal's thickness in some specimens. The male genital pore is wider than the female one.

The oocytes are formed within the ovaries that are mainly distributed dorsally. The eggs in the ovaries show shell granules in the cytoplasm while the eggs in the uterus show a thick layer of granules in the periphery.

The paired uterine ducts unite in a short and narrow common one (Fig. 3F) which opens ventrally into the *vagina interna* (Figs. 1E–F; 2B). The *vagina interna* extends posteriorly and sometimes can develop a rudimentary Lang's vesicle. Extending anteriorly, the *vagina media* is forming a reverse "question mark" (Figs. 1E–F; 2B). The *vagina media* is accompanied by a well developed mass of shell glands. The backward curve of the *vagina media* to the *vagina interna* (the reverse "question mark") is common in polyclads. Just before this backward turn, the

vagina media shows a spiral folded epithelium (Fig. 2B). The *vagina externa* (Fig. 2C) leads then to the female genital pore located in the midventral line. The female pore is about 2 mm behind the male pore. In some specimens, eosinophilous secretions were observed in the lumen of both *vagina externa* and *vagina media*. Other specimens showed a huge mass of spermatozoa in the *vagina externa* (Fig. 3D).

Reproduction and development in *Leptoplana mediterranea* (Bock, 1913) comb. nov.

Copulation and sperm transfer: Specimens were seen to copulate during both day and night. Live observations of the copulating animals show one of the two specimens lying on the other, putting its ventral side against the dorsal side of the partner (Figs. 2A–C). The upper animal fixes the lower one, very probably using its genital pit located between the two genital pores and then makes wave contraction movements with the posterior part of its body (Fig. 2C). The copulation process can be maintained for some hours, and is not interrupted by gliding or swimming movements.

White spherical structures were detected at the water surface of a dish in which two copulating animals had been placed. After microscopic observations, these structures were identified as spermatophores containing a mass of spermatozoa (Fig. 2E). The thread-shaped spermatozoon is wavy. A simple observation with light microscopy shows that in *L. mediterranea*, as in all acotyleans, the two flagella seem to be attached to the sperm shaft.

The histological revision of the material of two copulating exemplars shows the mass of spermatozoa in the vagina of each of the partners and even protruding from the vaginal pore, indicating deposition of spermatophores in the partner (Fig. 2D). This evidence suggests that the transfer of spermatophores is the result of a true copulation and not of hypodermic insemination as inferred, at first, by live observations of the mating behaviour.

During oviposition the rows of eggs pass from the uteri to the uterine ducts and to the vagina and are deposited as elongated egg masses, and glued to a free surface. No parental care was observed.

Early Development: In laboratory cultures, specimens laid egg plates from October to April. The deposited egg plates are ribbon-shaped (Fig. 2G). The cocoons are spherical and about 350 μm in diameter, usually with one, sometimes with two embryos inside (Fig. 4A). Just after constriction of the first polar body, the embryos develop pseudobodies ("blebbing", Fig. 4B) and a contortion in the shape of a crater-shaped impression of the eggs (Fig. 4C). The given time points are from the fastest developing embryos in a batch/a series of batches. The first cleavage occurs 18 HAL (Hours After Laying; average: 21.3 ± 2.4 , $n=18$) and two equal-sized blastomeres are formed (Fig. 4D). Four equal blastomeres are obtained after the second cleavage which occurs 30 HAL (average: 32.5 ± 6.1 , $n=16$) (Fig. 4E). The third and unequal division takes place 46 HAL (average: 48.8 ± 7.8 , $n=14$) (Fig. 4F). Division progresses and the 12-cell stage is attained 60 HAL (average: 63.2 ± 2.8 , $n=8$) (Fig. 4G). Segmentation progresses in a typical spiral pattern, with a very prominent presumptive mesentoblast (Fig. 4H), which is dividing laterally (Fig. 4I). Epidermis is observed to develop cilia at 12 DAL (Days After Laying; average: 14 ± 1.5 , $n=16$). From then on, rotational movements are recorded. The velocity of rotation of the embryos increases with developmental time.

Eye development: The formation of eyes is not synchronic. At 21 DAL (average: 23.9 ± 2.4 , $n=17$), two small eye spots appear at the animal pole of the still spherically-shaped embryos (Fig. 4J). The size of these spots increases and simultaneously the spherically shaped body becomes gradually flattened. At 24 DAL (average: 23.7 ± 3 , $n=18$), each eye divides into two and the four eye spot stage is attained (Fig. 4K). The newly formed eyes are more posterior and peripheral than the older ones. At 30 DAL (average: 31.7 ± 1.3 , $n=9$), the eyes divide again, developing four pairs of eyes (Fig. 4L). The most posterior eyes move posteriad a little and towards the midline of the body where they divide once more to form a posterior group of two pairs of eyes (Fig. 4M). At 37 DAL (average: 39.6 ± 2.1 , $n=8$), the posterior pair of the anterior group divides again to form a fourth anterior pair of relatively big eyes (Fig. 4N).

At 40 DAL (average: 43.8 ± 2.7 , $n=15$), hatching occurs. Juveniles possess six pairs of eyes which form two groups: an anterior first group including four pairs of eyes, arranged near the cerebral ganglions; and a second group located a bit more posteriorly than the first. The second group includes two pairs of eyes relatively smaller than those of the first group.

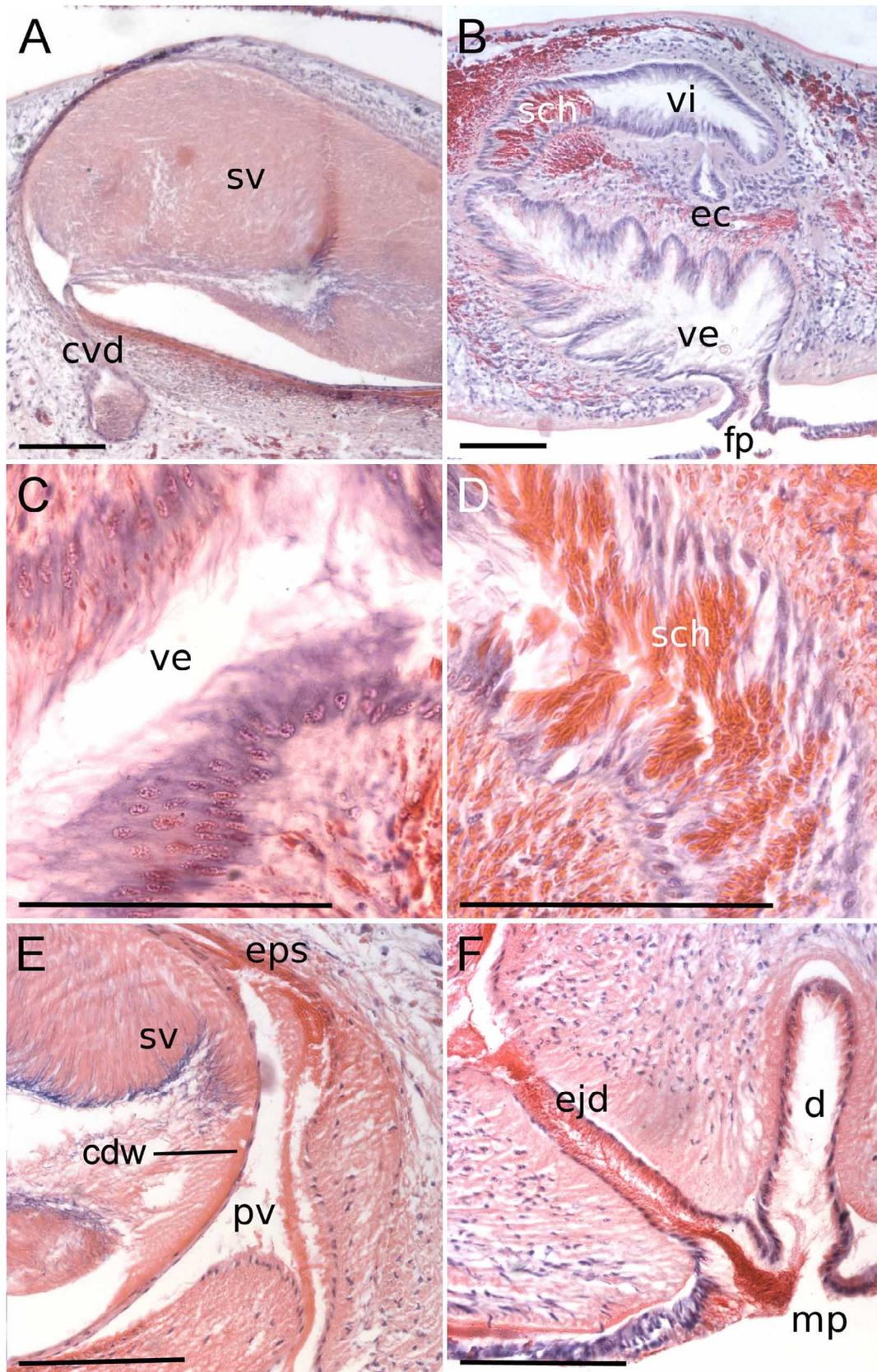


FIGURE 3. Histology of *L. mediterranea*. (A) Proximal part of seminal vesicle. (B) Vagina and common entrance of the uteri. (C) Vagina externa. (D) Shell chamber. (E) Entrance of the prostatic vesicle into the seminal vesicle. (F) Male atrium and distal part of ejaculatory duct. cvd: common vas deferens, cdw: common dividing wall, d: diverticulum, ec: entrance of common uterus, ejd: ejaculatory duct, eps: entrance prostatic vesicle in seminal vesicle, fp: female pore, mp: male pore, pv: prostatic vesicle, sch: shell chamber, sv: seminal vesicle, ve: vagina externa, vi: vagina interna. Scale bar: 0.1 mm

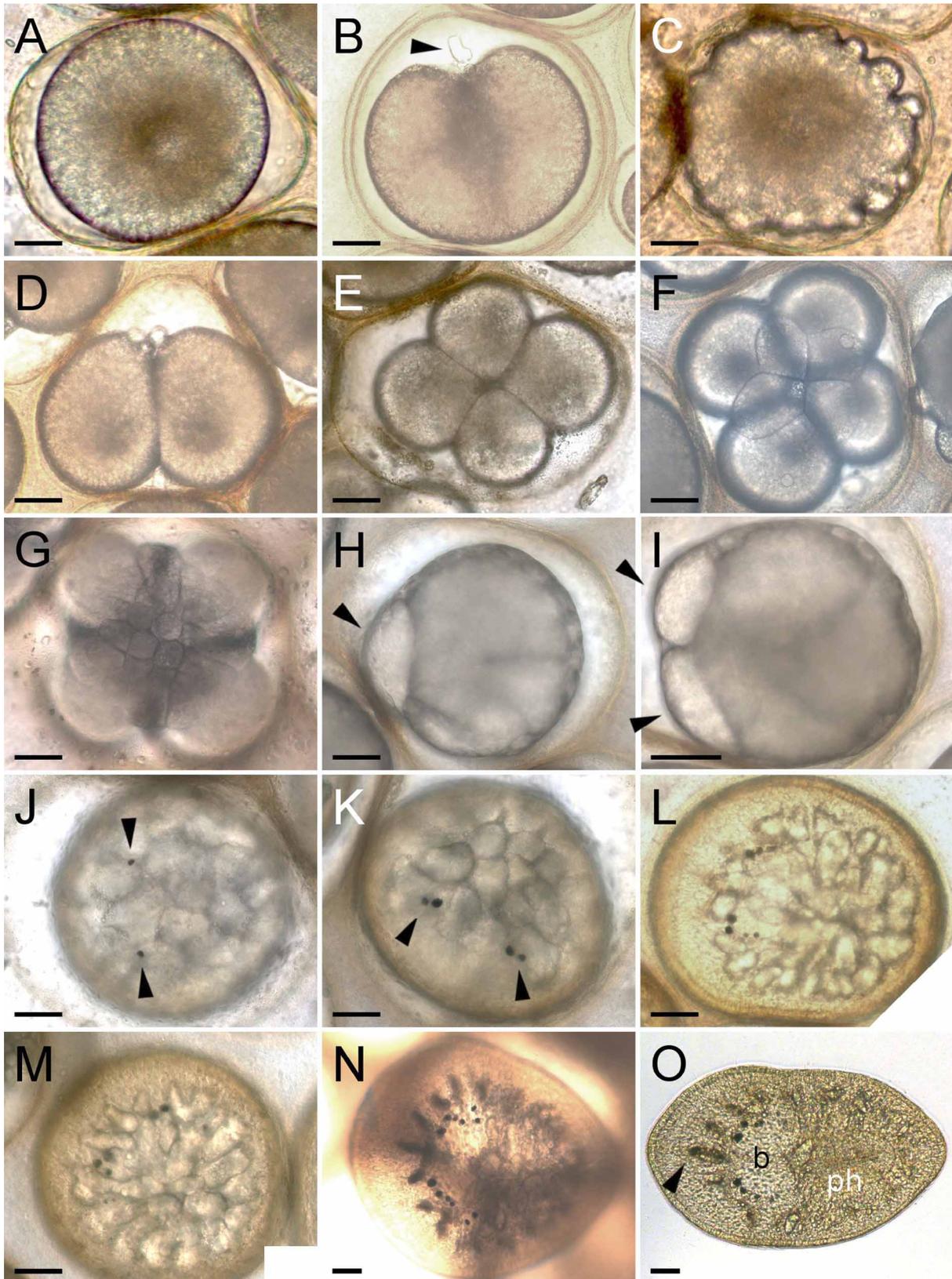


FIGURE 4. Embryonic development in *L. mediterranea*. (A) Embryo at the 1-cell stage. (B) Contortion of the egg during restriction of the polar bodies (arrowhead). (C) Contortions (blebbing) of 1-cell stage embryos. (D) 2-cell stage. (E) 4-cell stage. (F) 8-cell stage. (G) 12-cell stage. (H) Presumptive mesentoblast (arrowhead). (I) Divided presumptive mesentoblast (arrowheads). (J) Two-eyed (arrowhead) embryo. (K) Four-eyed (arrowhead) embryo. (L, M) Six-eyed and ten-eyed embryo (N, O) Hatched twelve-eyed juvenile with yolk-filled gut branches (arrowhead). Anterior is left. b: prominent brain, ph: posterior pharynx. Scale bars: 0.05 mm

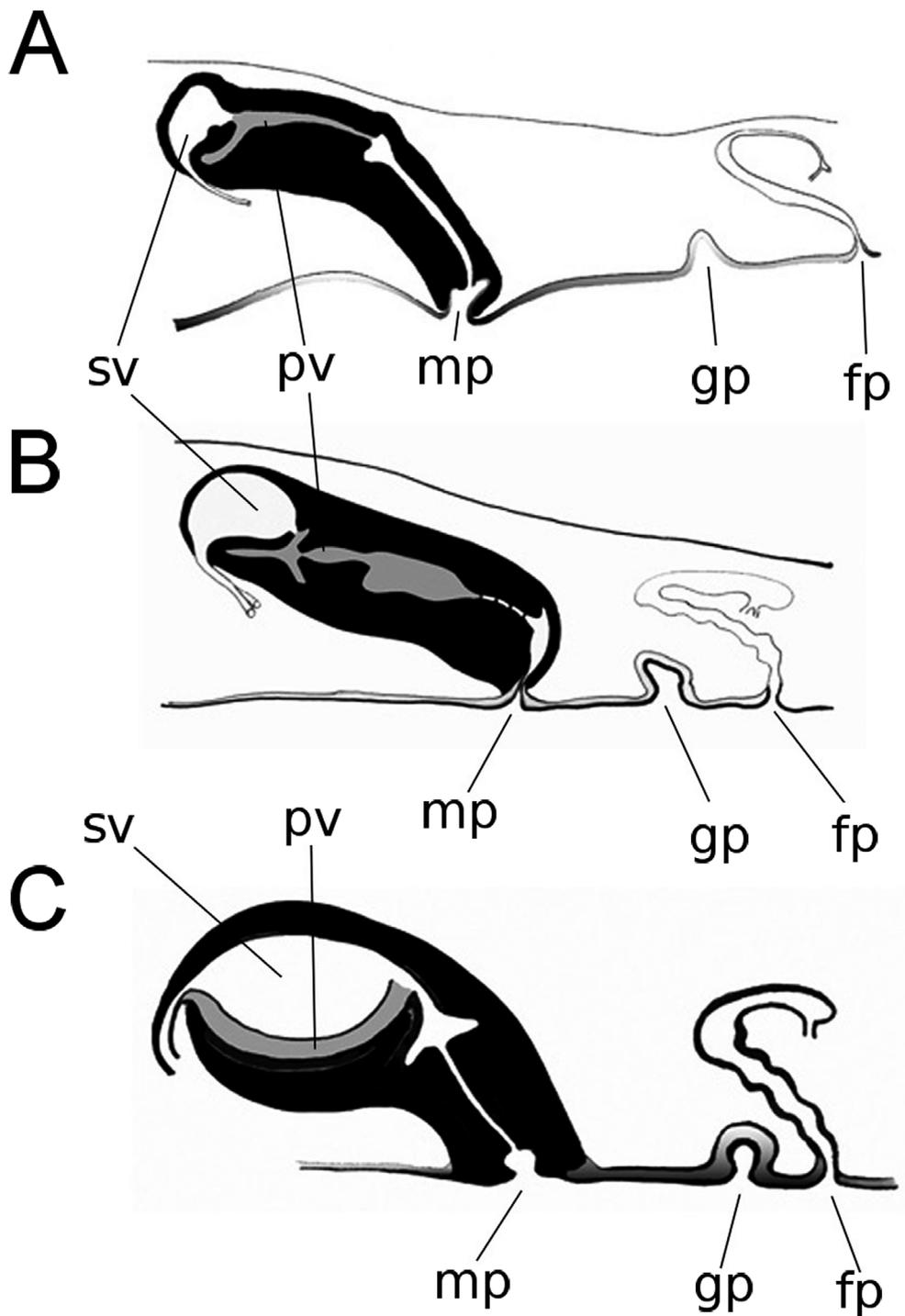


FIGURE 5. Diagrammatic representation of the copulatory apparatus of *L. tremellaris*. (A) Bock (1913), (B) Faubel (1983), (C) Lang (1884). fp: female pore, mp: male pore, pv: prostatic vesicle, gp: genital pit, sv: seminal vesicle.

The newly hatched juveniles are about 500 μm in length, dorsoventrally flattened and tapered at both ends, with cilia covering the external surface. Sensory cilia are observed along the body margin. The pharynx is situated in the posterior half of the body (Fig. 40).

The juveniles survived in Petri dishes at 15 $^{\circ}\text{C}$ for up to 3 weeks, without signs of feeding. In order to test a possible influence of temperature on the speed of eye development in *L. mediterranea* (e.g. juveniles of the same species may be hatching with only four eyes at higher temperatures and with 12 eyes at lower temperatures), 15 egg plates were followed at 22 $^{\circ}\text{C}$ (room temperature). All juveniles hatched with six pairs of eyes, regardless of the temperature.

Discussion

The genus *Leptoplana* is characterized by the absence of tentacles and the presence of a genital pit between the male and the female gonopores. The male copulatory apparatus shows a developed seminal vesicle and the ejaculatory duct acts as an eversible unarmed cirrus. The proximal section of the prostatic vesicle forms a ventral diverticulum.

The species *Leptoplana mediterranea* belongs to the genus *Leptoplana* due to the presence of an unarmed eversible cirrus and the prostatic vesicle with the ventral diverticulum. It differs distinctly from *L. trapezoglana* (Schmarda, 1859) and *L. vesiculata* Hyman, 1939. The latter, whose type locality is situated on the Pacific Northwest Coast of the United States, is characterized by the presence of a well-developed Lang's vesicle, and a dorsal diverticulum of the prostatic vesicle, which is absent in *L. mediterranea*. On the other hand, *L. trapezoglana* described by Stummer-Traunfels (1933) from the Indian Ocean lacks a well-developed ventral diverticulum of the prostatic vesicle.

Our species shows clear similarity with *Leptoplana tremellaris* (Müller O.F., 1773). Externally both species are almost impossible to separate. Only within the internal anatomy and through sagittally sectioned series significant differences can be found: before the common entrance into the seminal vesicle, the *vasa deferentia* form a characteristic bladder in *L. mediterranea*, which is not present in *L. tremellaris*. The average size of the seminal vesicle in mature specimens of *L. mediterranea* is clearly larger and with a different shape. The seminal vesicle of *L. tremellaris* is pear-shaped (Figs. 5A-B), while that of *L. mediterranea* is oval (Figs. 1D-E). The ventral diverticulum of the prostatic vesicle presents a common dividing wall with the seminal vesicle, and runs to the entrance of the common vas deferens (Figs. 1D-E; 3E; 5C) in contrast to the well-separated and muscle-surrounded prostatic vesicle of *L. tremellaris* (Fig. 5A-B). Furthermore, the opening of the seminal vesicle into the prostatic vesicle in *L. mediterranea* is small and dorso-caudally located; while in *L. tremellaris* it is wide and caudally positioned.

Further, the distinction of *Leptoplana mediterranea* is marked by the presence of a genital pit nearer to the male pore and not to the female one as in other species of the genus *Leptoplana* (Figs. 1F; 5A-C).

In respect to the embryonic development, the hatching of a 12-eyed juvenile (instead of a four-eyed one) supports strongly our anatomical findings that *Leptoplana mediterranea* is a species different from *L. tremellaris*. The development of *L. tremellaris* was described by many authors: Vaillant (1868), Keferstein (1868), Hallez (1879) and Selenka (1881). All the authors mentioned juveniles hatching with two pairs of eyes and they worked on specimens collected from localities different to the Mediterranean Sea. Vaillant (1868): The English Channel (between the British Island and France); Keferstein (1868): Saint Malo (North of France); Hallez (1879): Wimereux (North of France); Selenka (1881): Concarneau (North of France). On the other hand, Lang (1884) mentioned in his study of development some specimens of Mediterranean juvenile polyclads ("leptoplanids") with 6 pairs of eyes, possibly referring to juveniles of *L. mediterranea*.

In the present study, and based on new material, we consider the differences between both "formas" described by Bock (1913) as *Leptoplana tremellaris* forma *mediterranea* and *L. tremellaris* sensu stricto to be substantial enough to determine the "forma mediterranea" as a distinct species and to elevate it to the specific level.

According to the latest version of the "International Commission on Zoological Nomenclature" (ICZN) (articles: 15.1 and 45.6.4), *Leptoplana mediterranea* was made available by Bock in 1913, even though he only made a conditional proposal of a new name. Following articles 73.1.2 and 73.1.4 of the ICZN, the holotype of *Leptoplana mediterranea* (designated by Bock 1913 in the text) is the specimen drawn by Lang (1884) in figure 9 of table 14.

Conclusions

According to Faubel (1983) and Prudhoe (1985), there are only three valid species of the genus *Leptoplana*. These species are the following: *L. tremellaris* (Müller OF, 1773), *L. trapezoglana* (Schmarda, 1859) and *L. vesiculata* Hyman, 1939.

Within the Mediterranean basin only two species were cited: *Leptoplana tremellaris*, well distributed along the occidental and oriental coasts of the Mediterranean Sea and *L. diaphana* (Stummer-Traunfels, 1933), known from the Gulf of Naples, which Faubel (1983) considers as *nomen nudum* and which was described by Stummer-Traunfels inside the re-description of *L. trapezoglana*, where it has often gone overlooked. Marcus & Marcus (1968)

moved the species to the genus *Stylochoplana*, due to the lack of a genital pit and a ventral diverticulum in the prostatic vesicle, which are considered diagnostic characters of the genus *Leptoplana*. Although Faubel (1983) chose to consider *L. diaphana* as *nomen nudum*, the new location within the genus *Stylochoplana* is accepted by Prudhoe (1985).

Numerous observations of *L. tremellaris* over the years and along the coasts of the Mediterranean Sea have been undertaken on the basis of external features. The first reconstruction of the internal anatomy was given by Lang in 1884 who determined his specimens as *L. tremellaris* due to their external morphology.

Subsequent works that mentioned and described the internal anatomy of *L. tremellaris* in the Mediterranean area could be found in the 21st century (Novell 2001 and Gammoudi et al. 2009), but after the descriptions given in both works, the determined specimens definitely belong to *L. mediterranea* and not *L. tremellaris*.

Based on these data and the information reached in the present study we strongly support the zoogeographical hypothesis that the distribution of *L. tremellaris* is limited to the eulitoral areas of the North Atlantic, and within the Mediterranean basin the only representative of the genus *Leptoplana* is *L. mediterranea* (Bock, 1913).

Acknowledgements

The authors are indebted to Dr. Anno Faubel and two anonymous referees for their valuable discussions and comments. We would like to thank Bechir ben Rached, Raja ben Ahmed, Ío Almagro Padilla, Raimund Schnegg, Veronika Prantl, Stefanie Pontasch and Diana Delicado for their help collecting polyclads. M.G. was supported by the Spanish Agency for International Development (AECID) fellowship and the Tunisian ministry of High Education, Scientific Research and Technology. B.E. was supported by a Sparkling Science grant from the Austrian Ministry of Science and Research and a Leverhulme grant (F/07 134/DA) and a BBSRC grant (BB/H006966/1), and C.N. and B.E. were supported by the "Acciones Integradas - Wissenschaftlich-technisches Abkommen" funded by the Austrian Agency for International Cooperation in Education & Research (OeAD) and the Spanish Ministry for Science and Innovation (MICINN, project ref: HA2008–0020), as well as by the Spanish Agency for International Cooperation (AECI Project ref.: A/025685/09 and A/030038/10). This research received support from the SYNTHESYS Project <http://www.synthesys.info/> which is financed by European Community Research Infrastructure Action under the FP6 "Structuring the European Research Area" Programme and grant CGL2010-15786 from the Spanish Ministry of Science and Innovation..

References

- Arndt, W. (1943) Polycladen und maricole Tricladen als Giftträger. *Memórias e Estudos do Museu Zoológico da Universidade de Coimbra*, 148, 1–15.
- Bock, S. (1913) Studien über Polycladen. *Zoologiska Bidrag från Uppsala*, 2, 31–344.
- Ehrenberg, C.G. (1831) Phytozoa, Turbellaria africana et asiatica. In: Hemprich P. C. und Ehrenberg C.G. (Eds.) "*Symbolae physicae. Animalia evertibrata exclusis insectis recensuit Dr. CG Ehrenberg. Series prima cum tabularum decade prima*. Berolini, Berlin.
- Faubel, A. (1983) The Polycladida, Turbellaria. Proposal and establishment of a new system. Part I. The Acotylea. *Mitteilungen des hamburgischen zoologischen Museums und Instituts*, 80, 17–121.
- Faubel, A. (1984) The Polycladida, Turbellaria. Proposal and establishment of a new system. Part II. The Cotylea. *Mitteilungen des hamburgischen zoologischen Museums und Instituts*, 81, 189–259.
- Galleni, L. & Gremigni, V. (1989). Platyhelminthes-Turbellaria. In: Adiyodi KG & Adiyodi RG. (Eds.), *Reproductive biology of invertebrates*, Vol. IV, Part A. *Fertilization, development and parental care*. John Wiley and Sons, New York, pp. 63–89.
- Gammoudi, M. Tekaya, S & Noreña, C. (2009) Contribution to the knowledge of Acotylean Polyclads (Platyhelminthes Polycladida) from Tunisian Coasts. *Zootaxa*, 2195, 43–60.
- Grube, A.E. (1840) *Actinien, Echinodermen und Würmer des adriatischen und Mittelmeers, nach eigenen Sammlungen beschrieben*. Königsberg, 92pp.
- Hallez, P. (1879) Contributions à l'histoire naturelle des Turbellariés. *Travaux de l'Institut Zoologique de Lille et de la Station Maritime de Wimereux*, 2, 1–213.
- Hyman, L.H. (1939) New species of flatworms from North, Central and South America. *Proceedings of the United States National Museum*, 86, 419–439.
- Hyman, L.H. (1939) Some polyclads of the New England coast, especially of the Woods Hole region. *Biological Bulletin of the Marine Biological Laboratory, Woods Hole*, 76, 127–152.
- Keferstein, W. (1868) Beiträge zur Anatomie und Entwicklungsgeschichte einiger Seeplanarien von St. Malo. *Abhandlungen der Königlichen Gesellschaft der Wissenschaften zu Göttingen*, 14, 1–38.

- Lang, A. (1884) Die Polycladen (Seeplanarien) des Golfes von Neapel und der angrenzenden Meeresabschnitte. Eine Monographie. *Fauna und Flora des Golfes von Neapel* 11. ix + 688 pp. Leipzig.
- Lo Bianco, S. (1888) Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del golfo di Napoli. *Mitteilungen aus der zoologischen Station zu Neapel*, 8, 385–440.
- Lo Bianco, S. (1899) Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del golfo di Napoli. *Mitteilungen aus der zoologischen Station zu Neapel*, 13, 48–573.
- Marcus, Ev. & Marcus, Er. (1968) Polycladida from Curaçao and faunistically related regions. *Studies on the Fauna of Curaçao and other Caribbean Islands*, 26, 1–133.
- Micoletzky, H. (1910) Die Turbellarienfauna des Golfes von Triest. *Arbeiten aus dem Zoologischen Institut der Universität Wien*, 18, 167–182.
- Müller, O.F. (1773) Vermivm terrestrium et fluviatilium, seu animalium Infusoriorum, Helminthicorum et Testaceorum, non marinorum succincta historia. Havniae et Lipsiae: Apud Heineck et Faber, Typis Martinus Hallager, 1773-1774. Vol I, Part 1, 1–80
- Novell, C. (2001) Contribució al Coneixement dels Turbellaris Policlàdides del Litoral Català. Tesis doctoral, Universitat de Barcelona, 262 pp. http://www.tdr.cesca.es/ TESIS_UB/AVAILABLE/TDX-0107104-121621//Tesis.PDF
- Palombi, A. (1928) Report on the Turbellaria (Cambridge Expedition Suez Canal 1924). *Transactions of the Zoological Society of London*, 22, 579–631.
- Palombi, A. (1936) Policladi liberi e commensali raccolti sulle coste del Sud Africa, della Florida e del Golfo di Napoli. *Archivio Zoologico Italiano*, 23, 1–45.
- Prudhoe, S. (1985) *A Monograph on Polyclad Turbellaria*. Oxford University Press, Oxford, 259 pp.
- Prudhoe, S. (1989) Polyclad turbellarians recorded from African waters. *Bulletin of the British Museum of Natural History*, 55, 47–96.
- Pruvot, G. (1897) Essai sur les Fonds et la Faune de la Manche occidentale (Côtes de Bretagne) comparés à ceux du Golfe du Lion. *Archive de Zoologie Expérimentale et Générale*, 3 (5), 19–20, 617–660.
- Selenka, E. (1881) Zoologische Studien. II. Zur Entwicklungsgeschichte der Seeplanarien. Ein Beitrag zur Keimblätterlehre und Descendenztheorie, 4, 1–36.
- Steinböck, O. (1933) Die Turbellarienfauna der Umgebung von Rovigno. *Thalassia*, 1, 1–33.
- Stummer-Traunfels, R V. (1933). Polycladida. In: Bronn's Klassen und Ordnungen des Tierreichs, Band IV, Abt. 1c, Lief. 179: 3485–3596. Akademische Verlagsgesellschaft, Leipzig : C.F. Winter, 1880–1988.
- Vaillant, L. (1868) Remarques sur le développement d'une planaire dendrocèle, le *Polycelis laevigatus* Quatrefage. *Mémoires de l'Académie des Sciences et Lettres de Montpellier*, 7, 93–108.