

# ***Discoplana malagasensis* sp. nov., a New Turbellarian (Platyhelminthes: Polycladida: Leptoplanidae) Symbiotic in an Ophiuroid (Echinodermata), with a Cladistic Analysis of the *Discoplana*/*Euplana* species**

Gilles Doignon<sup>1\*</sup>, Tom Artois<sup>2</sup> and Dimitri Deheyn<sup>3†</sup>

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<sup>1</sup>Graduate student (Aspirant du Fonds National pour la Recherche Scientifique), Laboratoire de Biologie Marine (C.P. 160/15), Université Libre de Bruxelles, 50 ave. F.D. Roosevelt, B-1050 Bruxelles, Belgium

<sup>2</sup>Research Group Zoology, Dept. SBG, Limburgs Universitair Centrum, Universitaire Campus Gebouw D, B-3590 Diepenbeek, Belgium

<sup>3</sup>Laboratoire de Biologie Marine, Bâtiment des Sciences de la Vie, Université de Mons-Hainaut, 8 ave. du Champ de Mars, B-7000 Mons, Belgium

**ABSTRACT**—A new species of polyclad flatworm from Papua New Guinea is described. It is found symbiotic in the ophiuroid *Ophiothrix purpurea* von Martens, 1867 (Echinodermata: Ophiuroidea). Apparently it belongs to the taxon *Discoplana* Bock, 1913 and can be distinguished from the six previously described *Discoplana* species by its very short ejaculatory duct and a penial papilla covered with a penial sheath, but without any true sclerotised structures such as a stylet or spines. The cladistic analysis of the *Discoplana*/*Euplana* species, based on morphological features and including two outgroups, reveals that all species of *Discoplana*, except *D. pacificola*, form a monophyletic taxon, that is not a synonym of *Euplana* Girard, 1893. Therefore the name *Discoplana* is conserved and the new species will be described as *Discoplana malagasensis* sp. nov. A key for the *Discoplana*/*Euplana* group is provided. In this key the biogeographical distribution and possible synonyms are given.

**Key words:** cladistic analysis, Ophiuroidea, Polycladida, symbiosis, Turbellaria

## **INTRODUCTION**

The paraphyletic taxon "Turbellaria" (see Ehlers, 1985) includes mainly free-living species and only few that live in association with other organisms (Jennings, 1971). Most of these associations remain unclear in nature (commensalism, mutualism or parasitism), and mostly involve an echinoderm host (Jangoux, 1990; Jennings, 1997). Turbellarians symbiotic with echinoderms are found in four different taxa: Rhabdocoela (86 species), Acoela (8 species), Nemertodermatida (1 species) and Polycladida (4 species) (Jangoux, 1990; Lundin and Hendelberg, 1995; Jondelius, 1996; Kozloff, 1997). Within the Polycladida, *Ceratoplana colob-*

*ocentroti* Bock, 1925 and *Discostylochus parvus* Bock, 1925 live clung to the oral side of the test of sea-urchins, while *Discoplana takewakii* Kato, 1935 and *Notoplana comes* Cannon and Grygier, 1991 live in association with ophiuroids, being found in the genital bursae of *Ophioplocus japonicus* or on the oral side of the disc of *Ophiocoma scolopendrina*, respectively. Recently, a new symbiont attributed to the taxon *Discoplana* has been reported to live in association with *Ophiothrix purpurea*: it was found in the host disc, either in the coelomic cavity or bursal lumen (Deheyn *et al.*, 1998). In the present contribution, we formally describe this species from Hansa Bay, northern east Papua New Guinea.

The monophyly of the taxon *Discoplana* Bock, 1913 has been a matter of debate for a long time. The taxon was considered monophyletic by Prudhoe (1985, 1989) and Tajika *et al.* (1991), who recognised six species and distinguished them from the four species of the taxon *Euplana* Girard, 1893 by the fact that in the type species *E. gracilis* the Lang's vesicle is absent and the penial papilla is inconspicuous. However, in the other *Euplana* species, a Lang's ves-

\* Corresponding author: Tel. +32-2-6502970;

FAX: +32-2-6502796.

E-mail: gdoignon@ulb.ac.be

† Present address: Post Graduate Researcher, Marine Biology Research Division-0202, Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Dr, La Jolla CA 92093-0202, USA

icle may be present (*E. clippertonii*) and the penial papilla can be obvious (all species but the type species). Aware of these problems, Hyman (1939a, b, c, 1940a, b, 1953, 1954, 1955, 1959) and Marcus (1947) considered *Discoplana* a synonym of *Euplana*. Faubel (1983) did not consider *Discoplana* monophyletic and brought the species in four different genera within two separate families. The relationships within the taxon *Discoplana* were never assessed from a cladistic point of view, although an attempt was made by Tajika *et al.* (1991). In their "cladogram", which was based on morphological features only, a group consisting of *D. malayana* (Laidlaw, 1903) and *D. concolor* (Meixner, 1907) forms the sister group of a group formed by the other four species. Although they consider the group formed by *D. concolor* and *D. malayana* monophyletic, Tajika *et al.* (1991) fail to provide a synapomorphy for these two species. The only feature they say is shared by the two species is the posteriorly directed vagina, a feature they explicitly state as the primitive condition. The anteriorly directed vagina is considered the derived condition, and put forward as the apomorphy of the group formed by the four other species. Within this group, they propose two sister groups: one consisting of *D. pacificola* (Plehn, 1896) and *D. gigas* (Schmarda, 1859), the other one of *D. longipenis* Kato, 1943 and *D. takewakii* Kato, 1935. *D. longipenis* and *D. takewakii* are considered sister species because in both the penis is "armed", either with a stylet or with spines. This implies that Tajika *et al.* (1991) consider spines and stylet homologous to each other, a view highly doubtful by the different position and morphology of the structures involved. *D. pacificola* and *D. gigas* were considered sister species because the Lang's vesicle is "specialized", being enlarged or extending to both sides. Tajika *et al.* (1991) thus consider two different conditions of the Lang's vesicle (enlarged and extending to both sides) as homologous. This, however, is again a rather doubtful decision, which they take without any justification. There is no reason whatsoever that these two conditions of the Lang's vesicle should be considered as having the same origin and therefore they are better considered as separate character states in an analysis.

In this contribution we present a cladistic analysis of the *Discoplana* and *Euplana* species based on morphological features. With this we will try to assess whether the *Discoplana* taxon is monophyletic and to reveal the phylogenetic relationships within the taxon. Finally, a key to the *Discoplana*/*Euplana* species is presented.

## MATERIALS AND METHODS

### Collection and observation

Specimens of *Ophiothrix purpurea* were collected from the coral reefs surrounding Laing Island (4°10'S, 144°52'E), Hansa Bay and Durangit Reef, Papua New Guinea in July 1994 (D. Deheyn), 1995 (D. Deheyn) and 1996 (G. Doignon). All specimens were collected by SCUBA diving between 3 and 20 m deep and transported to the King Léopold III Biological Station on Laing Island in individual seawater filled zip-lock bags. There the collected ophiuroids

were kept alive in open circuit marine aquaria for 1 to 24 hr until investigation.

Upon dissection, the ophiuroids were anaesthetized in a 3.5% w/w MgCl<sub>2</sub> seawater solution. If symbiotic polyclads were found, they were counted, photographed and measured using micrometric paper, and afterwards fixed and dehydrated (if applicable). Some turbellarian specimens were fixed with lactophenol and whole mounted. They were studied with a Polyvar Reichert-Jung microscope using Nomarski interference contrast. Specimens intended for light microscopical sectioning were fixed in Bouin's fluid for 24 hr, then dehydrated in graded concentrations of ethanol, embedded in paraplast and sectioned into 7 to 10 µm thick sections using a Leica RM 2155 microtome. Sections were stained following the hematoxylin-eosin staining (Gabe, 1968) and studied using a Leitz Laborlux D light microscope. For a more detailed description, one adult specimen was fixed with 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.8) for 3 hr at 4°C, rinsed in the buffer and then postfixed for 1 hr with 1% osmium tetroxide in the same buffer. After a final buffer wash, it was dehydrated in graded ethanol, embedded in Spurr and sectioned into 0.5 to 3 µm semi-thin sections using a Reichert OmU2 ultramicrotome. Sections were stained in a 1:1 solution of 1% methylene blue/1% azure II according to the procedure of Richardson *et al.* (1960), then examined using a Leitz Laborlux D light microscope. Freehand drawings were made with camera lucida. Type material will be deposited in the collection of the Research Group Zoology of the Limburgs Universitair Centrum (LUC), Diepenbeek, Belgium.

### Phylogenetic analysis

In total, 13 species are included in the analysis. Apart from the six *Discoplana* species, the four *Euplana* species recognised by Prudhoe (1985) and the new species described in this contribution, two more species, *Notoplana comes* Cannon & Grygier, 1991 and *Stylochoplana aberrans* Kato, 1944 are included as outgroups. The analysis is based on morphological characters only. Due to the lack of good specimens, or lack of specimens altogether, most information for character delimitation was retrieved from literature (Bock, 1913; Cannon and Grygier, 1991; Diesing, 1862; Faubel, 1983; Hyman, 1939a, b, c, 1940a, b, 1953, 1954, 1955, 1959; Kato, 1934, 1935, 1943, 1944; Laidlaw, 1902, 1903; Marcus, 1947; Meixner, 1907; Plehn, 1896a, b; Prudhoe, 1985, 1989; Stummer-Traunfels, 1933; Tajika *et al.*, 1991).

The number and the localization of ocelli are characters often used by authors to discuss phylogeny within polyclads. In our phylogenetic analysis, we decided to discard these characters because they can vary so much within one species that interspecific differences may be more apparent than real. To make use of these characters, intraspecific variation should be better known than it is possible with the material now available. After examination of the literature, 13 characters appeared possibly important to reveal the phylogenetic relationships of the group under study. These characters are discussed in detail in the appendix at the end of this paper.

Sometimes a feature shows important variation in species where it is present, but is absent in other species. In such cases we prefer to code this with one character accounting for the presence of the feature and other characters accounting for the variation observed. Species lacking the feature are then coded inapplicable for that character ("—") in the matrix, Table 1). This is the so-called "Inapplicable data coding-missing" of Hawkins (2000). Although this way of coding introduces some logical dependency between characters, this will not lead to problems of overweighting (Wilkinson, 1995). All characters are considered unordered and of equal weight initially. Two characters were parsimony uninformative (characters 2 and 12). They are kept in the analysis, however, as they are important characters that could have a wider distribution if more taxa are added in future analyses. Addition of such characters may however inflate character fit measures as e.g. the ensemble consis-

tency index (CI) (Brooks *et al.*, 1986), and therefore we have calculated these values omitting the parsimony uninformative characters. Missing data are scored with a question mark. The data matrix can be found in Table 1.

**Table 1.** Datamatrix showing the distribution of the character states for the thirteen characters and thirteen species. For ease of reading, characters nrs. 1 and 10 are indicated.

	1	1	0
<i>Notoplana comes</i>	1	0	0 0 0 1 0 1 1 1 1 1 1
<i>Stylochlora aberrans</i>	0	–	1 0 0 1 1 1 1 1 1 1 0
<i>Discoplana concolor</i>	0	–	0 1 1 1 0 0 0 1 1 1 0
<i>Discoplana malayana</i>	0	–	0 1 1 1 0 1 0 1 1 1 0
<i>Discoplana pacificola</i>	0	–	0 0 0 1 1 1 0 1 0 1 0
<i>Discoplana gigas</i>	0	–	1 1 0 1 2 1 0 1 0 1 0
<i>Discoplana malagasensis</i>	1	1	1 0 1 0 0 0 0 0 1 0
<i>Discoplana takewakii</i>	1	1	1 0 1 0 0 0 1 1 1 0
<i>Discoplana longipenis</i>	0	–	1 1 0 1 0 1 0 1 0 1 1
<i>Euplana gracilis</i>	0	–	0 0 0 0 – 0 0 0 1 0 –
<i>Euplana carolinensis</i>	0	–	0 0 0 0 – 0 ? 0 1 1 0
<i>Euplana clippertoni</i>	0	–	0 0 0 1 ? 0 0 1 0 1 1
<i>Euplana hymanae</i>	0	–	0 0 0 0 – 0 0 1 1 1 0

The data matrix was analysed in a parsimony analysis using PAUP\* version 4.0b10 (Swofford, 2001) on a Macintosh PowerBook G4 computer. Because of the limited dimensions of the data matrix, it was possible to use a branch-and-bound search method. Internal zero-length branches were collapsed using "Amb-" option of PAUP\*. The analysis was done using an iterative reweighting of the characters (successive weighting of Farris (1969)) with the unit rescaled consistency index (rci) (Farris, 1989) as measure of homoplasy. Alternatively, the matrix was also analysed using Goloboff's (1993) implied weighting procedure. Drawback of this method is the subjective choice of the concavity constant *k*, for which we chose all values from two to ten. The use of too low *k*-values (<2) is strongly opposed to by Goloboff (1995) as they downweight the homoplastic characters too strongly; use of too high *k*-values gives the same results as an equally weighted analysis.

Character states were polarised by rooting the trees using outgroups (*Notoplana comes* and *Stylochlora aberrans*). All analyses were run preferring reversals over parallelisms when there is ambiguity in the data-matrix (ACCTRAN option in PAUP\*).

To estimate the relative degree of support for each node, Bremer values (so-called decay-indices) are calculated (Bremer, 1988, 1994). These values are the differences in length between the most parsimonious tree and trees resulting from a constraint analysis using the clade under consideration as a reversed constraint. To generate the constraint trees the freeware AutoDecay version 4.0 (Eriksson, 1998) was used. Cladograms with the Bremer support values indicated on the respective clades can afterwards be visualised in the computer program Treeview version 1.6.5 (Page, 2000). To rescale the Bremer support values of the cladograms obtained with successive weighting the factor *s<sub>w</sub>/s* was used, with *s<sub>w</sub>* the length of the tree under successive weighting and *s* the length of the same cladogram supposing weights of one, as was advocated by Bremer (1994).

## SYSTEMATICS

### *Discoplana malagasensis* sp. nov.

#### Material examined

**Holotype:** One sagittally sectioned specimen (3.6 mm long) (LUC nr. 215). Laing Island western reef (4°10'30"S, 144°52'47"E), Hansa Bay, Bismarck Sea, Papua New Guinea (14 vii. 1994, coll. D. Deheyn). Subtidal, found in the genital bursae of the ophiuroid *Ophiothrix purpurea*.

**Paratypes:** A whole mounted specimen (LUC nr. 216). Laing Island western reef (4°10'30"S, 144°52'47"E), Hansa Bay, Bismarck Sea, Papua New Guinea (14 vii. 1994, coll. D. Deheyn). One transversally sectioned specimen (LUC nr. 217). Laing Island eastern reef (4°10'30"S, 144°52'47"E), Hansa Bay, Bismarck Sea, Papua New Guinea (vii. 1996, coll. G. Doignon). One animal conserved in ethanol (LUC nr. 218). Laing Island northern reef (4°10'30"S, 144°52'47"E), Hansa Bay, Bismarck Sea, Papua New Guinea (vii. 1996, coll. G. Doignon). All subtidal, found in the genital bursae of the ophiuroid *Ophiothrix purpurea*.

**Other material:** Observations and measurements on live specimens.

#### Description

**Habitus:** Body flat, grossly ovoid and with a blunt posterior end (Fig. 1). Body margins slightly frilled. Creamy white to pale yellow in colour. Both ventral and dorsal body sides ciliated. Ventral epidermis opaque, dorsal epidermis semi-transparent, with pale brown digestive tract, whitish ovaries and numerous dark ocelli shining through. No tentacles. Size of living animals: 0.5 to 18.0 mm long, 0.4 to 4.0 mm broad.



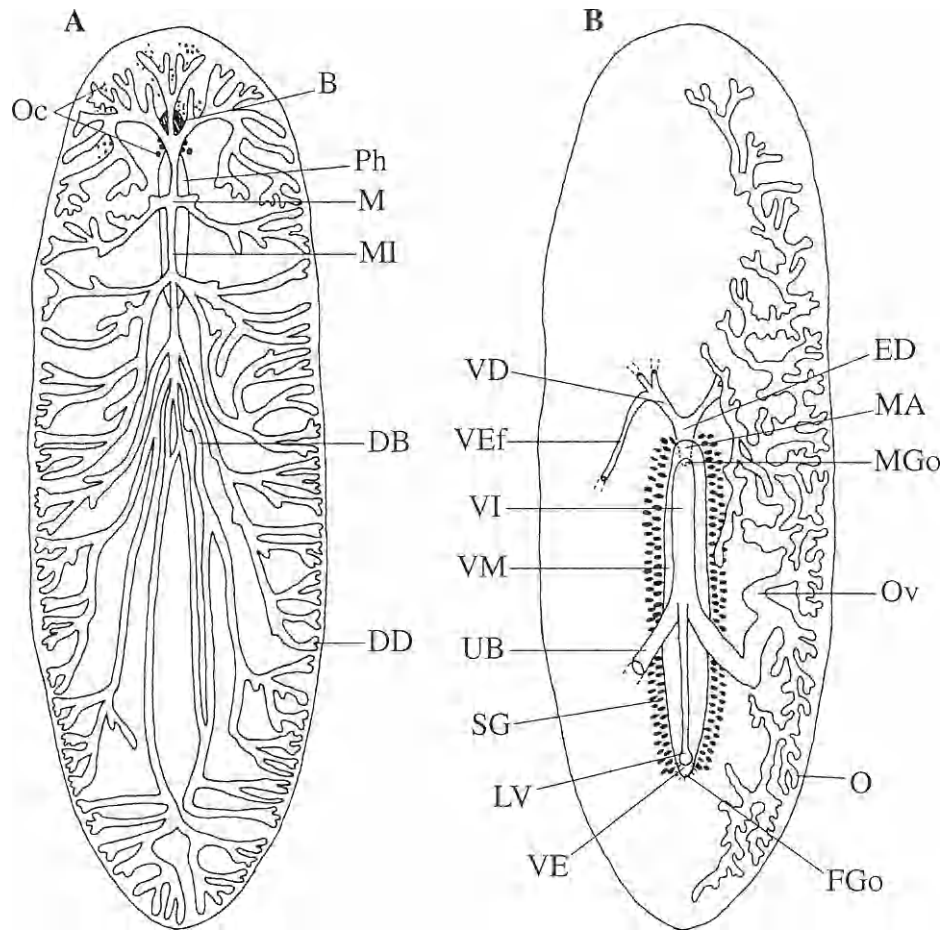
**Fig. 1.** *Discoplana malagasensis* sp. nov. Live adult specimen (dorsal view). Scale bar: 1 mm.

**Body wall:** Epidermis, about 20 µm thick, with a well-developed basal lamina. Epithelium cellular, with numerous rhabdites and gland cells. Underneath basal lamina lying several thick muscle layers: an outer circular and an inner longitudinal layer, with a diagonal muscle layer between them.

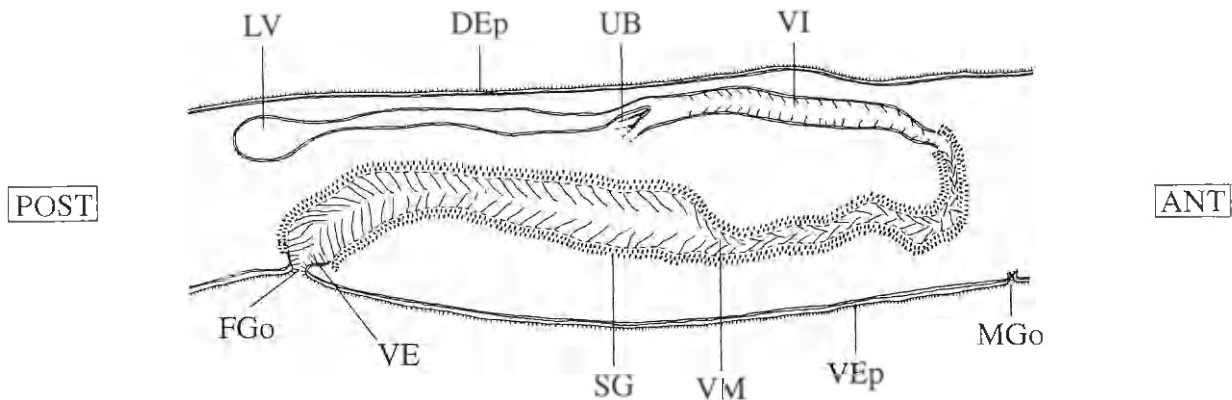
**Nervous system:** Small (ca 70 µm long) and bilobed

brain located antero-dorsally to pharynx, in anterior sixth part of body (Fig. 2A). One main nervous tract extends posteriorly from each lobe and radiates ventrally between

parenchymal cells. Ocelli located in parenchyma close to brain, clustered in two tentacular and two cerebral groups (tentacular ocelli located forward from brain, cerebral ocelli



**Fig. 2.** *Discoplana malagasensis* sp. nov. (A) Schematic drawing of the digestive system (paratype 2). (B) Schematic drawing of the reproductive system (paratype 2). Vagina interna and vagina media are connected to each other, which is not obvious on the drawing as it is seen from above. B, brain; DB, digestive branch; DD, digestive diverticulum; ED, ejaculatory duct; FGo, female gonopore; LV, Lang's vesicle; M, mouth; MA, male antrum; MGo, male gonopore; MI, medium intestine; O, ovary; Oc, ocelli; Ov, oviduct; Ph, pharynx; SG, shell glands and their secretions; UB, uterine branch; VD, vas deferens; VE, vagina externa; VEf, vas efferens; VI, vagina interna; VM, vagina media (= shell chamber).



**Fig. 3.** *Discoplana malagasensis* sp. nov. Reconstruction of the female reproductive system (holotype). DEp, dorsal epidermis; FGo, female gonopore; LV, Lang's vesicle; MGo, male gonopore; SG, shell glands and their secretions; UB, uterine branch; VE, vagina externa; VEp, ventral epidermis; VI, vagina interna; VM, vagina media (= shell chamber).



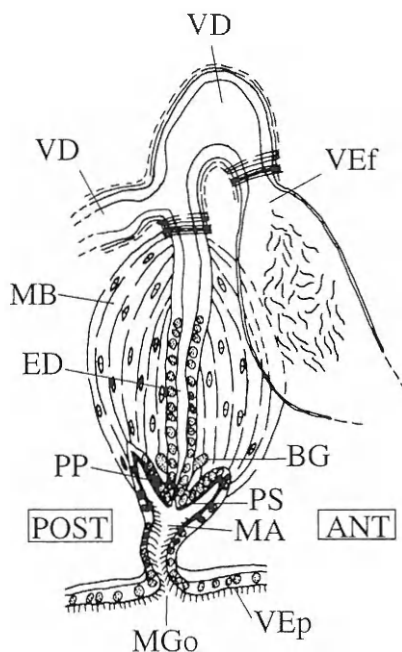
laying on its sides). Exact localization and number of ocelli vary between individuals (from 4 to 8 and from 5 to 8 per group for tentacular and cerebral ocelli, respectively) and from left to right. Marginal ocelli absent.

**Digestive tract.** Mouth located at 1/5 of body length, leading into a narrow pharyngeal chamber situated centrally in body, just behind brain (Fig. 2A). Pharynx small and muscular, cylindrical, with its internal epithelium ruffled. Medium intestine about 1/6 of body length, extending medio-dorsally, with seven pairs of lateral branches: three anterior to the mouth and four caudally from it (caudally of the male gonopore). Proximally, the lateral branches anastomose; distally, each of them splits up into numerous digestive diverticula.

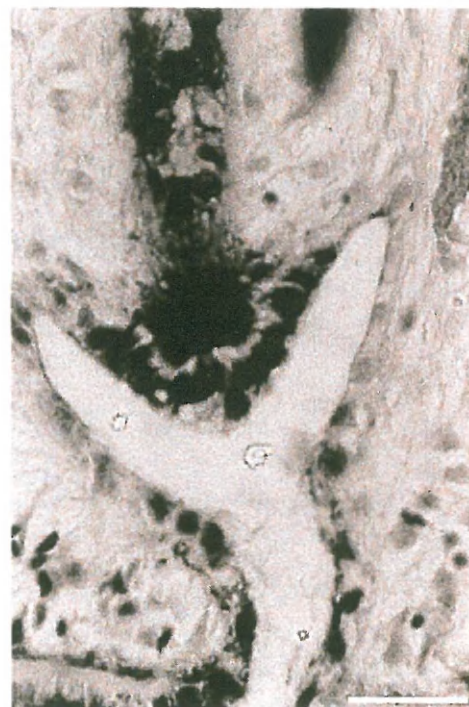
**Reproductive system.** Male and female gonopores situated ventrally, posterior to the pharynx (Fig. 2B) and separated from each other by a distance of 1/3 of body length. Female gonopore situated posteriorly to male gonopore, at about 5/6 of body length (Figs 2B and 3). Vagina well developed, extending forward from female gonopore and divided into three easily discernible parts. Vagina externa (the "antrum femininum" of Prudhoe (1985)) very short, moderately ciliated with long cilia about twice the length of those of the epidermis. Vagina media (also called "shell chamber" or "cement duct" by Prudhoe (1985)) highly ciliated with very long cilia. Shell glands discharge their droplet-like coarse-grained eosinophilic secretions in this part of vagina and over whole of its length. These glands fill up the entire middle half of body. Proximal part of vagina media makes a 180° turn at level of male gonopore. Vagina interna just

proximally from this turn. It is moderately ciliated with long cilia. Proximally it splits into three ducts: two lateral uterine branches ventrally and one long Lang's vesicle dorsally. Lang's vesicle very long and narrow distally, with a swollen, bulbous proximal end. Ovaries extend at both sides of body from anterior to the caudal end. They are divided into a large anterior and a smaller posterior part from each of which a short oviduct departs. Both oviducts distally join to form uterine branch. Latter from left and right do not fuse before entering vagina interna.

Male gonopore situated at about half of body length (Fig. 2B), and opens into a narrow and short (less than 50 µm long) male antrum. A distinct penial papilla, which is a protrusion of the ejaculatory duct, at proximal end of male antrum. Penial papilla conical, attached to distal end of ejaculatory duct proper by strong longitudinal muscles (Fig. 4). Entire surface of penial papilla and proximal half of male antrum covered by a thick penial sheath (Figs 4 and 5). Penial stylet or penial spines lacking. No real prostate organ, but numerous eosinophilic glands around male antrum, penial papilla and ejaculatory duct. Additionally, a small bundle of basophilic glands enters the most distal part of the ejaculatory duct, near opening of penial papilla. Distal part of ejaculatory duct very narrow and surrounded by inner longitudinal and outer circular muscles, and proximally bifurcates into two vasa deferentia. This bifurcation surrounded by a sphincter. Each vas deferens splits into three vasa efferentia. Latter proximally surrounded by strong circular muscles and lead to the thin-walled testes, which are scat-



**Fig. 4.** *Discoplana malagasensis* sp. nov. Reconstruction of the male reproductive system (holotype). Only one vas efferens shown. BG, basophilic glands; ED, ejaculatory duct; MA, male antrum; MB, muscle bulb; MGo, male gonopore; PP, penial papilla; PS, penial sheath; VD, vas deferens; VEF, vas efferens; VEp, ventral epidermis.



**Fig. 5.** *Discoplana malagasensis* sp. nov. Photomicrograph of the male antrum, showing the penial papilla and the pseudo-cuticularized penial sheath (holotype). Scale bar: 25 µm.

tered over the entire body length.

### Diagnosis

*Discoplana malagasensis* sp. nov. *Discoplana* species with male and female gonopores far from each other. Without real seminal vesicle. With a very short ejaculatory duct. Penial papilla covered by a thick penial sheath, without penial stylet or penial spines. Vagina extending anteriorly to the female gonopore. Without common uterine duct. Lang's vesicle bulbous.

### Etymology

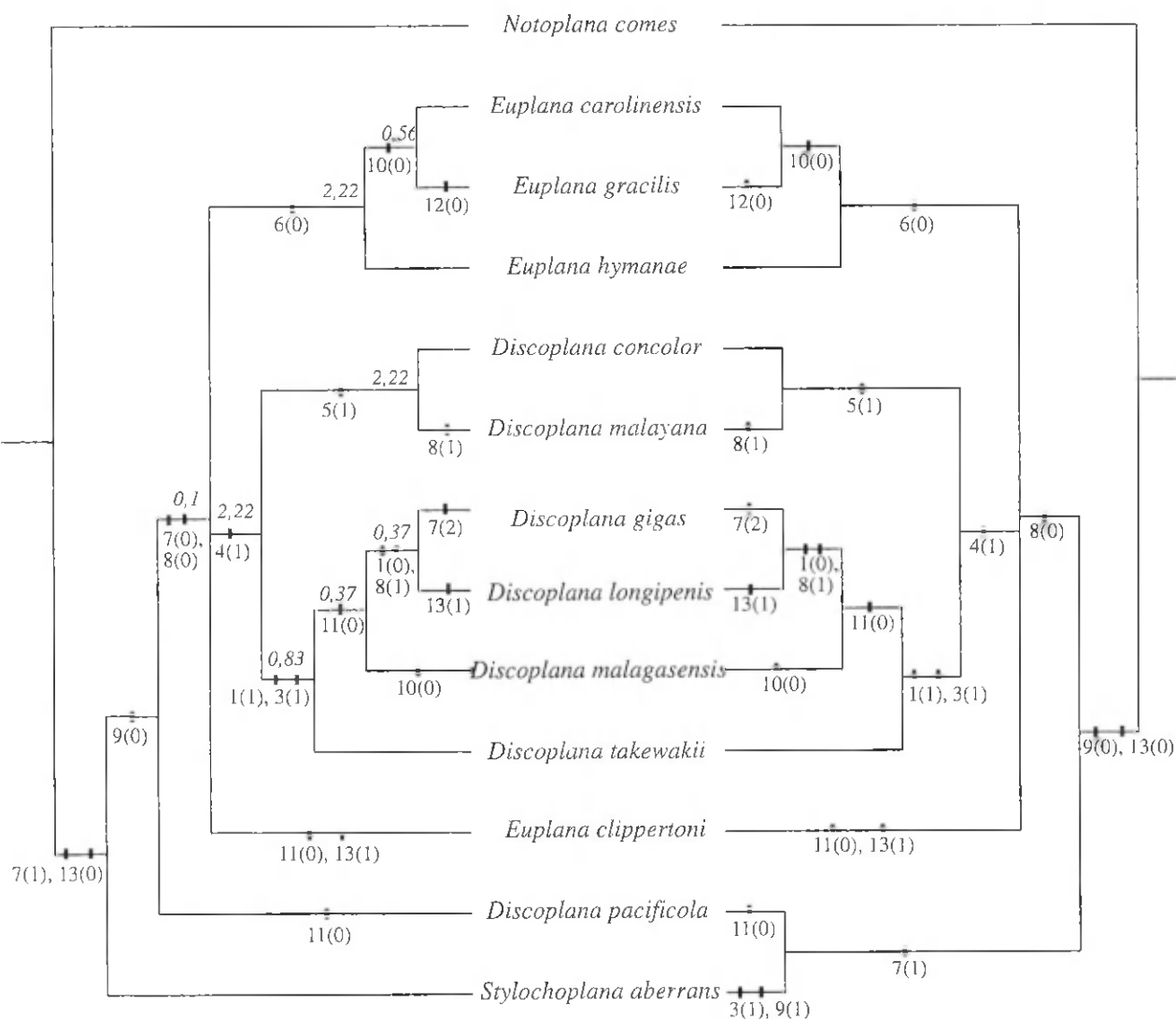
Named after the aboriginal name of the type locality Laing Island (Malagasa in Pidgin).

### Remarks

58 out of 198 ophiuroids dissected were infested by tur-

bellarians. A total of 116 symbionts were found, all of them in the genital bursae of their hosts. During dissection of the host, the worms were able to glide out of the ophiuroid bursal slits in a few seconds. The symbiont always stayed preferentially in contact with the host and no specimen has ever been found free-living, neither in the field nor in the aquarium. *D. malagasensis* shows a strong negative phototropism.

*Discoplana malagasensis* can be distinguished from related species by its very short ejaculatory duct and a penial papilla covered with a penial sheath, but without any true sclerotised structures such as a stylet or spines. This last character permits to distinguish it from *D. takewakii*, the only other species of the group associated with an ophiuroid but whose penial papilla is armed with spines. Moreover, the adults of *D. takewakii* are in average smaller than those of *D. malagasensis*. The ophiuroid host is also different for the two species, *Ophiothrix purpurea* for *D. malagasensis*, *Ophi-*



**Fig. 6.** The two most parsimonious cladograms resulting from the parsimony analysis using successive weighting of characters. Bremer supports above zero are given in italics at the respective nodes in the first cladogram (identical in the second cladogram). Character state change are given beneath each branch.

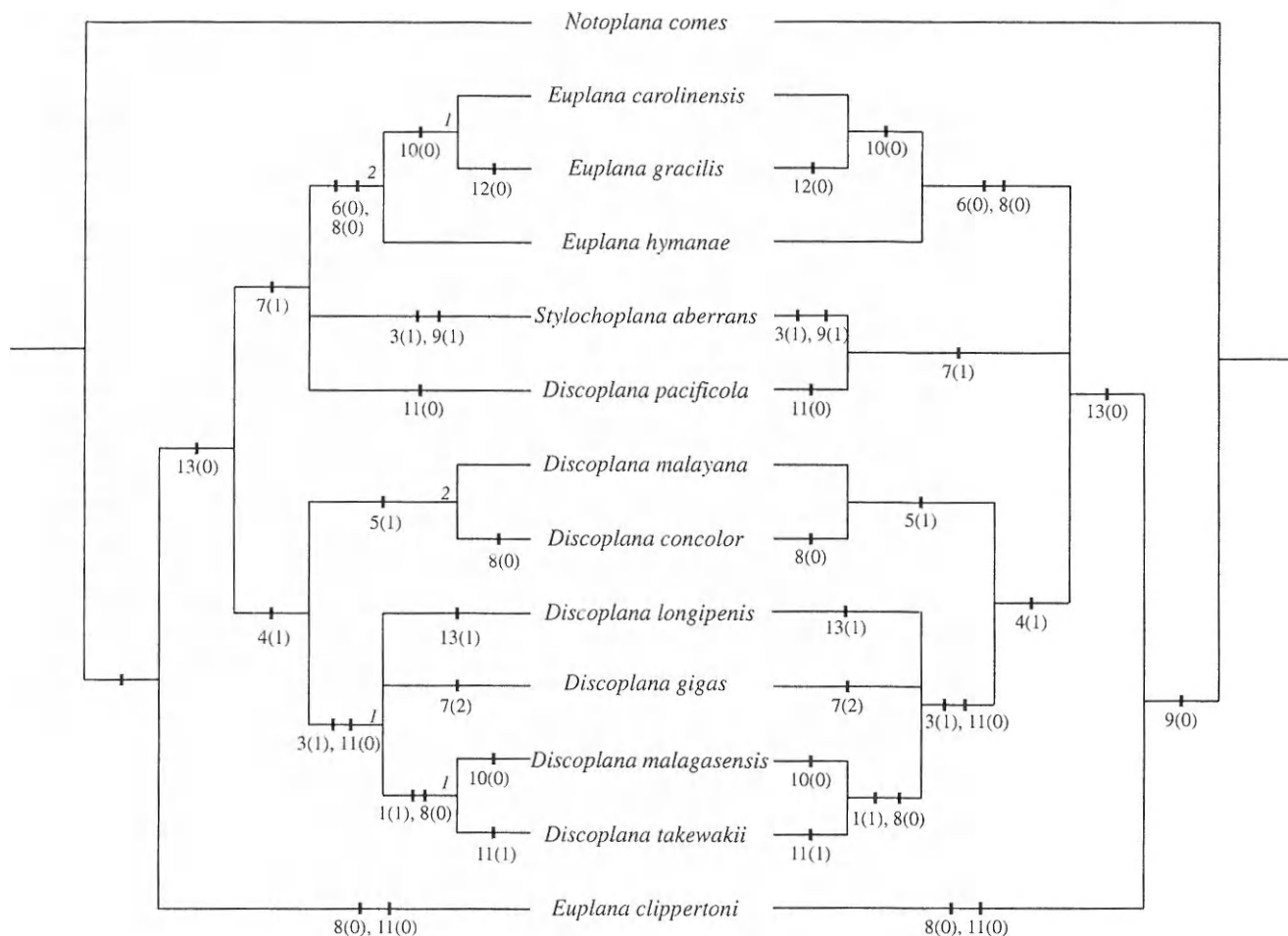


Fig. 7. The two most parsimonious cladograms resulting from the parsimony analysis using implied weights. Bremer supports above zero are given in italics at the respective nodes in the first cladogram (identical in the second cladogram). Character state changes are given beneath each branch.

*oplocus japonicus* for *D. takewakii*. The key presented at the end of the paper details all the morphological differences between *D. malagasensis* and the other *Discoplana* species.

### PHYLOGENETIC ANALYSIS

The analysis of the data matrix giving equal weights to the characters (weight of 1) results in 54 equally parsimonious cladograms of length 25 (CI=0.52; RI=0.62; RC=0.32). Three rounds of successive weighting using *rc* as a measure of homoplasy yielded two trees with a weighted length of 11.35 (CI=0.75; RI=0.83; RC=0.62) (Fig. 6). One of them could not be rooted using both *N. comes* and *S. aberrans* as outgroups, because of a sistergroup relationship between *S. aberrans* and *D. pacificola* in this tree. Therefore all trees were rooted using only *N. comes* as outgroup. In the other, *N. comes* and *S. aberrans* do have a basal position.

Implied weighting was performed with *k* values ranging from two to ten. In each case, the same three trees were retrieved, one of which was always equal to the strict consensus of the other two (Fig. 7). These two trees have fits

ranging between -9,14286 (for *k*=2) to -10,15476 (for *k*=10) and are identical to two of the trees found in the analysis with equal weights. In both trees, *S. aberrans* falls within the ingroup, and therefore these trees could be rooted only with *N. comes*.

### DISCUSSION

The fact that none of the cladograms obtained from the analyses can be rooted using both outgroup species clearly shows that the monophyly of the ingroup (*Discoplana*+*Euplana*) cannot be assigned. However, a well supported monophyletic group consisting of *E. hymanae*, *E. gracilis* and *E. carolinensis* (called the *Euplana*-clade in the rest of the discussion) is found, among them *E. gracilis* and *E. carolinensis* are sister species. Another well-supported clade retrieved in all the analyses is formed by the *Discoplana* species, with exclusion of *D. pacificola* (the *Discoplana*-clade). Within this clade, *D. concolor* and *D. malayana* always form a sistergroup relationship, a clade also found in the cladogram of Tajika *et al.* (1991).

Choice of weighting scheme mainly influences the position of the taxa that are found at the base of the cladogram: *E. clippertoni*, *D. pacificola* and *S. aberrans*. This also influences the distribution of the character states on the respective cladograms. In the cladograms resulting from successive weighting (Fig. 6), the *Euplana*-clade is characterised by the absence of a Lang's vesicle [6(0)], whereas the *Discoplana*-clade is characterised by a very long vagina [4(1)], which is directed posteriorly [5(1)] in *D. concolor* and *D. malayana*, a synapomorphy of both species. The other four species in the *Discoplana*-clade have the gonopores far from each other [3(1)]. In the cladograms resulting from the implied weighting analyses (Fig. 7), a second synapomorphy of the *Euplana*-clade is the absence of a common uterine duct [8(0)].

An important difference between both weighting schemes is that in the successive weighting analysis the endosymbiosis with ophiuroids has originated once [1(1)] in

the *Discoplana*-clade, but afterwards is lost [1(0)] in the clade consisting of *D. longipenis* and *D. gigas*. *D. malagasensis* is the sisterspecies of the monophyletic clade formed by these two species. The loss of the seminal vesicle [11(0)] is the synapomorphy of *D. gigas*, *D. longipenis* and *D. malagasensis*. In the cladograms obtained by implied weighting, endosymbiosis [1(1)] is a synapomorphy for a clade consisting of *D. malagasensis* and *D. takewakii*, and the sister-group relationship between *D. longipenis* and *D. gigas* is lost.

The presence of a seminal vesicle and its form are considered important characters by many authors and they are used for example by Prudhoe (1985) to distinguish taxa within the Leptoplanidae. We have carefully checked all descriptions of *Discoplana* and *Euplana* species and it appears that different authors used the terms "seminal vesicle" and "ejaculatory duct" in different ways. Sometimes it was used to denote the somewhat swollen bifurcation of the

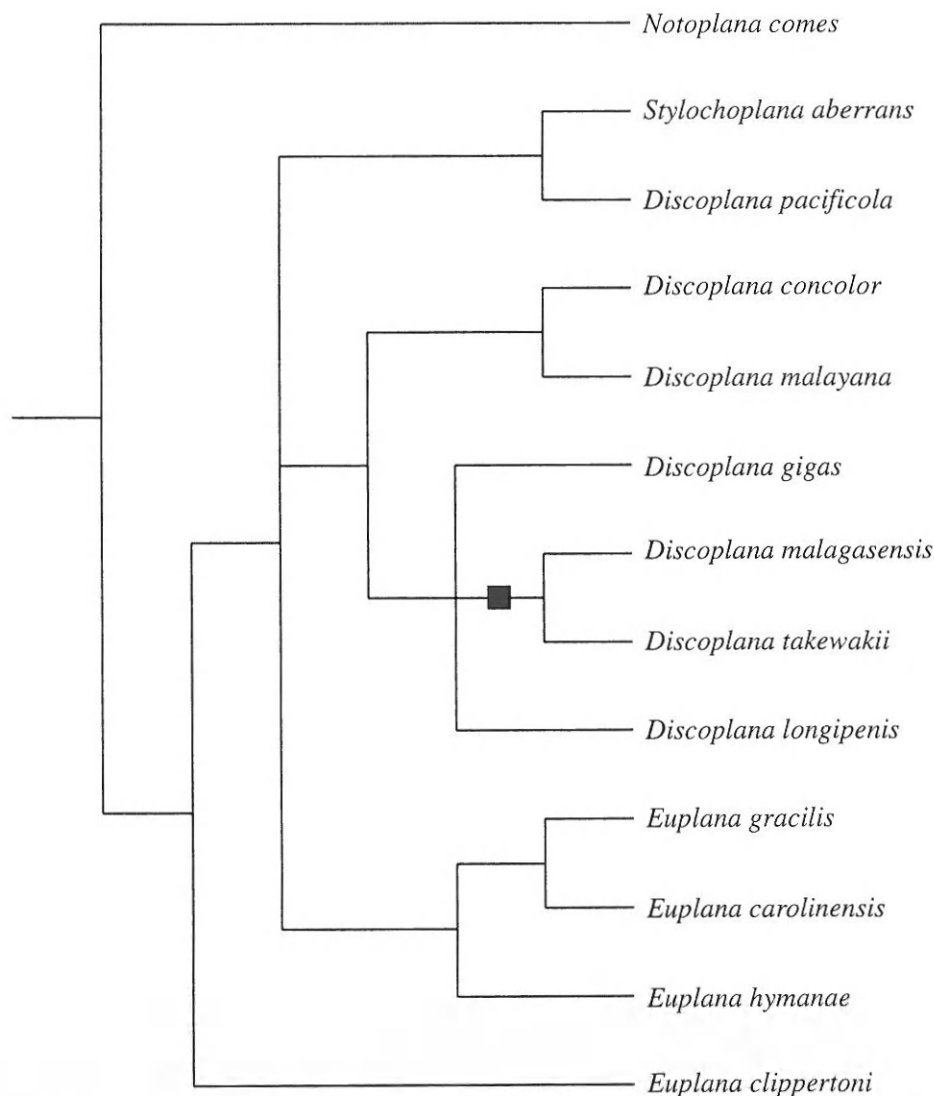
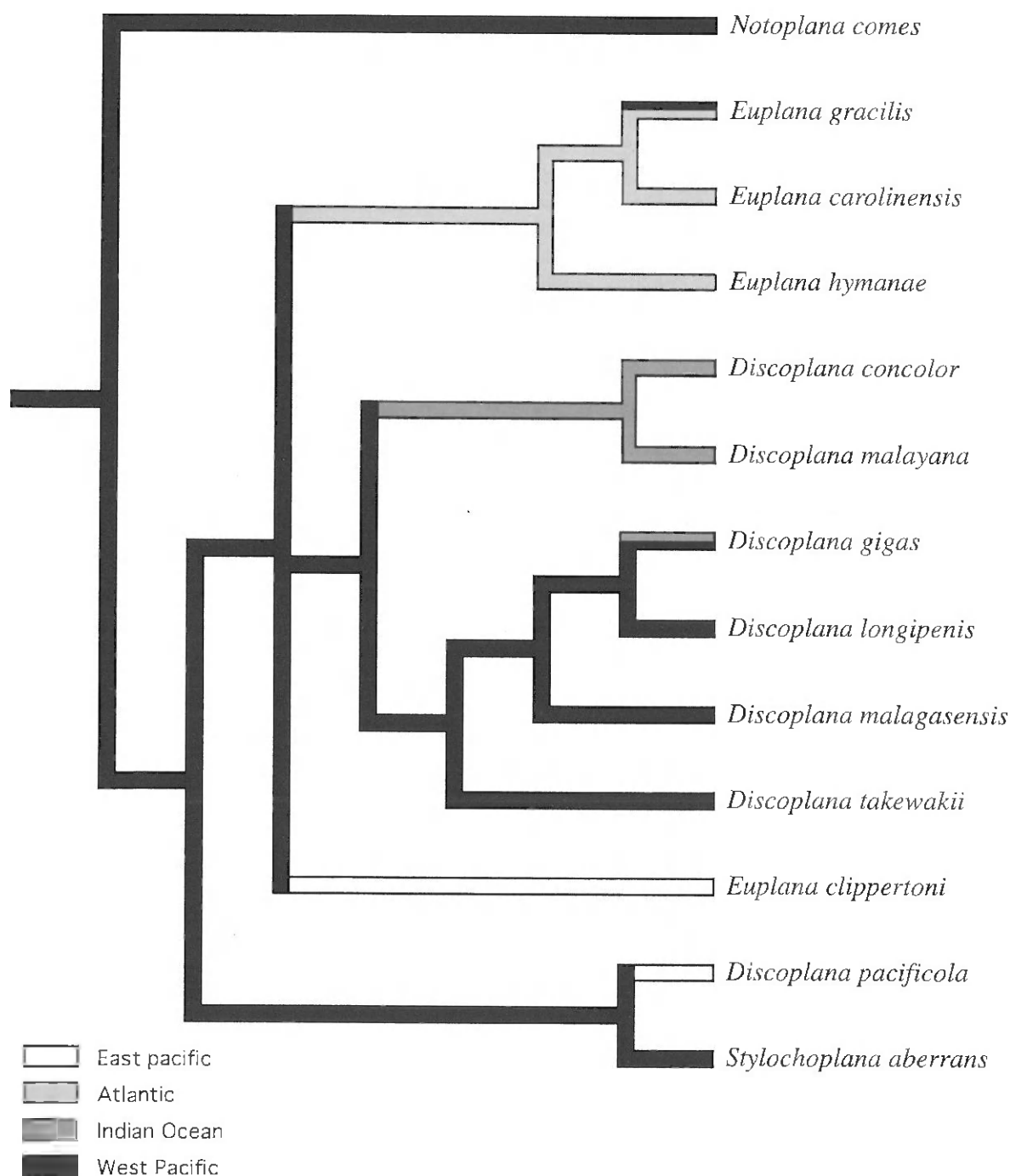


Fig. 8. Strict consensus cladogram of the two cladograms obtained by a successive weighting analysis, omitting the presence/absence of the seminal vesicle as a character. The black square indicates the origin of symbiosis.



ejaculatory duct into both vasa deferentia, in other cases it was used to denote some more or less swollen areas of the ejaculatory duct. In our definition, a seminal vesicle is a defined permanent organ where sperm are stored before copulation and is in fact a specialized area of the ejaculatory duct. Therefore, some species coded as lacking a seminal vesicle in our analysis were sometimes described as having such a vesicle in older literature. Due to this confusing ter-

minology, absence or presence of a seminal vesicle is often difficult to assess, especially from literature. Therefore we decided to reanalyse the matrix using the successive weighting procedure but omitting the seminal vesicle as a character. In the resulting two cladograms, the strict consensus of which is shown in Fig. 8, endosymbiosis is a synapomorphy for a monophyletic clade consisting of *D. malagasensis* and *D. takewakii*. The relationships between



**Fig. 9.** One of the most parsimonious cladograms resulting from the parsimony analysis using successive weighting of characters with the geographical distribution indicated.

this clade, *D. longipenis* and *D. gigas* remain unresolved. In other words, omitting of the doubtful character concerning the possible presence of the seminal vesicle cancels the differences between the different weighting procedures for what these four taxa are concerned. A more comprehensive research is needed to reveal the relationships between the different species, but our data suggest a sistergroup relationship between the two endosymbiotic species, both infesting ophiuroids and occupying the same anatomical compartment in their host (i.e. the genital bursae).

The taxonomical position of *D. pacificola* and *E. clippertoni* remains unclear. According to our data they do not belong to a monophyletic *Discoplana* and *Euplana* respectively. Some results even suggest a close relationship between *D. pacificola* and *S. aberrans*. Regarding *E. clippertoni*, the discussion made by Hyman (1939b) could refer to an immature specimen (see Prudhoe, 1985). More material is certainly required for a good discussion of that species. For the time being we refrain from any name changes regarding these species, because more research is surely needed, especially including more species of the taxon *Stylochoplana* and including more characters.

If the distribution of the species (detailed in the key presented hereafter) is plotted on the results of the cladistic analysis, a West Pacific origin of the *Discoplana/Euplana* group is apparent (Fig. 9). In this area still four species occur (*D. gigas*, *D. longipenis*, *D. malagasensis* and *D. takewakii*), as are the outgroup taxa (*S. aberrans*, *N. comes*). The *Euplana*-clade seems to result from an invasion into the Atlantic Ocean of a common ancestor that lost the Lang's vesicle. *E. gracilis* also occurs in the Western Pacific, but apparently this is a secondary distribution in that area. The Indian Ocean apparently has been invaded twice in the history of the group, once by the common ancestor of *D. concolor* and *D. malayana*, both species with the vagina orientated posteriorly, and a second time by *D. gigas*, which also still occurs in the Western Pacific. Only two species occur in the East Pacific. *E. clippertoni* is known from Clipperton Island and *D. pacificola* from the American West Coast. Judging from our cladograms, they have reached this distribution separately. However, because the phylogenetic position of both species is far from certain, this conclusion must be confirmed by future studies. Tajika *et al.* (1991) came also to the conclusion that the taxon *Discoplana* has an Indo-West Pacific origin rather than an Eastern Pacific one and their theory, based on the hypothetical evolutionary importance of the orientation of the vagina only, is confirmed by our findings.

**Emended diagnosis of *Discoplana*** (*D. pacificola* not included): Leptoplanidae without tentacles. With male and female gonopores separated, close or far from each other. Without real prostate organ. With distinct and muscular penial papilla, with or without a true stylet. Vagina long, extending anteriorly or posteriorly from the female gonopore. Without vaginal bursa or ductus vaginalis. Lang's vesicle present, proximally bulbous or extending to both sides

(anchor-shaped), without external aperture. Free-living or symbiotic in ophiuroids, relatively large oval forms.

**Emended diagnosis of *Euplana*** (*E. clippertoni* not included): Leptoplanidae without tentacles. Male and female gonopores separated and close to each other. Without real prostate organ. Distinct seminal vesicle present. With a short vagina extending anteriorly from the female gonopore. Common uterine duct absent. Vaginal bursa or ductus vaginalis absent. Lang's vesicle absent. Free-living, relatively small elongate forms.

#### Key to the *Discoplana/Euplana* species

- 1 Lang's vesicle absent.....2  
Lang's vesicle present.....4
- 2 Penial papilla present.....3  
Penial papilla absent.....***E. gracilis***  
Synonymy: *Prosthlostomum gracile* Girard, 1850  
*Conjuguterus parvus* Pearse, 1938  
Note: Up to 10 mm long.  
Distribution: Atlantic Ocean and Western Pacific Area.  
USA: Woods Hole, Boston Harbour, Massachusetts (Girard, 1850; Hyman, 1939a), Florida, North Carolina and Prince Edward Island (Pearse, 1938; Pearse and Littler, 1938; Pearse and Walker, 1939; Hyman, 1940a, b), Maryland (Christensen, 1971); Australia: Victoria (Prudhoe, 1982).
- 3 Ejaculatory duct very short, elongate pharynx.....***E. carolinensis***  
Synonymy: *Leptoplana angusta* Pearse & Littler, 1938  
Note: Up to 5 mm long.  
Distribution: Atlantic Ocean. USA: Beaufort, Bogue Sound, North Carolina (Pearse and Littler, 1938; Hyman, 1939c).  
Ejaculatory duct longer, compressed pharynx.....***E. hymanae***  
Note: Up to 8,5 mm long.  
Distribution: Atlantic Ocean. Brazil: Ilha das Palmas, Santos Bay (Marcus, 1947).
- 4 Vagina extends posterior to the female gonopore.....5  
Vagina extends forward from the female gonopore.....6
- 5 Common uterine duct absent.....***D. concolor***  
Synonymy: *Leptoplana concolor* Meixner, 1907  
*Euplana concolor* (Meixner, 1907) Hyman, 1954  
*Euplanoida concolor* (Meixner, 1907) Faubel, 1983  
Note: Up to 16 mm long.  
Distribution: Indian Ocean. Djibouti: "Iles Musha", Gulf of Tadjourah (Meixner, 1907).  
Common uterine duct present.....***D. malayana***  
Synonymy: *Leptoplana malayana* Laidlaw, 1903  
*Euplana malayana* (Laidlaw, 1903) Hyman, 1939a  
*Euplanoida malayana* (Laidlaw, 1903) Faubel, 1983

Note: Up to 35 mm long.

Distribution: Indian Ocean. Indonesia: Pulau Bidan, Penang, Straits of Malacca (Laidlaw, 1903).

6 Common uterine duct absent.....7

Common uterine duct present.....9

7 Symbiotic in ophiuroids, long vagina.....8

Free-living, penial papilla armed with a true stylet, short vagina.....**E. clippertoni**

Note: Up to 17 mm long.

Distribution: Eastern Pacific Area. Clipperton Island (Hyman, 1939b).

8 Penial papilla armed with a penial sheath.....**D. malagasensis**

Note: symbiotic of ophiuroid *Ophiothrix purpurea*. Up to 18 mm long.

Distribution: Western Pacific Area. Papua New Guinea: Laing Island, Hansa Bay (Deheyn et al. 1998, present contribution). In ophiuroid genital bursae.

Penial papilla armed with spines.....**D. takewakii**

Synonymy: *Euplana takewakii* (Kato, 1935) Hyman, 1953

*Euliyoida takewakii* (Kato, 1935) Faubel, 1983

Note: symbiotic of ophiuroid *Ophioplocus japonicus*. Up to 11 mm long.

Distribution: Western Pacific Area. Japan: Susaki near Shimoda, Misaki, Seto, Kii (Kato, 1935, 1944). In ophiuroid genital bursae.

9 Penial papilla devoid of sclerotic structures.....10

Penial papilla armed with a true stylet.....**D. longipenis**

Synonymy: *Euplana longipenis* (Kato, 1943) Hyman, 1953

*Aprostatum longipenis* (Kato, 1943) Faubel, 1983

Note: Up to 16 mm long.

Distribution: Western Pacific Area. Palao Islands: Ngatmel (Kato 1943, 1944).

10 Lang's vesicle enlarged.....**D. pacificola**

Synonymy: *Leptoplana pacificola* Plehn, 1896

*Euplana pacificola* (Plehn, 1896) Hyman, 1953

*Euplanoida pacificola* (Plehn, 1896) Faubel, 1983

Note: Up to 22 mm long.

Distribution: Eastern Pacific Area. Chile: Valparaiso (from the bottom of a boat) (Plehn, 1896a); Peru (Plehn, 1896a); Mexico: Guaymas, Lower California (Hyman, 1953).

Lang's vesicle extending to both sides.....**D. gigas**

Synonymy: *Leptoplana gigas* Schmarda, 1859

*Centrostomum gigas* (Schmarda, 1859) Diesing, 1862

*Leptoplana subviridis* Plehn, 1896

*Leptoplana pardalis* Laidlaw, 1902

*Discoplana subviridis* (Plehn, 1896) Bock,

1913

*Susakia badiomaculata* Kato, 1934

*Euplana gigas* (Schmarda, 1859) Hyman, 1955

*Ilyella gigas* (Schmarda, 1859) Faubel, 1983

*Euplanoida pardalis* (Laidlaw, 1902) Faubel, 1983

Note: Up to 65 mm long. Wide range of colour variation.

Distribution: Western Pacific Area and Indian Ocean. Djibouti: "Récif de la Clochetterie", near Obock (Meixner, 1907); Laccadive Islands (Laidlaw, 1902); Maldive Islands: Goidu, Goffurfehendu, Minikoi (Laidlaw, 1902); Sri Lanka: Trincomalee, Bellingham (Schmarda, 1859); Indonesia: Ambon, Moluccas (Plehn, 1896b; Bock, 1925b); Indonesia: Timor (Bock, 1913); Japan: Susaki near Shimoda, Izu (Kato, 1934, 1944); Bonin Island (Bock, 1925a); Gilbert Islands: Onotoa Atoll (Bock, 1925a; Hyman, 1955); Tuvalu: Funafuti Islands (Laidlaw, 1903); Fiji: Viti Levu (Tajika et al., 1991); also reported in Pacific Ocean: Caroline Islands: Kapin-gamarangi, Palao Islands and Guam (Hyman, 1955, 1959; Tajika et al., 1991).

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## APPENDIX

Characters used in the analysis:

1. Symbiotic: 0, no; 1, yes.
2. Location in the host: 0, ectosymbiotic; 1, endosymbiotic. Species that are ectosymbiotic are located at the oral side of the disc of the ophiuroid. Endosymbiotic species are found in the genital bursae.
3. Gonopores: 0, close to each other; 1, far from each other. This is proportional to the length of the animal. Close means they are very near to each other, i.e. less than 5% of the body length apart. Far means that they are much further apart than this.
4. Vagina: 0, short; 1, long. This is also taken proportional to the overall length of the animal.
5. Orientation of the vagina: 0, anterior; 1, posterior. In some species the vagina starts running anteriorly, ending or turning posteriorly after a while. In other species it starts running posteriorly immediately.
6. Lang's vesicle: 0, absent; 1, present. If a Lang's vesicle is absent, the vagina proximally starts where the two uterine ducts join each other.
7. Shape of Lang's vesicle: 0, bulbous; 1, enlarged; 2, extending to both sides. The Lang's vesicle can take different shapes. It can be long and narrow distally with a bulbous proximal swelling ("bulbous"), swollen over all of its length ("enlarged") or narrow with two very long sideway swellings proximally, giving it the shape of an anchor ("extending to both sides").
8. Common uterine duct: 0, absent; 1, present. Both uterine ducts can unite into one duct before entering the vagina. This is the common uterine duct. In other species, the uterine ducts enter the vagina separately.
9. Prostate vesicle: 0, absent; 1, present. Taxa coded absent can have some very small, inconspicuous glands at the distal end of the ejaculatory duct. These do however in no way resemble of a real prostate vesicle as it is known in many other Polyclads.
10. Ejaculatory duct: 0, very short; 1, longer. To avoid terminological problems, we will call the duct that extends proximally from the junction of the vasa deferentia towards the male antrum as ejaculatory duct. With a short ejaculatory duct we mean that proximally from the very muscular penial area, the ejaculatory duct almost immediately splits into the two vasa deferentia. If it is extending well beyond the penial area it is coded as being longer.
11. Seminal vesicle: 0, absent; 1, present. With seminal vesicle we mean a clear and obvious permanent proximal swelling of the ejaculatory duct in which sperm is stored.
12. Penial papilla: 0, absent; 1, present. The penial papilla is an obvious and permanent very muscular protrusion of the ejaculatory duct in the male antrum. It can be lined with a thin sclerotic layer (penial sheath). In these cases mostly the proximal part of the male antrum is also covered with such a thin layer.
13. Stylet: 0, absent; 1, present.



