Boninia neotethydis sp. nov. (Platyhelminthes: Polycladida: Cotylea)—the first lessepsian flatworm

M. Curini-Galletti* and P. Campus

Dipartimento di Zoologia e Genetica Evoluzionistica, Università di Sassari, via Muroni 25, I-07100 Sassari, Italy.
*Corresponding author, e-mail: curini@uniss.it

Based on specimens collected in the Mediterranean and Red Sea coasts of Israel, the first lessepsian flatworm (*Boninia neotethydis* sp. nov.) is described. The genus *Boninia* includes cotylean polyclads characterized by the presence of multiple accessory (prostatoid) organs. The three species described are distributed in the western Pacific (*B. mirabilis*) and in the Caribbean (*B. antillarum* and *B. divae*). The new species differ from congenerics in the number and arrangement of the prostatoid organs, and in the number of marginal eyes. Specimens of the new species feed on mussels, and their possible effects on Mediterranean mussel cultures should be monitored.

INTRODUCTION

There is a growing concern about the problem of allochtonous marine species. Introduced species may in fact affect the structure of native communities, and, in the worst case scenario, become serious pests, affecting local levels of biodiversity and the economy of entire areas (Ruiz et al., 1997).

The opening of the Suez Canal resulted in a massive migration of exotic species into the Mediterranean, the so-called 'lessepsian migrants' (see Galil, 2000, for a recent overview). Reports of these migrants, however, are mostly limited to conspicuous taxa, such as fish and molluscs, with few records of 'lesser', inconspicuous groups, raising suspicions that our understanding of the actual extent of lessepsian migration may be based on non-representative data.

Members of the Polycladida (Platyhelminthes), mostly with pelagic larval development, appear as potential candidates to yield lessepsian migrants—yet, both Mediterranean and Red Sea polyclads have received limited (if any) attention in recent decades. However, since many polyclads are serious pests to bivalve cultures (especially oysters and mussels, but even to farmed *Tridacna gigas* (L., 1758) (Newman et al., 1993)), the taxon deserves closer attention.

Research on interstitial flatworms on both the Red Sea and the Mediterranean coasts of Israel revealed the presence of a new species of cotylean polyclad, representing the first documented case of lessepsian migrant among Platyhelminthes.

MATERIALS AND METHODS

Specimens were collected from sandy habitats or from shell grit by scooping the superficial layer of sediment. Extraction of the animals from the sediment was through $MgCl_2$

decantation (see Martens, 1984). For microscopical analysis, specimens were fixed in Bouin's fluid, embedded in 56°C paraplast, and serial sections were cut at 4 μm, stained with Mayer's haematoxylin and eosin and mounted in Depex. Three specimens were maintained alive in an aquarium, and were fed weekly with specimens of the mussels *Mytilaster minimus* (Poli, 1795).

Karyological techniques were as described by Curini-Galletti et al. (1989). Relative lengths (r.l.=length of chromosome×100/total length of haploid genome) and centromeric indices (c.i.=length of short arm×100/length of entire chromosome) were obtained from measurements of camera lucida drawings of five metaphaic plates from two larvae. Karyometrical data (means and standard deviation) are presented in the karyotype formula, as follows: haploid genome absolute length (in μm); relative length and centromeric index of each chromosome; chromosome nomenclature between parentheses (m=metacentric; sm=submetacentric).

Cladistic analysis was based on the following morphological characters: (1) prostatoid vesicle; (2) muscular coating of the seminal vesicle; (3) ciliated penis papilla; (4) ciliated ejaculatory duct; (5) ciliated male antrum; (6) prostatoids connected to the male antrum; (7) prostatoid organs arranged into one girdle (0), two girdles (1), three girdles (2); (8) prostatoid stylet straight (0); tapering into a distinct point (1); (9) embedded portion of the stylet longer than the protruding part; (10) Lang's vesicle non-ciliated (0), ciliated (1), partly ciliated (2). Except in the case of multistate characters and when otherwise indicated, absence of the character described is marked in Table 1 as 0; presence as 1. All characters were unweighted and unordered. *Paraboninia caymanensis* Prudhoe, 1944 was chosen as the outgroup. The matrix was edited in MacClade (Maddison & Maddison, 1992), and the parsimony analysis performed in Paup
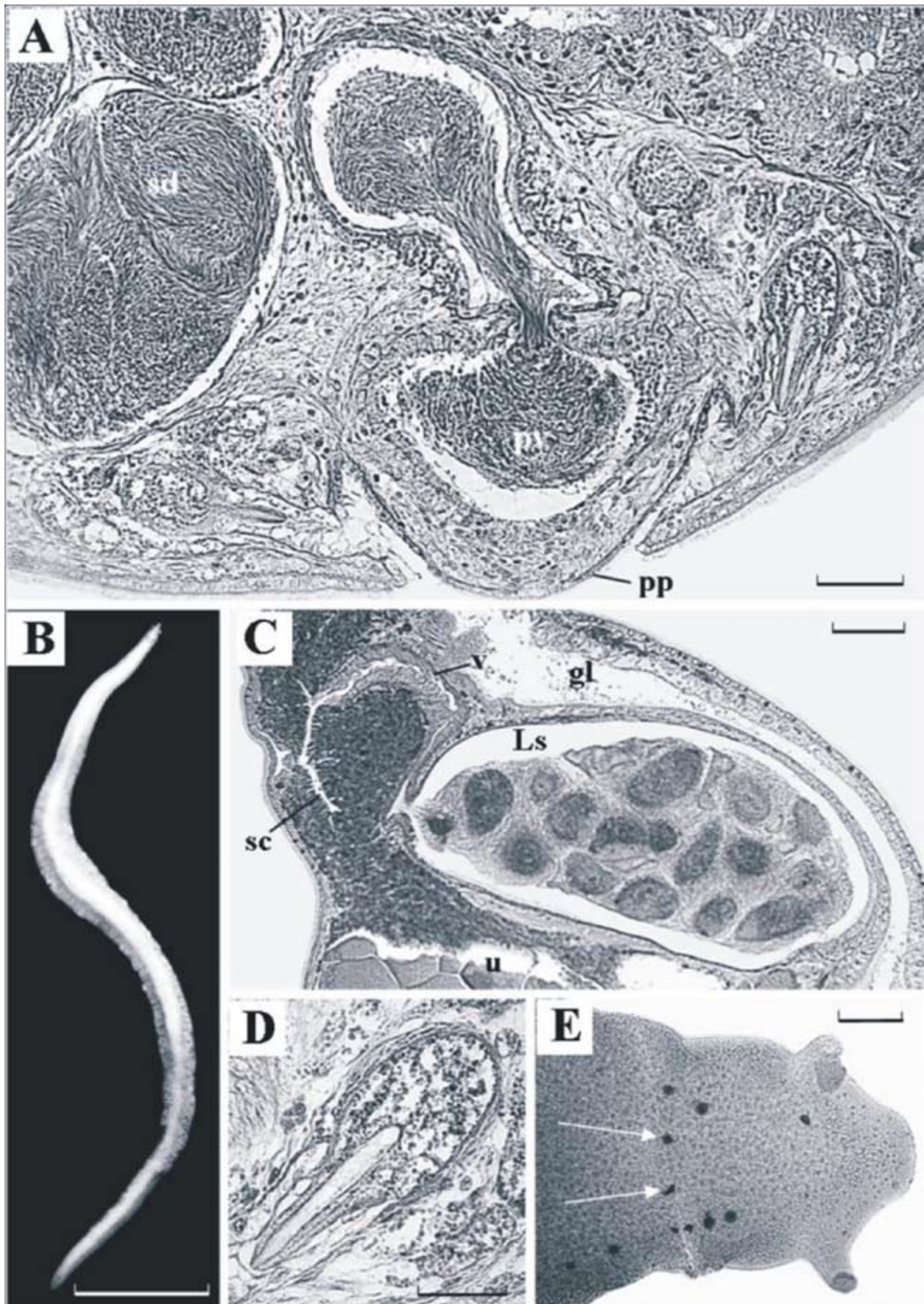
Figure 1. (A–E) *Boninia neotethydis* sp. nov. (A) Holotype (SMNH 6345 f), male genital organs; (B) living specimen; (C) holotype, female genital organs; (D) holotype, prostatoid organ; (E) cephalic region of a juvenile specimen, whole mount (Akko); arrows point to the cerebral eyes. Scale bars: A, 50 μm; B, 10 mm; C, E, 100 μm; D, 25 μm.

4.0b (Swofford, 1998). Exhaustive search (with collapse of branches if maximum length is zero) was applied, and all minimal trees were kept. Bremer support values were calculated with AutoDecay (Eriksson, 1998).

Voucher specimens of *Boninia mirabilis* Bock, 1923, holotypes of *B. divae* du Boys-Reymond Marcus & Marcus, 1968, and syntypes of *B. antillarum* (Hyman, 1955) were loaned by the Swedish Museum of Natural History (SMNH), where the type material of the new species is deposited. The remaining material is stored in the Zoological Museum at the Dipartimento di Zoologia e Genetica Evoluzionistica, University of Sassari (Italy).

Abbreviations used in figures:

cg, cement glands; cm, circular muscles; e, egg; ed, ejaculatory duct; fp, female pore; gl, gut lumen; lm, longitudinal muscles; Ls, Lang's vesicle; lu, left uterine vesicle; luc, left uterine canal; ma, male antrum; mp, male pore; pg, prostatoid glands; po, prostatoid organ; pog, prostatoid organ glands; pp, penis papilla; pv, prostatoid vesicle; ruc, right uterine canal; s, stylet; sc, 'shell chamber'; sd, sperm duct; sp, sperm; sv, seminal vesicle; u, uterine vesicle; v, vagina.

SYSTEMATICS
Order POLYCLADIDA Lang, 1884
Suborder COTYLEA Lang, 1884
Family BONINIDAE Bock, 1923
Genus *Boninia* Bock, 1923
Boninia neotethydis sp. nov.
(Figures 1A–E, 2B & 3)

Type material

Holotype: posterior half of a mature specimen, sagittally sectioned and mounted on 11 slides (SMNH 6345 a-k) (Israel: Eilat (Red Sea), in front of the Interuniversity Institute of Eilat, H. Steinitz Marine Biology Laboratory, 29°30'09"N 34°55'05"E), in coarse sediment (shell and madrepore fragments) pockets, on a limestome platform, at a depth of about 15 cm (collected on 8 October 1998).

Other material

- Same data as holotype: two specimens studied alive and processed for karyology; several juveniles observed alive from intertidal coarse sand, beach in front of the Steinitz Marine Biology Laboratory; February 1987.

- Same data as holotype: several specimens, observed alive, March 1988.

- Israel: Akko (Mediterranean Sea), at the base of the northern walls of the old town; in shell grit, mostly consisting of valves of the bivalve *Brachidontes pharaonis* (Fischer P., 1870), in shallow water pools among rocks: posterior half of one specimen, tangentially sectioned and mounted on two slides; four specimens sagittally sectioned (one on one slide, two on two slides, one on three slides); three whole mounts: two posterior halves, one cephalic region (juvenile). All collected on 13 October 1998.

- Israel: Shiqmona (Mediterranean Sea), in front of the National Institute of Oceanography, in shell grit in crevices of rocky pools: posterior half of one specimen, sagittally sectioned, collected on 14 October 1998.

Comparative material examined

Boninia mirabilis Bock, 1923: SMNH 15141; 15139; 15138a,b, from Samboanga (Philippines); *B. divae* du Boys-Reymond Marcus & Marcus, 1968: SMNH 5074a,b (holotype), from Curacao, Piscadera Baai; *B. antillarum* (Hyman, 1955); SMNH 5075a; 5075b, 5075c, 5075b, from Curacao.

Etymology

The specific epithet refers to the Neo-Tethys, the term introduced by Por (1990) to indicate the new biogeographical province resulting from the resumed connection, via the Suez Canal, between the Mediterranean and the Red Sea, severed since the closure of the Tethys Sea during the Miocene.

Diagnosis

Boninia with very few marginal eyes (3–8 per side) and four cerebral eyes, and with a penis papilla surrounded by a single girdle of 10–18 prostatoid organs, bearing a stylet tapering into a distinct point.

Description

Living worms (Figure 1B) appear milky-white, slender and elongate, variable in shape. When crawling, an adult specimen may extend to 60 mm long, and about 5 mm broad. The pointed tentacles are located at the sides of the head, and appear flexible and mobile in living specimens.

The small marginal eyes are few in number, ranging 3–8 at each side of the head (Figure 1E). In most specimens, a pair of marginal eyes was distinctly anterior to the others, and placed at the base of the tentacles. Two cerebral eyes lie at each side of the brain. Members of this pair lie very close to each other, and point to different directions.

Dorsally, the epidermis is ciliated (cilia length: 6–7 μm) and columnar, 10–11 μm high in the holotype, with numerous ovoid rhabdites, about 10 μm long at their longest axis, and provided with very numerous mucous glands. The ventral epithelium is ciliated (cilia length: 4–7 μm), cubical, about 6 μm high, with fewer and finer rhabdites and very few mucous glands.

The basal lamina is very thick dorsally, poorly developed ventrally. The two sides of the body also differ for the development of the subepidermal longitudinal musculature, which is very strongly developed ventrally, much weaker dorsally.

A small (about 250 μm long in the holotype) adhesive area ('sucker') lies ventrally, at the extreme caudal end of the body. Its columnar epithelium (about 13 μm high) is non-ciliated and provided with densely packed adhesive glands. Subepidermally, numerous transverse muscle fibres are present, overlying a very thick layer of longitudinal fibres. Whilst the latter fibres continue into the subepidermal longitudinal musculature of the ventral side of the body, transverse fibres disappear outside of the adhesive area.

The ruffled pharynx is extremely long, and occupies more than one third of the length of the body, extending into its posterior half. The mouth is nearly central. The main-gut is median, and extends from some distance behind the brain to near the posterior end of the body, giving off numerous lateral branches throughout its length.
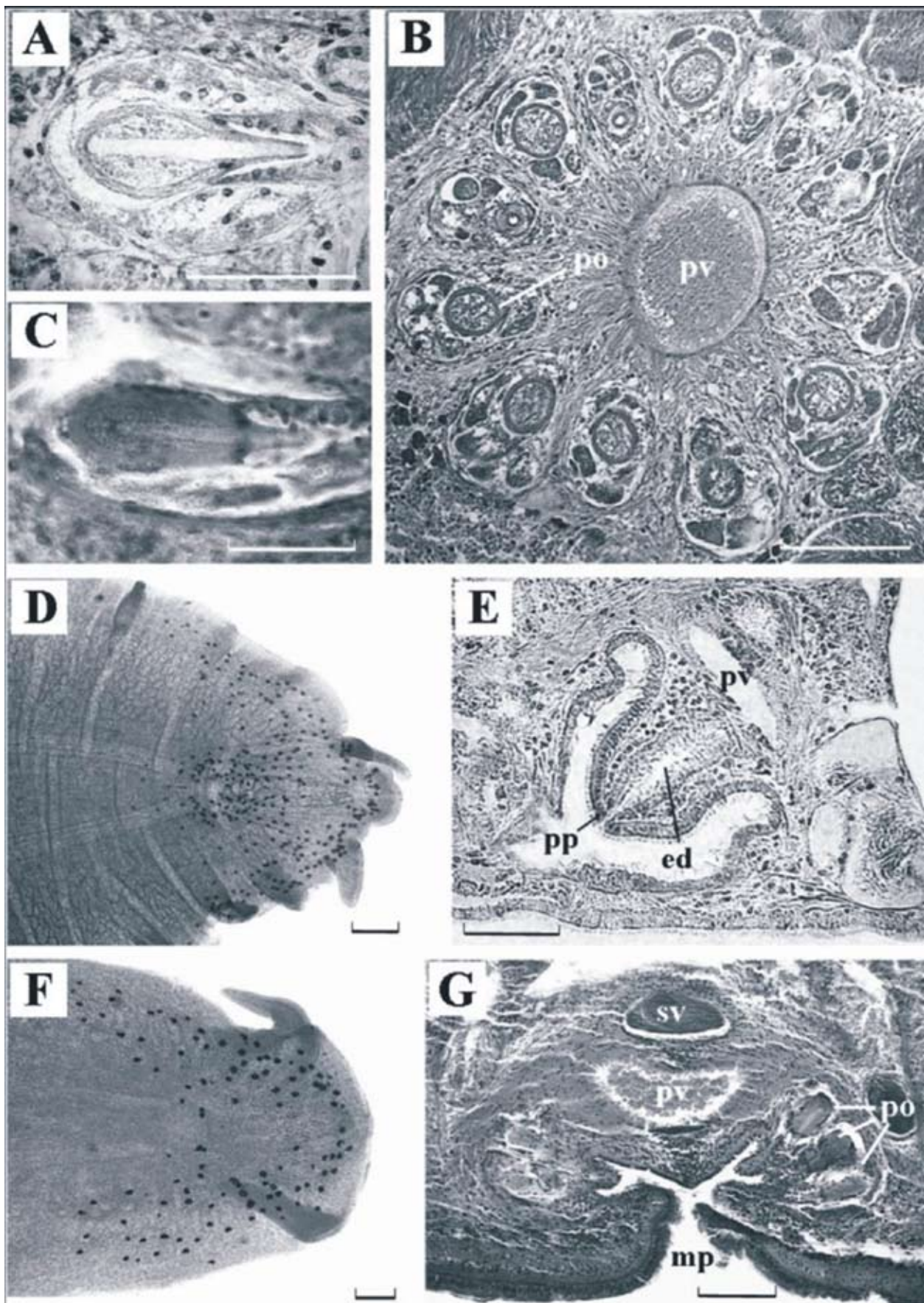
Figure 2. (A) *Boninia* sp. (from Samboanga) (SMNH 15141), prostatoid organ; (B) *Boninia neotethydis* sp. nov., tangential section of the male genital organs (Akko); (C) *Boninia divae*, holotype (SMNH 5074 a), prostatoid organ; (D) *Boninia divae*, holotype (SMNH 5074 b) cephalic region, whole mount; (E) *Boninia* sp. (from Samboanga) (SMNH 15141), male genital organs; (F) *Boninia antillarum* (SMNH 5075a) cephalic region, whole mount; (G) *Boninia divae*, holotype (SMNH 5074 a), male genital organs. Scale bars:, A, C, E, G, 50 μm; B, 100 μm; D, F, 200 μm.
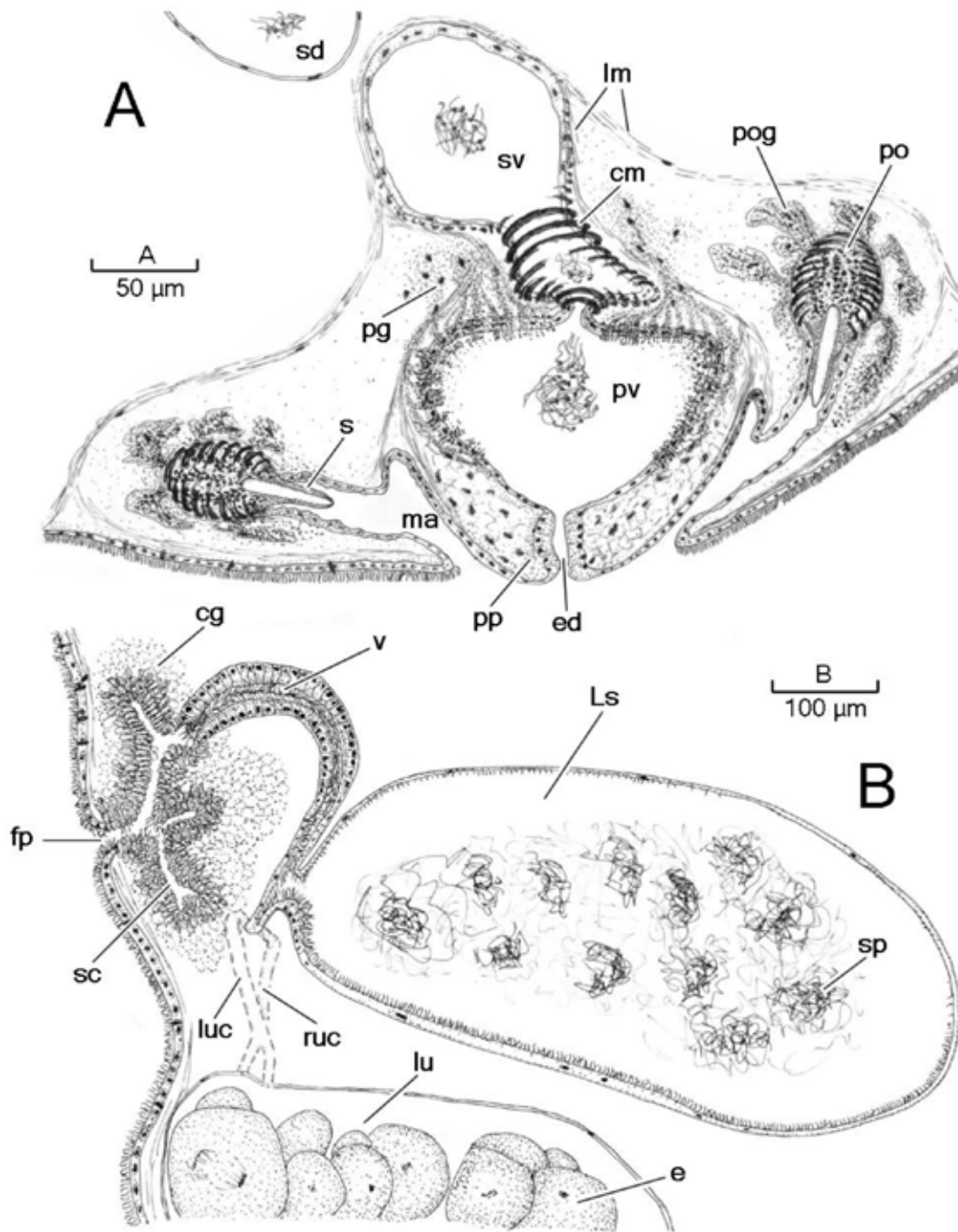
Figure 3. Reconstruction of male (A) and female (B, ventral side to the left) genital organs of the holotype of *Boninia neotethydis* sp. nov. The courses of the uterine canals, which are more laterally placed with respects to the vagina, are dashed.

Male genital organs

The male pore lies a short distance behind the posterior level of the pharyngeal chamber in the posterior third of the body. The numerous follicular testes are dorsally and laterally distributed, and extend from just behind the brain to the posterior end of the body. Two vasa deferentia run ventrally to the testes. These thin-walled ducts become convoluted and widen markedly just behind the pharynx, acting as large sperm reservoirs, and open into the somewhat ovoid, vertically elongate, seminal vesicle. The latter is lined by a flat, nucleated epithelium, and surrounded by a very strong coat of circular muscle fibres, particularly in its lower half. Only few longitudinal muscles, placed externally to the circular fibres, could be observed. Distally, the seminal vesicle opens into an ovoid, weakly muscular, prostate (granular) vesicle. This vesicle is lined with a high epithelium, pierced by very numerous terminations of prostate glands, which are in pits in the surrounding parenchyma (Figures 1A, 3A). The prostate vesicle is connected distally to a slender, non-ciliated ejaculatory duct, which runs through the penis to open into the male antrum. The muscular, non-ciliated papilla-like penis projects into the male antrum and is covered by an epithelium that is continuous with the lining of the antrum. Its length is about 120 µm in the holotype (Figure 1A). The

Table 1. *Character matrix of the species of the genus* Boninia; *P. caymanensis is chosen as the outgroup.*

Characters	1	2	3	4	5	6	7	8	9	10
Parabonia caymanensis	0	0	1	0	1	0	2	1	1	1
Boninia divae	1	0	0	0	1	1	2	0	1	1
Boninia antillarum	1	1	1	1	1	1	2	0	1	0
Boninia sp. (Samboanga)	1	1	1	1	0	1	0	0	1	0
Boninia mirabilis	1	1	0	1	0	1	1	1	0	1
Boninia neotethydis	1	1	0	0	0	1	0	1	0	2

Figure 4. Cladogram depicting the phylogenetical relationships among *Boninia* species. Bremer support values are indicated at nodes.

male antrum is non-ciliated, and is surrounded by circular muscular fibres only in its proximal portion. The few, outer longitudinal fibres are connected to the ventral longitudinal musculature of the body.

The individual ducts of 10–18 prostatoid organs (18 in the holotype), radially arranged into a single girdle around the copulatory organ (Figure 2B), open into the inner area of the male antrum. Each prostatoid organ is pyriform in shape (Figures 1D & 3A), about 55 μm long in its longest axis, and consists of a thick muscular coating containing a fine granular secretion, produced by extracapsular glands. Prostatoid organs bear a sclerotised, hollow stylet, about 40 μm long. The stylet is about 5 μm in diameter, coated by a cellular lining about 4 μm thick, and tapers into a distinct point, about 2 μm in diameter.

Female genital organs

The ovaries are dorsally arranged in two rows, one on either side of the body, between the testes and the median line, extending from behind the brain to almost the posterior end of the body. The two uterine canals, posteriorly to the male copulatory organ, are connected through short side branches to several uterine vesicles. Each uterine canal widens distally to form a very large posterior dilatation, filled with eggs in all sectioned specimens, and then bends sharply inward and opens separately into the vagina, at its junction with the Lang's accessory vesicle (Figure 3B). The latter is an elongate receptacle (Figures 1C & 3B), placed between the two dilatations, lined with cilia at its ventral side. Dorsally, cilia are sparse and short, and vast dorsal areas appear wholly non-ciliated. The narrow ciliated vagina runs anteriorly for a short distance, and then turns ventrally to dilate and form a shell chamber, which is surrounded by numerous cement glands. After forming the shell chamber, the vagina continues its course to open externally through the female genital pore. This pore lies on the ventral surface of the body, posteriorly to the male pore.

Karyology

Chromosome number: 2n=20. Most chromosomes are metacentric, slightly differing in size: the smallest pair of the set is about half the length of the largest. Karyotype formula: 14.9 ±0.8; Chromosome I: 7.01 ±0.28; 45.6 ±2.16 (m); Chromosome II: 6.29 ±0.41; 47.05 ±4.32 (m); Chromosome III: 5.82 ±0.21; 45.51 ±0.82 (m); Chromosome IV: 5.35 ±0.44; 44.63 ±2.70 (m); Chromosome V: 5.33 ±0.37; 28.58 ±5.04 (sm); Chromosome VI: 4.92 ±0.26;
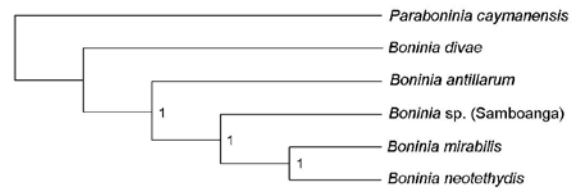
46.56 ±1.49 (m); Chromosome VII: 4.24 ±0.61; 33.94 ±6.22 (sm); Chromosome VIII: 4.16 ±0.07; 46.06 ±3.93 (m); Chromosome IX: 3.69 ±0.11; 43.95 ±3.77 (m); Chromosome X: 3.19 ±0.31; 39.71 ±4.56 (m).

Phylogenetic relationships

Assessment of relationships was limited by the poor original descriptions of the possible outgroups of *Boninia*, which led to the elimination of *Traunfelsia*. Furthermore, the specimens of *Boninia mirabilis* from Samboanga (Philippines) belonged to a yet undescribed species (see below), and are reported as a distinct terminal taxon in Table 1; however, only few characters, mostly related to genital organs, could be retrieved from these slides and this reduced the choice of characters in the data matrix. The analysis yielded one most parsimonious tree (tree length=16 steps; consistency index=0.750; retention index=0.667) (Figure 4). *Boninia neotethydis* sp. nov. belongs to a clade including the Indo-Pacific species (based on the non-ciliated male antrum and the prostatoid organs arranged into one to two girdles) and results as the sister species of *B. mirabilis* (based on the stylet tapering into a point, with the embedded portion shorter than the protruding part). However, it should be noted that Bremer support values are very low throughout.

Reproductive behaviour

Three specimens were kept in an aquarium, fed with crushed mussels, at a temperature of about 24°C. They were active and voracious, and all died by dehydration, crawling above the water's edge. This may be a common behaviour of the species, given its habitat in shallow, somewhat isolated rocky pools. The longest lived specimen was kept in the aquarium for about two years. Copulations were often observed, and they resulted in the deposition of few (3–5 per specimen) adhesive, disk shaped cocoons, each containing 10–20 eggs, and provided basally with a pore through which larvae were released. Within a week from the deposition, Müller larvae were seen actively swimming in the aquarium. Attempts to feed the larvae with commercial food for planktivores failed, and all larvae died after a few days.

DISCUSSION

The family Boniniidae Bock, 1923 includes cotylean polyclads with narrow and elongate body, two pointed tentacles on the antero-lateral margins of the head, and a male copulatory complex with unarmed penis papilla, provided

with a single prostatoid organ with a stylet, or surrounded by several of such organs opening into the male antrum or on the ventral surface of body. Female system with Lang's vesicle developed, and with uterine canals bearing several rounded vesicles and opening separately into the vagina (Prudhoe, 1985).

The family comprises three genera, as redefined by Prudhoe (1985):

- *Traunfelsia* Laidlaw, 1906, with a single prostatoid organ posterior to the penis-papilla, and opening into a depression on the ventral surface of the body. Type-species: *Traunfelsia elongata* Laidlaw, 1906, from the Cape Verde Islands.

- *Paraboninia* Prudhoe, 1944, with numerous prostatoid organs irregularly distributed around the male complex and opening independently on the ventral surface. Type-species: *Paraboninia caymanensis* Prudhoe, 1944, from the Cayman Islands.

- *Boninia* Bock, 1923, with several prostatoid organs arranged in two symmetrical girdles around the penis papilla, and opening into the male antrum. Type-species: *Boninia mirabilis* Bock, 1923 from the Bonin Islands.

The new species, with several prostatoid organs opening into the male antrum, cannot be placed in the former two genera, but does not fit the diagnosis of *Boninia* because it does not have the double girdle of prostatoids presumed to be characteristic for the genus. Yet, the validity of the definition of the genus as given by Prudhoe (1985) might be questioned. Besides the type species, the genus *Boninia* includes two Caribbean species (see below) in which there are three prostatoid girdles, each slightly displaced with respect to the others, so that, in sagittal sections, one to three prostatoids may be seen at each side of the penis papilla (Figure 2G). Furthermore, in none of the Bock's specimens from Samboanga (Philippine Islands), conserved at the SMNH and labelled as *B. mirabilis*, a double girdle of prostatoids is clearly visible (see Figure 2E and below for a further discussion of these problematic specimens). Present data therefore suggest the existence of a number of closely related species with many prostatoid organs opening into the male antrum and arranged radially around the penis papilla, and that the number and disposition of the girdles of prostatoids may be a species-specific feature. The definition of the genus *Boninia* should therefore be emended to encompass this species heterogeneity and should include a statement as 'with one or more girdles of prostatoid organs, opening into the male antrum' and thus may satisfactorily accommodate the new species.

Number, arrangement and morphology of prostatoid stylets allow discrimination among *Boninia neotethydis* sp. nov. and congeneres. *Boninia divae* du Boys-Reymond Marcus & Marcus, 1968 (type locality: Curaçao) has many more prostatoids, at least 48 in specimen SMNH 5074, spirally arranged into three girdles (Figure 2G) and a somewhat smaller penis papilla (about 100 μm long) with a ciliated ejaculatory duct. Furthermore, prostatoid organs (Figure 2C) in this species are ovoid-elongate, about 70 μm long in their longest axis, and bear a straight stylet, not distinctly tapering into a point. In *B. divae* the portion of the stylet embedded into the prostatoid organ is much longer than the protruding area, which is about 25 μm long, whereas

it is decidedly shorter in *B. neotethydis* (Figure 1D). *Boninia antillarum* (Hyman, 1955) (type locality: Curaçao) has about 30 prostatoids, spirally arranged into three girdles. Each prostatoid organ is ovoid, about 65 μm long, and bears a nearly straight stylet, protruding for about 35 μm (Hyman, 1955; du Boys-Reymond Marcus & Marcus, 1968, and observations on SMNH 5075).

The original description of *B. mirabilis* reports the presence of about 40 prostatoids, arranged in two girdles, with prostatoids neatly arranged one above the other (Bock, 1923; figure 8; plate 1). Number and arrangement of the prostatoids are thus very different in the new species. Furthermore, *B. mirabilis* was described as having a wholly ciliated Lang's vesicle and ejaculatory duct, and with characteristically stiff tentacles. Bock (1923) based his detailed description of *B. mirabilis* on specimens from the Bonin Islands (southern Japan), mentioning the fact that the additional specimens he collected at Samboanga (Philippine Islands) could not be studied, since 'owing to delay they have yet to reach me. Accordingly I am not able to verify the identity by microscopical observation, but as the species has a very characteristic habitus I am quite sure that my determination is right' (Bock, 1923, p.1). These specimens, which evidently arrived eventually, were sectioned, and are the only ones currently available (SMNH 15141; 15139; 15138a,b). They differ from the original description for the very small number of prostatoids (6 in SMNH 15141; 7 in 15138, 3 in 15139), arranged into a single girdle, the presence of a completely non-ciliated Lang's vesicle, and a nearly straight prostatoid stylet (Figure 2A), about 30–35 μm long (described as 'tapering into a point' by Bock, 1923). Furthermore, although the Samboanga specimens appeared mature, no posterior dilatation of uteri was seen. The lack of measurements in the original description of *B. mirabilis* impedes further comparisons, but it appears not unreasonable to assume that these specimens may not be conspecific with *B. mirabilis*. *B. neothetydis* sp. nov. is anyway distinct from these specimens, as they possess a smaller (65–90 μm high), ciliated penis papilla, with a ciliated ejaculatory duct, and with a non-ciliated Lang's vesicle, in contrast to the new species. Furthermore, although size and general shape of the prostatoid organs are similar, they are more numerous in *B. neotethydis*, whose stylets are distinctly tapering into a point, and whose embedded portion is markedly shorter than the protruding part, in contrast to the specimens from Samboanga.

Boninia neotethydis sp. nov. is also distinct from its congeneres in other characters. The specimens examined had very few eyes (6–16 marginal eyes; 4 cerebral eyes) (Figure 1E). There are usually many more eyes in other *Boninia* species (110–120 marginal eyes and about 90 cerebral eyes in *Boninia divae* (Figure 2D); about 100 marginal eyes and 8 cerebral eyes in *Boninia antillarum* (Figure 2F); about 100 marginal eyes and 12–14 cerebral eyes in *Boninia mirabilis*). Unfortunately, the number of eyes could not be observed in the Samboanga specimens.

Information on the karyology of polyclads is very limited, and present data are the first on a species of the family Boninidae. In the Cotylea, the haploid chromosome numbers range from six to ten, and a haploid set with ten isobrachial

chromosomes is widespread across the Acotylea (Galleni & Puccinelli, 1984, 1985). The karyotype of *B. neotethydis* confirms the prevalence of sets with N=10 and isobrachial chromosomes, which may represent the plesiomorphic condition for the Polycladida.

Assessment of the phylogenetic relationships within the genus *Boninia* is problematical, owing to the few morphological characters available, and their apparent mozaic distribution. These facts contributed to the very low Bremer support values of the cladogram (Figure 4) and highlight the need for more research in the group. Therefore, the phylogenetical relationships depicted, pointing to a shared ancestry with the Pacific species, albeit congruent with biogeography, should be cautiously considered as the best hypothesis available at the moment. An Indo-Pacific origin of the species is further supported by the history of its records. Samplings in 1998 revealed the species as present both in the Red Sea, and along the Mediterranean shores of Israel. However, research carried in 1987 and 1988 failed to find the species in the Mediterranean, whilst numerous specimens were collected from the Red Sea. It can thus be suggested that, whenever the migration of the species into the Mediterranean occurred, its colonization success along northern Israelian shores dates from only after 1988. In 1998 the species was common in the Mediterranean, particularly in shelly, very coarse sediments at the bottom of rocky pools, and among clumps of the mussel *Brachidontes pharaonis*. It is worth mentioning that *B. pharaonis* itself is a well known lessepsian species (Rilov et al., 2004), and may constitute its abitual prey in the original faunistic region. However, specimens of *Boninia neotethydis* showed feeding plasticity, as they fed, in the laboratory, on the Mediterranean autochtonous mussel *Mytilaster minimus*. The predatory behaviour of the species may thus pose a further threat to mussel cultures in the Mediterranean, already subject to predation by other polyclads (Galleni et al., 1980).

REFERENCES

Bock, S., 1923. *Boninia*, a new polyclad genus from the Pacific. *Nova Acta Regiae Societatis Scientiarum Upsaliensis* (4), **6**, 1–32.

Curini-Galletti, M., Puccinelli, I. & Martens, P.M., 1989. Karyometrical analysis of ten species of the subfamily Monocelidinae (Proseriata, Platyhelminthes) with remarks on the karyological evolution of the Monocelididae. *Genetica*, **78**, 169–178.

du Boys-Reymond Marcus, E. & Marcus, E., 1968. Polycladida from Curaçao and faunistically related regions. *Studies on the Fauna of Curaçao and other Caribbean islands*, **26**, 1–106.

Eriksson, T., 1998. *AutoDecay v. 4.0.1 (program distributed by the author)*. Stockholm: Department of Botany, Stockholm University.

Galil, B.S., 2000. A sea under siege – alien species in the Mediterranean. *Biological Invasions*, **2**, 177–186.

Galleni, L. & Puccinelli, I., 1984. Karyology of five species of Turbellaria from the Øresund, Denmark. *Ophelia*, **23**, 141–148.

Galleni, L. & Puccinelli, I., 1985. Karyology of *Yungia aurantiaca* (Turbellaria: Polycladida). *Transactions of the American Microscopical Society*, **104**, 122–128.

Galleni, L., Tongiorgi, P., Ferrero, E. & Salghetti Drioli, U., 1980. *Stylochus mediterraneus* (Turbellaria: Polycladida) predator on the mussel *Mytilus galloprovincialis*. *Marine Biology*, **55**, 317–326.

Hyman, L.H., 1955. Some polyclad flatworms from the West Indies and Florida. *Proceedings of the United States National Museum*, **104**, 115–150.

Maddison, W.P. & Maddison, D.R., 1992. *MacClade. Version 3.0.1 (Computer software and manual)*. Sunderland, Massachussets: Sinauer Associates.

Martens, P.M., 1984. Comparison of three different extraction methods for Turbellaria. *Marine Ecology Progress Series*, **14**, 229–234.

Newman, L.J., Cannon, L.R.G. & Govan, H., 1993. *Stylochus (Imogene) matatasi* n. sp. (Platyhelminthes, Polycladida) – pest of cultured giant clams and pearl oysters from Solomon Islands. *Hydrobiologia*, **257**, 185–189.

Por, F.D., 1990. Lessepsian migration. an appraisal and new data. *Bulletin de l'Institut Océanographique*, **7**, 1–10.

Prudhoe, S., 1985. *A monograph on polyclad Turbellaria. British Museum (Natural History)*. Oxford: Oxford University Press.

Rilov, G., Benayahu, Y. & Gasith, A., 2004. Prolonged lag in population outbreak of an invasive mussel: a shifting-habitat model. *Biological Invasions*, **6**, 347–364.

Ruiz, G.M., Carlton, J.T., Grosholtz, E.D. & Hines, A.H., 1997. Global invasions of marine and estuarine habitats by non indigenous species: mechanisms, extent and consequences. *American Zoologist*, **37**, 621–632.

Swofford, D.L., 1998. *PAUP*. Phylogenetic Analysis Using Parsimony* and other methods*. Sunderland, Massachussets: Sinauer Associates.