Zoologica Scripta, Vol. 19, No. 1, pp. 13–29, 1990
Printed in Great Britain

0300–3256/90 $3.00+.00
Pergamon Press plc
© 1990 The Norwegian Academy of Science and Letters

A monograph of the Dimarcusidae (Platyhelminthes, Seriata, Tricladida)

RONALD SLUYS

Accepted 6 April 1989

Sluys, R. 1990. A monograph of the Dimarcusidae (Platyhelminthes, Seriata, Tricladida).—*Zool. Scr. 19:* 13–29.

The triclad family Dimarcusidae contains the species *Rhodax evelinae, Opisthobursa mexicana, O. josephinae, Balliania thetisae* and *Mitchellia sarawakana.* A comprehensive description is given of each of these species, covering external features, alimentary system, reproductive system, life cycle, ecology and distribution. Postulated apomorphies of the Dimarcusidae are (1) a penis bulb with gland cells, (2) horizontal orientation of female genital duct or bursal canal, combined with a dorsal opening of the common oviduct or diverticulum and (3) location of the ovaries at some distance posterior to the brain. The genera *Rhodax, Opisthobursa* and *Mitchellia* form a monophyletic group, the sistergroup being *B. thetisae.* The genera *Rhodax* and *Opisthobursa* share a sistergroup relationship. The Dimarcusidae should not be classified among the Maricola, Terricola or Paludicola. The family represents a fourth lineage within the Tricladida and is more closely related to the Paludicola and Terricola than to the Maricola.

R. Sluys, Institute of Taxonomic Zoology, University of Amsterdam, P.O. Box 4766, 1009 AT Amsterdam, The Netherlands.

Introduction

Over the last 40 years five planarians have been described which defy the taxonomic schemes developed by triclad systematists, viz. *Rhodax evelinae* Marcus, 1946, *Opisthobursa mexicana* Benazzi, 1972, *O. josephinae* Benazzi, 1975, *Balliania thetisae* Gourbault, 1978, and *Mitchellia sarawakana* Kawakatsu & Chapman, 1983. Four of these species have been assigned to the infraorder Maricola, albeit with much reservation, while *R. evelinae* has tentatively been placed among the paludicolans. The authors of these five species and also a few other workers (cf. Ball 1974*a*, 1977*a*; Kawakatsu & Mitchell 1984) have discussed the unusual characteristics of the animals and the difficulty of placing these species in the existing classifications.

In the most recent review of the phylogenetic relationships of the triclads (Sluys 1989*a*) it has only been attempted to assess the genealogical relationships of the well known groups of planarians, the Maricola, Terricola and Paludicola. The five species discussed in the present paper were omitted in the hope that the resulting phylogenetic tree would facilitate an evaluation of the phyletic position of the genera *Rhodax, Opisthobursa, Balliana* and *Mitchellia.* The most recent monograph on the marine triclads (Sluys 1989*b*) does not include these four genera because these taxa do not have the apomorphic characteristic of the infraorder Maricola, nor do they possess the derived features of the more restrictive groups of maricolans.

Re-examination of the type material of the species in the Dimarcusidae revealed that previous descriptions had to be supplemented and corrected. These new interpretations of the morphology are here incorporated in a standardized, comprehensive description of each species.

The nomenclature of the Dimarcusidae has been subject to some controversy concerning the criteria of availability, as specified in the International Code of Zoological Nomenclature. Mitchell & Kawakatsu (1972) claimed that their name *Dimarcus villalobosi* had priority over *Opisthobursa mexicana* which Benazzi (1972) had coined in a short note for what was evidently the same species. However, Benazzi (1973) presented ample arguments for the priority of the name *O. mexicana.* In subsequent studies other planarian specialists have followed Benazzi (cf. Ball 1977*a*), while Kawakatsu & Mitchell (1983: 293, 296) have accepted the name *O. mexicana* as the senior synonym.

In their paper Mitchell & Kawakatsu (1972) not only described a new genus and species but also erected the family Dimarcusidae, despite the fact that they realized that ". . . monotypic triclad families have not fared well". This unfortunate action now results in the situation that the family Opisthobursidae Benazzi & Giannini, 1973 is the junior synonym of Dimarcusidae, in accordance with article 40 of the International Code of Zoological Nomenclature (ed. 1985) and pointed out by Kawakatsu & Mitchell (1983: 296–297).

The aim of this paper is (1) to provide comprehensive taxonomic descriptions of *Rhodax evelinae, Opisthobursa mexicana, O. josephinae, Balliania thetisae* and *Mitchellia sarawakana,* (2) to analyze the phylogenetic relationships of these species, and (3) to discuss the historical biogeography of this monophyletic group of species. The present paper builds on the two aforementioned studies of the writer (Sluys 1989*a, b*) and argues that the genera *Rho-*

dax, *Opisthobursa*, *Balliania* and *Mitchellia* should all be placed in the family Dimarcusidae Mitchell & Kawakatsu, 1972. The possible relationships of the Dimarcusidae are discussed in the light of the previous study on the phylogenetic relationships among triclads (Sluys 1989*a*).

Material and methods

The material used for this study is listed under the appropriate species headings; additional material consisted of one series of sagittal sections of *Eviella hynesae* Ball, 1977.

The phylogenetic relationships have been analyzed according to the principles formulated by Hennig (1966) (see also Wiley 1981; Ax 1984). Relative degree of apomorphy and plesiomorphy was assessed through outgroup comparison (Watrous & Wheeler 1981; Maddison *et al.* 1984). Saether (1983) discussed an important phylogenetic concept not treated in the publications mentioned above, viz. underlying synapomorphy, but which is here considered to be of great value for phylogenetic reconstructions.

Systematic account

Family Dimarcusidae Mitchell & Kawakatsu, 1972

Opisthobursidae Benazzi & Giannini, 1973

Tricladida in which the common oviduct or diverticulum is oriented perpendicular to the horizontal bursal canal or female genital duct, and the penis bulb is provided with glandular elements; ovaries generally located at some distance posterior to the brain; vasa deferentia unite to extra-bulbar common vas deferens or penetrate separately the penis bulb; testicular follicles fused or discrete.

Genus *Rhodax* Marcus, 1946

Dimarcusidae with eyes, fused testes, and without a primary copulatory bursa; female genital duct communicating with the intestine; oviducts with caudal dichotomy; vasa deferentia unite to common vas deferens before penetrating the penis bulb.

Type species. *Rhodax evelinae* Marcus, 1946

Rhodax evelinae Marcus, 1946 (Figs. 1, 2)

Type locality. Near São Paulo, Brazil.

Type material. No specific specimen has been designated as the holotype by Marcus (1946), nor is such indicated on the labels of the original material.

Material examined. The redescription is based on the original serial sections and whole mounts as well as newly prepared sections of the type material. Original slides: two series of sagittal sections, each on 1 slide; two series of horizontal sections, each on 1 slide; 12 whole mounts on several slides. Newly sectioned material: preparation Rh.A, transverse sections of one specimen, mounted on 4 slides; preparation Rh.B, sagittal sections of one animal on 3 slides; preparation Rh.C, sagittal sections of the region of the copulatory apparatus of one animal, mounted on 2 slides; preparation Rh.D, transverse sections of the pharynx region of one specimen, on 2 slides; preparations C80-1, C80-2, C80-3, sagittal sections of the front end, each series mounted on a separate slide. Additional, sectioned type material was obtained from the Royal Ontario Museum, Toronto, Canada.

Diagnosis. Characteristic for *Rhodax evelinae* are the fused testes follicles, the course of the vasa deferentia and the various large, false seminal vesicles. With respect to the female reproductive system the species is characterized by (1) the absence of a bursa and (2) a female genital duct which opens into the digestive tract, and by (3) the caudal dichotomy of the oviducts.

Redescription

Habitus. Preserved and mounted specimens are up to about 5.5 mm long and 1.25 mm wide; live specimens may reach a length of 9 mm and a width of 1–1.5 mm (Marcus 1946). The front end is rounded, the hind end pointed; the body margins run more or less parallel. The median region of the front end forms a highly glandular adhesive area. The colour of the animals "is pink or brownish, due to red, brown or black pigment cells that are smaller and scarce on the ventral side" (Marcus 1946), while the eyes are situated in white patches.

Alimentary system. The pharynx is short, about one-tenth of the body length. The inner circular muscle layer of the pharynx is considerably thicker than the outer circular muscle layer. The mouth opening is at the middle of the pharyngeal pocket.

The anterior ramus of the intestine gives rise to 7–10 pairs of lateral diverticula and terminates behind the brain. Each posterior ramus gives off 10–20 lateral diverticula; the branches usually do not communicate (Marcus 1946).

Male reproductive system. The testes extend from the level of the ovaries into the posterior end of the body. The numerous follicles are fused and form a tube with a continuous lumen; this tube occupies the entire space between ventral and dorsal body surface. At the level of the pharyngeal pocket each tube gives off an efferent duct (or vas deferens) which runs postero-medially. The ducts traverse the row of vitellaria and cross the ventral nerve cords and, subsequently, expand to wide false seminal vesicles. From the posterior surface of these vesicles arise rather narrow ducts which unite just behind the pharyngeal pocket to form another large false seminal vesicle. Before the vasa deferentia unite to form the last-mentioned seminal vesicle, the ducts already communicate through a narrow commissure.

From the hind wall of the common seminal vesicle arises a narrow duct that penetrates the penis bulb, runs through the papilla and, eventually, opens at the tip of the latter. In some specimens this duct increases in diameter to form a small seminal vesicle just after having entered the penis bulb. This duct, the ejaculatory duct, follows the course of the penis papilla. This implies that the major portion of the ejaculatory duct runs more or less parallel to the body surface and that its distal section curves rather sharply towards the ventral surface in order to open at the ventrally facing tip of the penis papilla. The ejaculatory duct is lined with a cuboidal, nucleated epithelium which bears cilia. These cilia extend from the beginning of the duct up to the point where it bends towards the ventral body surface; cilia are absent posterior to this point, while the lining epithelium becomes somewhat taller. The ejaculatory duct is surrounded by a thin layer of circular muscles.

The penis bulb is provided with strong circular, and more or less interwoven muscle fibres. These circularly running fibres also extend on the penis papilla, where they form a sort of layer traversing the parenchyma of the papilla, but which is distinct from the thin circular muscle layer that underlies the lining epithelium of the penis. This last-mentioned circular muscle layer is internally bounded by an equally thin layer of longitudinal fibres.

The male atrium is lined with a tall, villose epithelium which is underlain with a thin layer of circular and longitudinal muscles, respectively.

Female reproductive system. The highly developed vitellaria occupy the entire space between dorsal and ventral body surface. The vitelline follicles lie medially to the testes tubes and extend from anterior to the ovaries into the hind end of the body.

The ovaries are located dorsally to the ventral nerve cords at about one-third of the distance between the brain and the root of the pharynx. The oviducts arise from the ventro-medial surface of the ovaries. A short branch of the oviducts may extend anterior to the ovaries but could not be observed in all specimens examined. In running backwards, the oviducts stay just medially to the ventral nerve cords. At the level of the gonopore the oviducts bifurcate, one branch curves dorso-medially while the other branch runs to the hind end of the body, medially to the ventral nerve cords. The dorso-medially curving branches from either side of the body unite to a common oviduct. The latter shows a pronounced loop before its distal, ventrally facing section opens into the female genital duct. The common oviduct is lined with a cuboidal, nucleated epithelium which is provided with cilia. Highly developed and extensive shell glands discharge their secretion into the common duct.

The female genital duct communicates with the male atrium via a narrow and unciliated section; the posterior, ciliated part of the female genital duct meets the intestine. A copulatory bursa is absent. The female genital duct is surrounded by a thin layer of circular muscles and a thin layer of longitudinal muscle fibres.

Eyes. The eyes consist of a unicellular pigment cup containing a relatively large, though not precisely known number of retinal cells. Eye lenses are absent. In several specimens each eye was accompanied by an extra eye, so that these animals had four eyes in total (contra Kawakatsu & Chapman 1983: 29).

Life cycle. According to Marcus (1946) the animals lay unstalked, spherical cocoons from March to June; two cocoons present with preserved type material were about 0.75 mm in diameter. The species also reproduces asexually by means of fission and according to Marcus (1946) this forms its principal mode of reproduction.

Ecology and distribution. Rhodax evelinae was "found in dirty ponds and clear water brooks near the city of São Paulo" (Marcus 1946: 171). The species is known only from its type locality.

Remarks

The description given above, which is based on a re-examination of the type material, differs in some respects from Marcus (1946).

According to Marcus, the female genital duct first widens to a funnel-shaped bursa before communicating with the intestine. Neither in the original sections, nor in the newly sectioned specimens, could I find evidence of such a bursa. In all cases the female genital duct opened directly into the digestive tract; in one horizontally sectioned specimen this occurs via a slightly enlarged, funnel-shaped section which possesses cilia, as is the case with the entire female duct.

The newly sectioned specimens clearly showed common oviducts with a loop-like course and the same could be reconstructed from the original material, although this is not mentioned in Marcus' description. The histology and the diameter of the common oviduct are not different from those of the oviducts, as suggested by Marcus (1946: fig. 149). On the basis of Marcus' description, Ball (1974*a*) concluded that the "common ovovitelline duct" was actually equivalent to the diverticulum of the bursal canal or female genital duct. Although this conclusion may still be correct, the re-examination revealed that it is equally valid to consider the structure to be a common oviduct.

Ball (1974*a*: 352) also interpreted Marcus' description such that part of the female genital duct actually forms a part of the atrium. Ball defined these parts in relation to the gonopore. I prefer, however, to use the opening of the common oviduct into the copulatory apparatus as reference point. The section anteriorly to this opening then may correspond with the atrium, which is lined with a villose epithelium that lacks cilia (cf. Marcus 1946: fig. 152). The portion of the female genital duct posterior to the opening of the common oviduct, corresponds with the female genital duct *sensu stricto* and is equivalent to the bursal canal of other triclads. This section of the female genital duct is lined with a cuboidal and ciliated epithelium.

The female genital duct is surrounded by an inner layer of circular muscles and an outer layer of longitudinal fibres.

Kawakatsu *et al.* (1985) published on the chromosome portrait of asexual *Rhodax* ? sp. collected from fish tanks in Japan.

Genus *Opisthobursa* Benazzi, 1972

Dimarcus Mitchell & Kawakatsu, 1972

Dimarcusidae without eyes and with a secondary copulatory bursa which opens ventrally to the exterior; testicular follicles fused or discrete; vasa deferentia separately penetrating the penis bulb.

Type species. *Opisthobursa mexicana* Benazzi, 1972

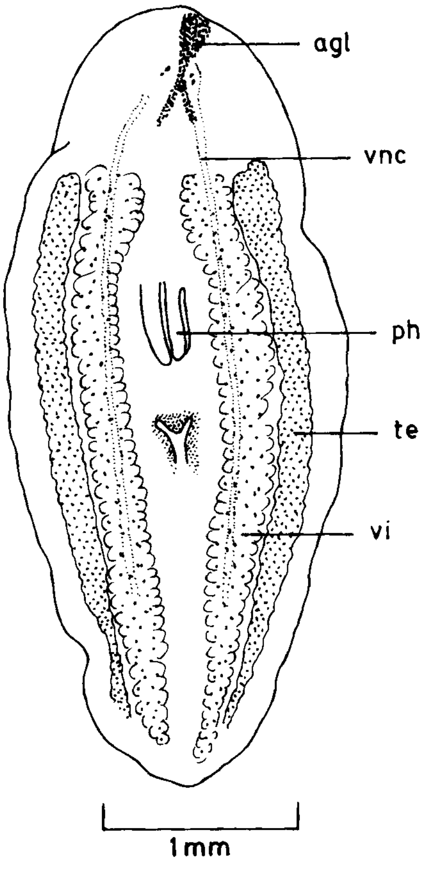
Opisthobursa mexicana Benazzi, 1972 (Figs. 3, 4, 13)

Dimarcus villalobosi Mitchell & Kawakatsu, 1972

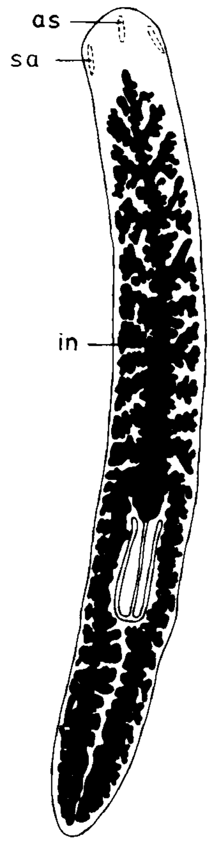
Type locality. The cave "Grutas de Coconá", Tabasco, Mexico.

Type material. The type series consists of 5 sets of sagittal and one set of transverse serial sections, deposited in Benazzi's collection in the Department of Zoology, University of Pisa, Italy.

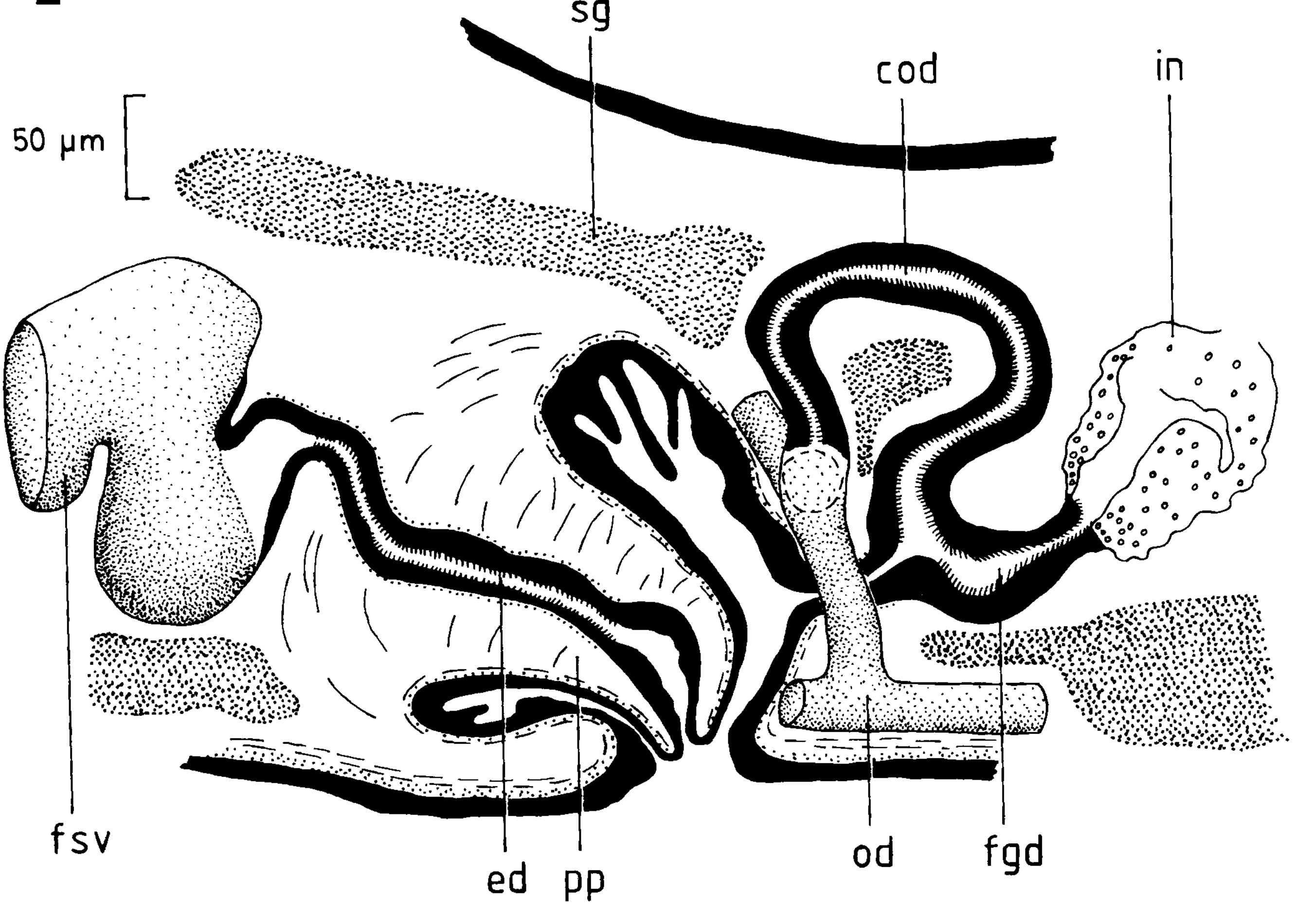
1



3



2



Figs. 1–3.—1. Rhodax evelinae. Dorsal view of whole mount.—*2. Rhodax evelinae.* Sagittal reconstruction of the copulatory apparatus.—*3. Opisthobursa mexicana.* Dorsal view of live specimen (after Mitchell & Kawakatsu 1972). Mature specimens vary between 6 and 14 mm in length.

Material examined. United States National Museum, Washington: USNM 50085, Las Grutas de Coconá, Tabasco, Mexico, 26.08.1972, sagittal sections on 2 slides; USNM 50086, sagittal sections on 2 slides; USNM 50087, sagittal sections on 2 slides; USNM 50088, horizontal sections on 3 slides.

Diagnosis. Opisthobursa mexicana may be recognized by the following features: (1) a copulatory bursa that is surrounded by unicellular glands and which opens ventrally to the exterior, (2) a characteristic structure of the penis bulb, consisting of gland cells and muscle fibres, (3) dorsally directed diverticulum of the bursal canal.

Redescription

Habitus. Mature, live specimens vary between 6 and 14 mm in length and 0.8–3 mm in breadth. The body is slender, with the margins running almost parallel. The hind end is obtusely pointed, and the front end rounded or slightly protruded. The animals are unpigmented and eyeless (Mitchell & Kawakatsu 1972; Benazzi & Giannini 1973).

In addition to the normal gliding way of movement, *O. mexicana* is capable of a very rapid, leech-like type of locomotion: the body is drawn forwards, then the anterior portion of the animal is fixed to the substratum and, subsequently, the hind part of the body is pulled forwards (Mitchell & Kawakatsu 1972).

Alimentary system. The pharynx is about one-eighth of the body length and is situated in the posterior portion of the body. The inner circular muscle layer of the pharynx is much more developed than the outer layer of circular muscle fibres. The mouth opening is situated at the hind end of the pharyngeal pocket.

The anterior ramus of the intestine terminates at a short distance behind the brain. This anterior trunk gives rise to 10–15 irregularly branched, lateral diverticula; each of the posterior main intestinal trunks gives off 15–20 diverticula. There are no commissures between the posterior rami (Mitchell & Kawakatsu 1972).

Male reproductive system. The numerous testicular follicles are arranged in a lateral row on either side of the body. The follicles are situated laterally to the gut or extend only between the tips of the intestinal diverticula. The small testes are situated ventrally, but frequently they extend to well beyond the mid-line of the body or even approach the dorsal body surface. The rows of testicular follicles run from the level of the ovaries into the hind end of the body. In the horizontal sections I counted about 100 follicles in each row; their number, however, may be 300 or more (Mitchell & Kawakatsu 1972).

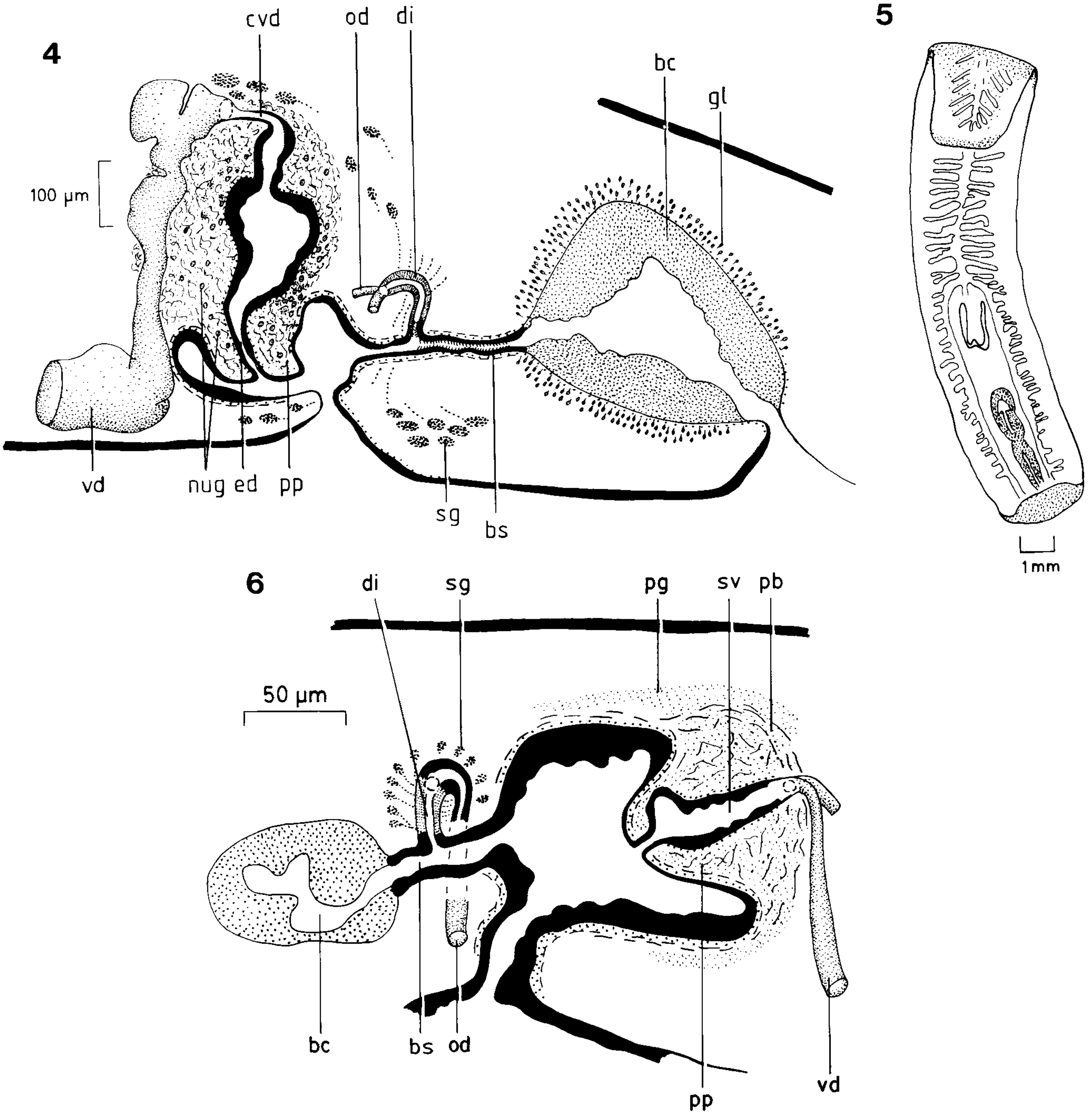
At the hind end of the pharyngeal pocket the vasa deferentia enlarge to form false seminal vesicles. They enter the ventro-lateral portion of the penis and, narrow and expand several times before they unite in the dorsal part of the penis bulb to a narrow common vas deferens. This common duct turns towards the ventral surface and opens, by gradually enlarging in diameter or by means of a more abrupt transition, into the broad lumen of the penis bulb. In the blunt, short penis papilla this bulbar lumen narrows to form the ejaculatory duct. The lumen of the bulb is lined with tall, nucleated and glandular cells. Similar, albeit smaller cells line the ejaculatory duct. The large oval-shaped penis bulb consists of large glandular cells, and loosely and irregularly arranged muscle fibres (I was unable to confirm the statement of Mitchell & Kawakatsu (1972) that "The fibres of the muscular coat of the bulb are arranged in concentric layers"). These gland cells are filled with a fine granular secretion which may also be found in the lumen of the penis bulb. Bulbar cavity and ejaculatory duct are surrounded by a thin layer of circular muscle fibres. The penis papilla is lined with a thin, nucleated epithelium. The small atrium into which the papilla projects is lined with tall, nucleated cells.

Female reproductive system. The small ovaries are situated just above the ventral nerve cord about 0.4 mm behind the brain, i.e. about one-fifth of the distance between the brain and the root of the pharynx. The oviducts arise from the posterior surface of the ovaries and run backwards slightly medially to the ventral nerve cords. At the level of the penis the oviducts turn dorsomedially and, subsequently, open separately into the distal part of the diverticulum of the bursal canal. The diverticulum arises from the antero-dorsal part of the bursal canal and its distal portion is curved anteriad. No musculature could be discerned around the diverticulum, which receives the openings of well-developed shell glands. This diverticulum could well be considered to constitute a common oviduct, its histology being quite like that of the oviducts (but also like the bursal canal).

The copulatory bursa is an oval to sac-shaped vesicle of which the postero-ventral part communicates with the exterior through a small, ventral pore. The bursa is lined with very tall, nucleated cells, which are filled with a granular secretion. The bursa is surrounded by a thin muscle layer and a zone of pear-shaped gland cells. The bursal canal arises from the anterior surface of the bursa; the canal runs forwards either more or less parallel to the body surface, or with a slightly oblique, ventral orientation, and meets the hind wall of the atrium. The rather narrow bursal canal is lined with a nucleated epithelium and is surrounded by a subepithelial layer of circular muscles and a layer of longitudinal muscles. The proximal portion of the canal, i.e. from the bursa to the point of emergence of the diverticulum, is ciliated.

Vitellaria are well-developed; they are situated between the intestinal diverticula and extend from just anterior to the ovaries into the hind end of the body. The vitelline follicles occupy the entire space between dorsal and ventral body surface.

Body wall. The dorsal epidermis is thicker than the ventral and has more rhabdites. Dorsal and ventral body surfaces are ciliated with the cilia much more developed on the ventral epidermis. The front end of the animal shows a mid-ventral slit and a lateral sensory area on either side of the head. According to Mitchell & Kawakatsu (1972) the adhesive slit is merely an indentation of the body wall lacking cilia. I was unable to confirm this observation since the slit could not be observed unequivocally in the material examined. However, I did observe an indentation of the normal, thus ciliated, ventral body wall



Figs. 4–6.—4. Opisthobursa mexicana. Sagittal reconstruction of the copulatory apparatus. —*5. O. josephinae.* Ventral view of preserved specimen. —*6. Balliania thetisae.* Sagittal reconstruction of the copulatory apparatus.

at the point where frontal, erythrophilous adhesive glands open to the exterior; perhaps this indentation corresponds with the adhesive slit. The erythrophilous glands extend dorsally to the brain.

Karyology. The diploid number of chromosomes is 12. Each haploid set consists of two large chromosomes, one metacentric and the other one telocentric, and four small chromosomes which are submetacentric or subtelocentric (Benazzi & Giannini 1973).

Cocoon. The cocoons are spherical and unstalked (Benazzi & Giannini 1973).

Ecology and distribution. Opisthobursa mexicana is known only from the type locality, which is a cave 65 m above sea level (Benazzi & Giannini 1973); according to Kawakatsu & Mitchell (1983) the cave entrance is at an altitude of approx. 40 m. Experiments revealed that *O. mexicana* is restricted to freshwater for it cannot survive even in diluted sea water (Benazzi & Giannini 1973).

Opisthobursa josephinae Benazzi, 1975 (Figs. 5, 7, 10, 12)

Type locality. Pozza Casa Bell, San Cristóbal de las Casas, Chiapas, Mexico.

Type material. Holotype: Benazzi's collection at the Department of Zoology, University of Pisa, Italy, sagittal sections on 7 slides. Paratypes: see below; Benazzi's collection, sagittal sections of one specimen. The original sections, housed in the Department of Zoology in Pisa, are said to be "heavily discoloured" (Benazzi *in litt.*).

Material examined. Newly sectioned paratypes from Benazzi's collection: preparation OJ-1, Mexico, Chiapas, San Cristóbal de las Casas, 7.10.1973, sagittal sections on 24 slides; preparation OJ-2, sagittal sections of hind end on 4 slides; preparation OJ-3, sagittal sections of front end on 8 slides.

Diagnosis. Opisthobursa josephinae can be distinguished from other species in the Dimarcusidae by the copulatory bursa with its highly folded lining epithelium and ventral pore.

Redescription

Habitus. Preserved specimens are up to 21 mm long and 3 mm wide. Anterior and posterior body ends are somewhat truncated or broadly rounded in appearance, while the body margins run parallel. The body is unpigmented and the animals lack eyes.

Alimentary systsm. The short pharynx is between one-fourteenth and one-thirteenth of the body length and is inserted at about the middle of the body. The inner circular muscle layer of the pharynx is considerably thicker than the outer circular muscle zone. The mouth opens at the hind end of the pharyngeal pouch.

The anterior ramus of the intestine extends for a short distance anterior to the brain.

Male reproductive system. The numerous testicular follicles extend from a short distance posterior to the ovaries into the most posterior end of the body, occupying the entire space between dorsal and ventral body surfaces. The follicles are interconnected, thus forming a testes tube on either side of the body.

The vasa deferentia expand to form large false seminal vesicles but diminish in diameter before separately penetrating the penis bulb. Within the bulb the vasa deferentia unite to a common section which communicates with the spacious penis lumen. The common vas deferens is lined with more or less cuboidal, nucleated cells and is surrounded by a thin layer of circular muscles.

The penis bulb is large, consisting of loose parenchymatic tissue, being bounded by a well developed layer of interwoven muscle fibres. An orange-red staining granular secretion accumulates within the bulb and is discharged into the anterior section of the penis lumen. This lumen is lined with relatively tall, nucleated cells, which are underlain with a subepithelial layer of circular muscles and a layer of longitudinal muscles. The first-mentioned layer is much thicker on the penis papilla than underneath the bulbar section of the penis lumen. The penis papilla is a plump structure, which fills the entire atrium.

Female reproductive system. The relatively small, paired ovaries are situated closely to the brain, medially to the ventral nerve cords. The oviducts arise from the posterior wall of the ovaries and run medially to the ventral nerve cords. Just behind the atrium the oviducts unite to a short common oviduct which opens in the mid-dorsal section of the bursal canal. Shell glands discharge their secretion into the common oviduct, and in addition a few glands also penetrate the posteriormost section of the oviducts. The bursal canal runs ventrally parallel to the body surface, connecting the atrium with the copulatory bursa. The canal is lined with a cuboidal, nucleated epithelium and is surrounded by a layer of interwoven muscle fibres.

The copulatory bursa is a large vesicle which at its posterior end opens ventrally to the exterior. The bursa is lined with a densely and intricately folded epithelium where many cells contain vesicles filled with relatively large globules. The bursa is surrounded by a well-developed layer of interwoven muscle fibres which follow the contour of the bursa but also penetrate the folds or septa. Benazzi (1975) observed a spermatophore in the bursa of his specimen(s) examined, but this could not be found in the newly sectioned animals.

Vitelleria are present throughout the body length, extending from dorsal to ventral body surface and being situated medially to the testes.

Ecology and distribution. Opisthobursa josephinae is known only from its type locality, a freshwater biotope in a cave with the entrance at a height of 2115 m (Benazzi 1975).

Remarks

In Benazzi's (1975) specimens the testes, a dozen follicles, were confined to the posterior end of the body, while the ovaries were absent. This peculiar situation, however, may be due to a particular phase in the sexual cycle since the newly sectioned specimens have both testes (tubes) and ovaries.

In the description given above it was assumed that the oviducts unite to from a common oviduct which opens into the bursal canal. However, it is equally valid to assume that the oviducts open into a diverticulum of the bursal canal.

***Opisthobursa*? sp. Kawakatsu & Mitchell, 1983**

Description

Specimens of this planarian were collected from the cave Grutas de Languin, Departamento de Alta Verapaz, Guatemala (approx. 15°34'25"N 89°58'50"W), which is at an altitude of approx. 352 m. The type localities of *Opisthobursa mexicana* and *O. josephinae* are within 400 km of the sampling locality of *Opisthobursa*? sp. (cf. Kawakatsu & Mitchell 1983: fig. 4).

The planarian is white due to lack of pigment and lacks eyes. The body dimensions range from 6 × 0.9 mm to 3 × 0.5 mm. The front end lacks tentacles. The posteriorly situated pharynx is about one-seventh of the body length. All animals examined by Kawakatsu & Mitchell (1983) were immature, so that no information could be obtained on the ovaries, testes, vitellaria and copulatory apparatus. According to these authors *Opisthobursa*? sp. resembles in general appearance *O. mexicana*.

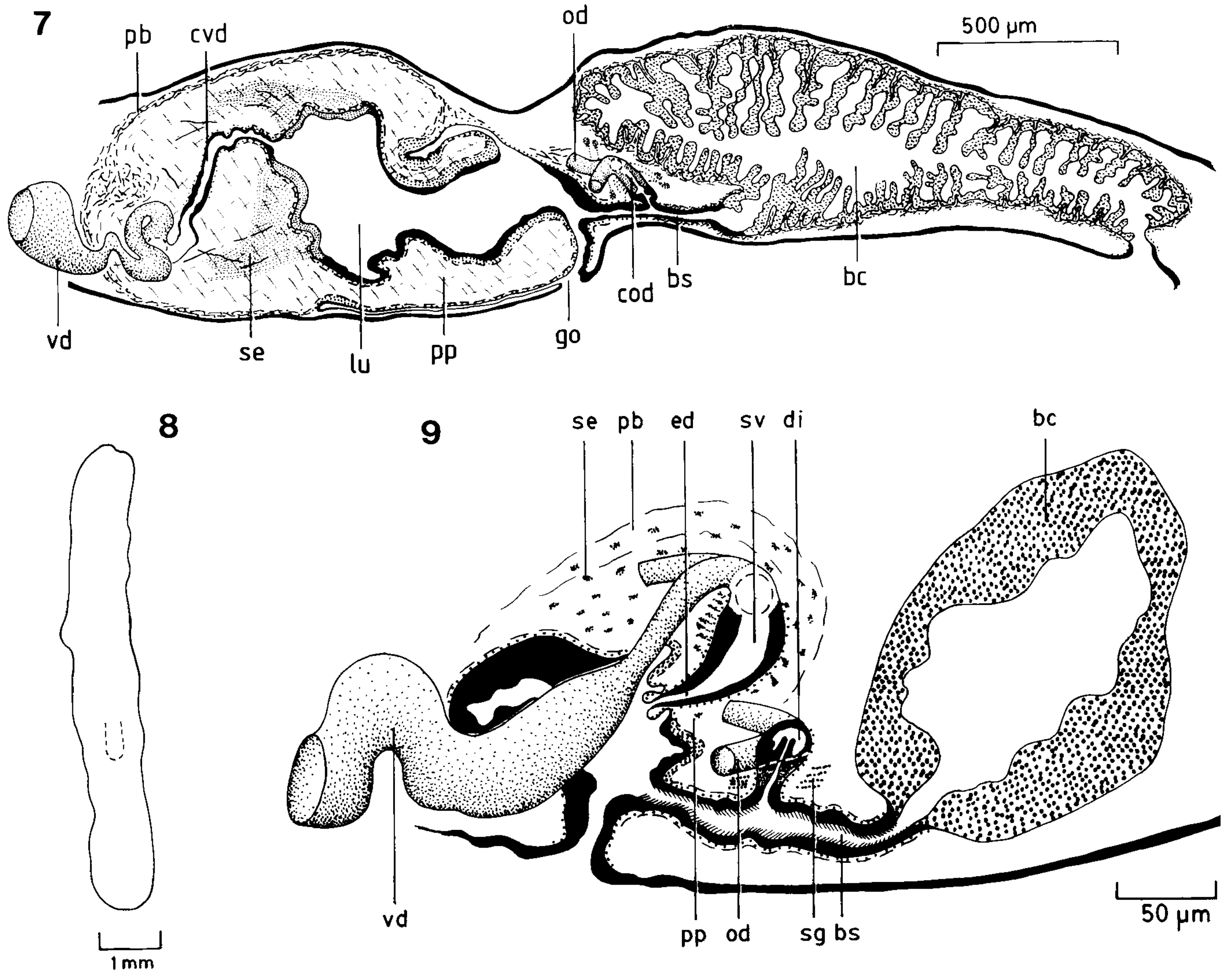
Genus *Ballianía* Gourbault, 1978

Dimarcusidae without eyes and with a primary copulatory bursa posterior to the gonopore; testes few and discrete; vasa deferentia separately penetrating the penis bulb.

Type species. *Ballianía thetisae* Gourbault, 1978

***Ballianía thetisae* Gourbault, 1978** (Figs. 6, 11)

Type locality. Maraa, Paea, Tahiti.



Figs. 7–9.—7. Opisthobursa josephinae. Sagittal reconstruction of the copulatory apparatus.—*8. Mitchellia sarawakana.* External features of living specimen (after Kawakatsu & Chapman 1983).—*9. M. sarawakana.* Sagittal reconstruction of the copulatory apparatus.

Type material. Holotype: MNHN, Paris: AJ 885–886, sagittal sections on 2 slides. Paratypes: see above; MNHN, Paris: AJ 770–773, serial sections.

Material examined. Museum National d'Histoire Naturelle, Paris: Paratypes: AJ 887, horizontal sections on 1 slide; AJ 888–889, sagittal sections on 2 slides (immature animal); AJ 890–891, sagittal sections on 2 slides; AJ 892–AJ 894 transverse sections on 3 slides.

Diagnosis. Balliania thetisae may be distinguished from other species in the Dimarcusidae by the following combination of features: (1) small number of testes, of which the anteriormost follicles are situated at a considerable distance behind the brain, (2) presence of ciliated pits, (3) presence of a primary copulatory bursa.

Redescription

Habitus. Living specimens may reach a length of 6.5 mm and a width of 1 mm. The truncated anterior body margin is slightly convex, while laterally the head possesses two rounded auricles. The margins of the elongate body run parallel and terminate in an obtusely pointed hind end. The body is unpigmented; eyes are absent.

Alimentary system. The pharynx is situated in the posterior half of the body and measures about one-tenth of the body length. The inner circular muscle layer of the pharynx is somewhat thicker than the outer zone of circular muscles. The mouth opening is situated in the middle of the pharyngeal cavity.

The anterior ramus of the intestine terminates behind the brain and, according to Gourbault (1978), gives rise to 15–16 diverticles. Each posterior intestinal trunk gives off about 12 lateral diverticula. Just behind the copulatory apparatus the posterior rami communicate through a commissure.

Male reproductive system. The very small, rounded testes are situated ventrally between the tips of the intestinal diverticula. There are 5–7 follicles on either side of the body, extending from a considerable distance behind the ovaries to somewhat behind the pharyngeal cavity, but not posteriorly to the gonopore.

The vasa deferentia narrow before penetrating the midsurface of the penis bulb. Immediately within the bulb both ducts open into a broad duct or seminal vesicle which is lined with a tall, nucleated epithelium surrounded by a layer of circular muscles. The seminal vesicle expands in the distal section of the penis papilla, after which it opens at the tip of the papilla through a short and narrow duct.

The penis glands discharge into the seminal vesicle. This secretion is present throughout the penis bulb and papilla and also occurs outside the bulb. The penis bulb consists of loosely interwoven muscle fibres which radiate over the proximal section of the penis papilla. The latter is a short, blunt cone which is covered with a low, nucleated epithelium.

The spacious male atrium is lined with a very tall, nucleated epithelium which is underlain with a circular and a longitudinal muscle layer, respectively.

Female reproductive system. The small ovaries are situated at some distance behind the brain. The oviducts arise from the lateral wall of the ovaries and run backwards dorso-laterally to the ventral nerve cords. Behind the gonopore the oviducts turn dorso-medially and open separately into the dorsal section of the diverticulum of the bursal canal. This dorsally directed diverticulum also receives the openings of the shell glands.

The short bursal canal runs more or less parallel to the body surface, connecting the atrium with a sac-shaped copulatory bursa. The bursa is lined with tall, nucleated cells which contain a granular substance.

The bursal canal is lined with nucleated cells and, according to Gourbault (1978), surrounded by a subepithelial layer of circular muscles, interiorly bounded by a layer of longitudinal muscles.

The vitellaria are only sparsely developed; they are situated between the intestinal branches, and occupy more or less the space between dorsal and ventral body surface.

Sense organs. The head of the animal is provided with a pair of sensory organs, consisting of shallow invaginations of the dorsal body surface (Fig. 11), being situated at about 150 μm from the anterior body margin. The ciliated lining epithelium of the invaginations lacks rhabdites and has nuclei only at the entrance of the pit. According to Gourbault (1978) these pits are similar to those described by Ball (1974*b*: fig. 2b) for *Reynoldsonia reynoldsoni*. However, the ciliated pits of *Balliania* are not flask-shaped, as is the case in *Reynoldsonia*. The pits in *Balliania* are simple invaginations and in general appearance similar to the sensory fossae of *Spathula fontinalis* (cf. Ball 1977*b*: fig. 34).

Karyology. The diploid complement consists of 22 chromosomes which decrease gradually in size. The chromosomes are metacentric or submetacentric (Gourbault 1978).

Life cycle. The unstalked, spherical cocoons are 0.6–0.8 mm in diameter. In general, cocoons are deposited every 2 months. Embryonic development takes about 2 weeks, after which 3–6, but usually 4, young hatch from the cocoons. It takes 2 months before these young planarians have matured and are capable of producing cocoons. Gourbault (1978) found evidence for a certain reproductive cycle since in April the animals were devoid of a copulatory apparatus, while no cocoons were laid from May to November.

Ecology and distribution. Balliania thetisae was collected from its only known locality: underground, phreatic waters on the isle of Tahiti.

Remarks

The drawing of Gourbault (1978: fig. 2) suggests that the diameter of the oviducts is very small as compared with the width of the diverticulum of the bursal canal. However, in the paratypes AJ 890–891, AJ 892–894, and AJ 887 differences in diameter were less pronounced, and it is difficult to decide whether the diverticulum represents a proper expansion of the bursal canal or is just a common oviduct.

On the basis of the available evidence I conclude that *Balliania* possesses a copulatory bursa (see above), which contrasts with Gourbault's opinion that the bursal canal opens into a branch of the intestine. According to Gourbault (1978) the histology of the bursa is similar to that of the intestine, and probably for that reason she considers the presumed bursa to be part of the digestive system. However, I found these cells to be more granular and less vacuolated than the cells lining the gut trunks. In the horizontally sectioned specimen the lining of the bursa stained differently from that of the intestine, which even may be observed in Gourbault's Plate I, fig. 4 (the rounded vesicle between OV and 4 is the bursa). Moreover, in none of the specimens examined could I trace a connection between the bursa and the intestine.

The dorsal body surface is underlain with extensive glandular elements, which occur from behind the brain into the posterior end of the body. Gourbault (1978) apparently considered these glands to be vitelline follicles.

Genus *Mitchellia* Kawakatsu & Chapman, 1983

Dimarcusidae without eyes and with a primary copulatory bursa posterior to the gonopore; testicular follicles discrete; vasa differentia separately penetrating the penis bulb.

Type species. *Mitchellia sarawakana* Kawakatsu & Chapman, 1983

Mitchellia sarawakana **Kawakatsu & Chapman, 1983** (Figs. 8, 9)

Type locality. Water Polo Cave, Gunung Mulu National Park, 4th Division, Sarawak, East Malaysia (4°00′N 114°51′10″E).

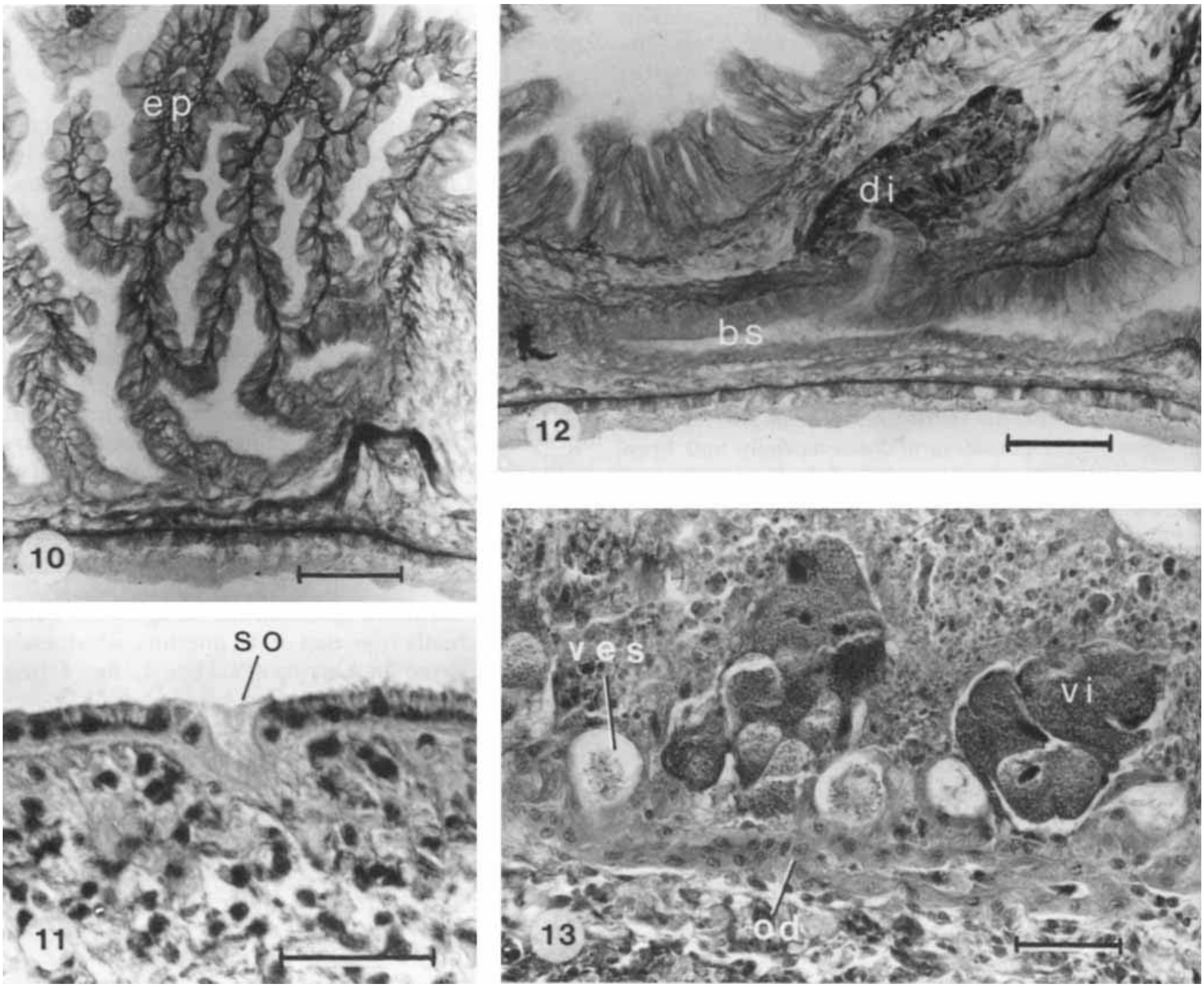
Type material. Holotype: NSMT, Tokyo (Kawakatsu's specimen 1657-o), sagittal sections on 1 slide. Paratypes: NSMT-Pl. 2967 (see below); Kawakatsu's private collection: nos. 1657-a, -c, -d, -e, -h.

Material examined. National Science Museum, Tokyo: Paratype: NSMT-Pl. 2967 (Kawakatsu's specimen 1657-g), sagittal sections on 1 slide (front end missing).

Diagnosis. Mitchellia sarawakana is characterized by a well developed bursa posterior to the male copulatory apparatus, and a very small penis papilla with a preputial fold.

Redescription

Habitus. Preserved, sexually mature specimens up to



Figs. 10–13.—10. Opisthobursa josephinae. Sagittal section through lining epithelium of copulatory bursa.—*11. Balliania thetisae.* Sagittal section of sensory organ.—*12. O. josephinae.* Sagittal section of bursal canal and common oviduct.—*13. O. mexicana.* Sagittal section of oviduct, vitellaria and resorptive vesicles. Scale bars: 10, 12, 13: 50 μm; 11: 30 μm.

7 mm long and 1 mm wide; body ends obtuse. The animals are unpigmented and eyes are missing (Kawakatsu & Chapman 1983).

Alimentary system. The pharynx is situated somewhat behind the middle of the body and is about one-fifth of the body length; the mouth opening is at the hind end of the pharyngeal cavity (Kawakatsu & Chapman 1983). The inner circular muscle layer of the pharynx is well developed, being much thicker than the outer circular muscle layer (cf. Kawakatsu & Chapman 1983: fig. 4A).

According to Kawakatsu & Chapman (1983) the anterior intestinal trunk gives rise to 10–12 pairs of lateral diverticula, while each posterior trunk gives off about 10 short diverticula. Unfortunately, Kawakatsu & Chapman (1983) only stated that the anterior intestinal branch extends anterior to the ovaries (see also their fig. 4B), but they did not mention whether this branch extends over the brain or ends behind the latter, and the material examined could not resolve this issue.

Male reproductive system. The small testes occur both at the dorsal and ventral body surface. According to Kawakatsu & Chapman the follicles are arranged in 5–7 longitudinal rows, extending from just behind the ovaries into the very hind end of the body, while behind the pharynx there also are a number of inter-intestinal testicular follicles.

Posterior to the pharyngeal cavity the vasa deferentia expand to false seminal vesicles, which narrow again before separately entering the penis bulb. Within the bulb the vasa deferentia open separately into the proximal portion of a seminal vesicle which may either (1) narrow gradually to form the ejaculatory duct, or (2) may be set off from the last-mentioned duct by a constriction (cf. Kawakatsu & Chapman 1983: fig. 5). Seminal vesicle and ejaculatory duct are surrounded by a thin layer of circular muscle fibres.

The musculature of the rounded penis bulb is weak. In the specimen examined the major part of the bulb appeared to contain blotches of accumulated secretion, which seemed to be discharged into the seminal vesicle and the ejaculatory duct. According to Kawakatsu & Chapman, however, penis glands only open into the distal portion of the ejaculatory duct.

The penis papilla is a short and blunt structure, with a preputial fold at its tip. The lining epithelium of the papilla is underlain with a relatively thick outer layer of circular muscles and a thin inner layer of longitudinal muscles.

The tall, nucleated epithelium of the common atrium is underlain with a thin outer layer of circular muscle fibres and an equally thin layer of longitudinal muscles.

Female reproductive system. In their description Kawakatsu & Chapman (1983) only mentioned that the two small ovaries are situated between the second and third, or between the third and fourth lateral branches of the anterior gut trunk, while in the genus diagnosis they stated that the ovaries are in their "usual anterior position". Unfortunately, these statements do not provide very precise information on the location of the female gonads. The oviducts run medially to the ventral nerve cords.

The relatively large, sac-shaped copulatory bursa is lined with tall, vacuolated cells with nuclei at their base. The bursal canal arises from the antero-ventral surface of the bursa and it runs anteriad, parallel to the body surface, in order to communicate with the genital atrium. At about half-way its length, the bursal canal gives rise to a dorsally directed diverticulum, the latter being surrounded by circular muscles. The oviducts increase somewhat in diameter before they open, separately, into the distal section of this diverticulum. The diverticulum is pierced by the openings of shell glands ventrally to the oviductal openings. The diverticulum is lined with nucleated cells (non-ciliated, according to Kawakatsu & Chapman), some of which form a tiny, dorsally directed, papilla-like projection in the distal portion of the diverticulum. The bursal canal is lined with nucleated cells bearing well developed cilia. This lining epithelium is underlain with a subepithelial layer of circular muscles and a layer of longitudinal muscles.

Ecology and distribution. Mitchellia sarawakana is known only from its type locality, a limestone cave, where the species was collected from very small, shallow pools (1–3 cm deep), "floored with floccular yellow-brown silt and bat droppings overlying calcareous gravel". The water temperature of the sampling locality was about 23.5°C, and similar pools had a pH of 7.0 (Kawakatsu & Chapman 1983).

Remarks

Some remarks are appropriate with respect to the structure of the diverticulum of the bursal canal. There is a discrepancy as regards the openings of the oviducts between my observations and fig. 5 of Kawakatsu & Chapman (1983). I found the oviducts to increase gradually in diameter before meeting the diverticulum and not being the narrow oviducts opening into the dorsal portion of the diverticulum described by Kawakatsu & Chapman (1983). It is debatable whether the dorsal section of the diverticulum actually corresponds with the fused posterior ends of the oviducts, or represents the distal section of the diverticulum itself. One other possibility is that the diverticulum actually is a true common oviduct.

Examination of more material will be necessary in order to ascertain whether the papilla-like projection inside the diverticulum is a constant feature or only represents an artifact.

Phylogeny

Monophyly of the Dimarcusidae

Marcus (1946: 172) described the genus *Rhodax* as "a collective type", thus expressing the situation that *R. evelinae* possesses a complex mixture of primitive features. The same holds true for the other genera in the Dimarcusidae, which obviously complicates the assessment of phylogenetic affinities. For example, in the Dimarcusidae the testes are (1) situated ventrally, or (2) occupy the entire space between dorsal and ventral body surface, or (3) occur along the dorsal as well as the ventral body surface. Ventral testes are primitive within the Tricladida (Sluys 1989*b*). The second and third condition of the testes, as mentioned above, may well have been secondarily derived from an initially completely ventral position of the testicular follicles. For the Maricola ventral testes in combination with a prepharyngeal distribution represents the primitive condition (Sluys 1989*b*). However, this does not imply that testes throughout the body length may be invoked as a derived feature for the Dimarcusidae. This is not difficult to see if it is realised that the Dimarcusidae probably is more closely related to the Paludicola and the Terricola than it is to the Maricola (see below). In the two first-mentioned infraorders both character states are found, viz. testes being confined to the prepharyngeal region as well as testes extending throughout the body length. This implies that the ancestral condition for the Dimarcusidae becomes equivocal (cf. Maddison *et al.* 1984), i.e. the ancestor may have had prepharyngeal testes or may have been characterized by testes throughout the body length.

Other examples of primitive characters are: nucleated epidermis, absence of adhesive papillae, presence of rhabdites, nucleated female genital duct or bursal canal, absence of a precerebral intestinal branch.

It is here proposed that there are three features suggesting the monophyletic status of the Dimarcusidae, viz. (a) the penis bulb with gland cells (Fig. 14, character 1), (b) the horizontal orientation of the bursal canal or female genital duct, combined with the dorsal opening of the common oviduct, or diverticulum (Fig. 14, character 2), (c) the location of the ovaries at some distance posterior to the brain (Fig. 14, character 3).

In all members of the Dimarcusidae, excepting *R. evelinae*, the penis bulb consists of interwoven muscle fibres being interspersed with gland cells (character 1), a situation which was already noticed by Gourbault (1978) in *B. thetisae*, by Benazzi & Giannini (1973) in *O. mexicana*, and by Benazzi (1975) in *O. josephinae*. In other triclads the glandular elements of penial glands are usually situated outside the penis bulb. Absence of character 1 in *Rhodax* may either be explained as secondary loss or as retention of the primitive character state. The second alternative implies that character 1 should be regarded as an underlying synapomorphy, which is expressed in *Balliania*, *Opisthobursa* and *Mitchellia*.

The second postulated apomorphous character for the Dimarcusidae concerns the combination of a horizontally running bursal canal or female genital duct and a dorsal opening of the common oviduct or diverticulum (Fig. 14, character 2), thus resulting in the characteristic T-junction. It must be noted that for none of the species is there unequivocal morphological evidence that the vertical part of the T-junction represents a common oviduct or the diverticulum of the bursal canal/female genital duct. But it is here assumed that these short vertical sections are homologous structures within the Dimarcusidae. It is here also assumed that the bursal canal (in the case of a primary or secondary copulatory bursa) and the female genital duct (in the case of absence of a bursa) share a homologous relationship.

A horizontal bursal canal is already a rare phenomenon among triclads, while the combination with a vertically oriented diverticulum or common oviduct represents a unique characteristic.

The groundplan or ancestral condition for the Tricladida is characterized by ovaries being situated directly posterior to the brain. Sluys (1989*b*) suggested that within the Tricladida deviations from this groundplan condition should be considered as the result of secondary change instead of retention of the primitive character state. This implies that the condition with the ovaries positioned at some distance posterior to the brain may in principle be assessed as a derived character state. However, this derived state apears to have evolved independently in various lineages within the Tricladida. Within the Maricola, for example, there are two lineages which are characterized by ovaries being located somewhere between the brain and the root of the pharynx (Sluys 1989*b*). But a posterior position of the ovaries, i.e. between the brain and the pharynx, is also known from various freshwater triclads, e.g. *Cura pinguis* and *C. foremanii* (pers. obs.), *Romankenkius pedderensis* (Ball 1974*b*), *Dendrocoelum lychnidicum* (Kenk 1978), *Dugesia burmaensis* (Kaburaki 1918). It is here suggested that a posterior position of the ovaries has also independently evolved in the Dimarcusidae, and supports the proposed monophyletic origin of the group (Fig. 14, character 3). This character is clearly expressed in *B. thetisae*, *R. evelinae* and *O. mexicana*, while for *M. sarawakana* present information is somewhat ambiguous (see above). Character 3 is not expressed in *O. josephinae*, in which the ovaries are situated closely to the brain; this situation is here interpreted as retention of the primitive character state.

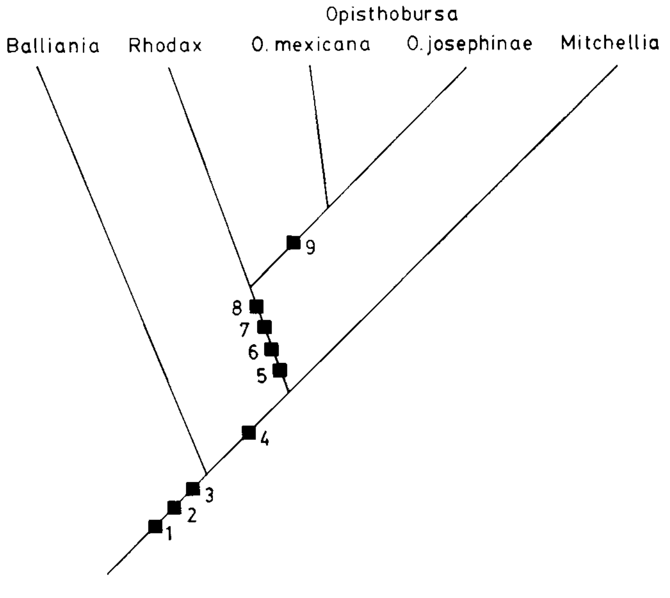


Fig. 14. Phylogenetic tree of the Dimarcusidae. Postulated apomorphous characters: 1, penis bulb with gland cells; 2, T-junction bursal canal/female genital duct and common oviduct/diverticulum; 3, ovaries at some distance posterior to the brain; 4, oviducts medially to ventral nerve cords; 5, posterior part bursal canal/female genital duct ciliated; 6, vitellaria medially to the testes; 7, absence of primary copulatory bursa; 8, testes tubes; 9, two gonopores (for further explanation, see text).

Relationships within the Dimarcusidae

In *Rhodax, Opisthobursa* and *Mitchellia* the oviducts run medially to the ventral nerve cords, whereas in *Balliania* the oviducts follow a course latero-dorsally to the nerve cords. There is only scant information on the course of the oviducts in the Proseriata, Terricola and Paludicola (see Sluys 1989*b*). In the Maricola the oviducts generally run laterally to the ventral nerve cords, a course medially to the nerve cords being very rare (Sluys 1989*b*). It is here hypothesized that oviducts running medially to the ventral nerve cords represent a derived character linking the genera *Rhodax, Opisthobursa* and *Mitchellia* (Fig. 14, character 4). However, it is desirable to have more information on the course of the oviducts in the relevant outgroups of the Dimarcusidae.

The proposed unique common ancestry of the genera *Rhodax* and *Opisthobursa* is supported by four apomorphous characters, viz (a) ciliation being confined to the posterior section of the bursal canal or female genital duct (Fig. 14, character 5), (b) vitellaria being situated medially to the testes (Fig. 14, character 6), (c) loss of the primary copulatory bursa (Fig. 14, character 7), (d) testes tubes (Fig. 14, character 8).

With respect to *R. evelinae,* Ball (1974*a*) in essence suggested that the non-ciliated section of the female genital duct anterior to the opening of the common oviduct forms part of the atrium, which is non-ciliated (see above). The same situation applies to *O. mexicana* and *O. josephinae,* in which also only the posterior part of the bursal canal—the section posterior to the opening of the diverticulum/common oviduct—is provided with cilia. In the outgroups, *Mitchellia* and *Balliania,* the bursal canal is ciliated over its entire length, i.e. posteriorly as well as anteriorly to the opening of the common oviduct/diverticulum. Moreover, in triclads the bursal canal or female genital duct generally is ciliated. Therefore, a partly ciliated bursal canal/female genital duct is here presumed to be an apomorphous character (character 5).

Vitelline follicles which are situated medially to the testes (character 6) are rare among triclads. In the Proseriata the vitellaria generally occur laterally to the testes and in the Maricola vitelline follicles either may be situated laterally to the testes or the distribution of the vitellaria coincides with that of the testes (Sluys 1989*b*). To the best of my knowledge paludicolans are characterized by vitelline follicles being situated laterally to the testes or have follicles of which the distribution coincides with that of the testicular follicles. In view of the distribution of the various character states, medially located vitellaria are here postulated as a synapomorphy (character 6) for the genera *Rhodax* and *Opisthobursa.*

A primary copulatory bursa, lined with vacuolated cells, is a characteristic structure in many triclads, although quite frequently it has secondarily been lost. By outgroup comparison it can be established that presence of a primary copulatory bursa represents a primitive, plesiomorphous character for the triclads in general and for the Dimarcusidae in particular. Such a primary bursa is present in *B. thetisae* and *M. sarawakana*. The primary bursa has been lost, however, in *Rhodax* and *Opisthobursa*, which is here hypothesized as a synapomorphy for these two genera (character 7).

There is ample evidence that the copulatory bursae of *O. mexicana* and *O. josephinae* are not primary bursae but secondarily evolved structures, a possibility already pointed out by Ball (1977*a*). An obvious difference between a primary copulatory bursa and the situation in *O. mexicana* and *O. josephinae* is that in these two species the bursae open ventrally to the exterior. In triclads the primary bursa does not have a ventral pore, while occasionally it may have a dorsal pore, as is the case in the marine triclad *Nesion arcticum* Hyman, 1956. But there are also histological differences suggesting a secondary origin for the bursae in *Opisthobursa*. In *O. mexicana* the bursa is lined with tall cells which are filled with a granular secretion, originating from gland cells that lie around the copulatory bursa; such a situation is unknown from primary bursae. The bursa of *O. josephinae* is lined with an intricately folded epithelium, thus forming irregularly shaped septa which are penetrated by muscle fibres (Fig. 10). This is quite unlike a primary bursa, which is a rounded or sac-shaped vesicle lined with vacuolated cells and is surrounded by a usually feeble muscle coat. It is not known whether the numerous vesicles with globules in the epithelium of the bursa result from temporary resorptive activity of the cells or represent a structural characteristic of the bursa of *O. josephinae*. But I have never observed such vesicles in the copulatory bursae of other triclads.

Testes tubes, i.e. interconnected testicular follicles (character 8), are only present in *R. evelinae* and *O. josephinae*. It is here hypothesized that the tendency of the testicular follicles to fuse forms an underlying synapomorphy, asserting the unique common ancestry of the genera *Rhodax* and *Opisthobursa* (character 8). This is based on the assumption that testes tubes have independently arisen in various platyhelminth lineages, and on the knowledge that fused testes are rare among triclads. Another hypothesis could be that testes tubes are postulated as a strict synapomorphy for the genera *Rhodax* and *Opisthobursa*, and that absence of this character in *O. mexicana* is interpreted as the result of secondary loss.

According to Karling (1940) rhabdocoels frequently show anastomoses between the testes. However, within the Tricladida I only know of the following species in which the testes are united or show a tendency to fusion: *Phagocata velata* (Ball 1974*a*; Ball *et al.* 1981), *Eviella hynesae* (Ball 1977*a*), *Spathula foeni* (Ball 1977*b*) and *Romankenkius bilineatus* (Ball & Tran 1979). The presence of testes tubes in the three last-mentioned species is best explained as derived independently of the situation in *R. evelinae* and *O. josephinae* because the genera *Eviella*, *Spathula* and *Romankenkius* are part of a monophylum (see de Vries & Sluys in press) which is only very distantly related to the Dimarcusidae. The species *P. velata* belongs to the Planariidae, a family with only distant affinities to the Dimarcusidae (see below).

The unique common ancestry of *O. mexicana* and *O. josephinae* is indicated by the presence of two gonopores (Fig. 14, character 9), which relates to the ventral pores of the secondary copulatory bursae.

Phyletic position of the Dimarcusidae

Starting from the phylogeny of the triclads as proposed by Sluys (1989*a*) the question to be asked is whether the Dimarcusidae belongs to one of the triclad infraorders, viz. Maricola, Paludicola or Terricola, or forms a separate phylogenetic unit.

Evaluation. Most of the species in the Dimarcusidae have previously been assigned to the Maricola or were, at least, thought to be marine relicts. However, there are several points which argue against inclusion of the Dimarcusidae in the monophylum Maricola. The foremost reason for doubting maricolan affinities is that the taxon Dimarcusidae does not have the apomorphous characteristic of the Maricola, nor the derived features of more restrictive groups of marine triclads. Members of the Dimarcusidae lack the annular zone of adhesive papillae which forms a synapomorphy for the marine triclads (Sluys 1989*a*, *b*). There are marine triclads in which the adhesive papillae have been lost, but for these species there is usually other evidence suggesting that they belong to the Maricola. Such is not the case for the species in the Dimarcusidae; on the contrary, other evidence suggests that the family should not be included in the Maricola.

Presence of eyes in epigean *R. evelinae* and absence of eyes in the four other, subterranean species suggests that loss of eyes has been induced by the cavernicole biotope. Therefore, the eyes of *R. evelinae* are here considered to be representative for the Dimarcusidae, which may gain some support from the fact that within the Tricladida eye structure may differ between taxa but is usually stable within groups.

In marine triclads the eyes consist of a unicellular pigment cup with 1–3 retinal cells. There is only one marine triclad with multiretinal eyes, *Stummeria marginata*, but in this species the eyes are provided with a lens, a structure that only occurs in the Maricola. The eye structure characteristic of *R. evelinae*, unicellular pigment cup with many retinal cells, is not found in the Maricola, but neither is it known from the Terricola or the Paludicola (see Sluys 1989*b*).

With respect to the eyes there is another point that argues against maricolan affinities of the Dimarcusidae. In several specimens of *R. evelinae* each eye was accompanied by an extra eye cup. Marine triclads never have more than two eye cups, in contrast to some freshwater triclads with multiple eyes and to the land planarians, which may have numerous eyes.

Benazzi (1975) reported the presence of a spermatophore in his specimens of *O. josephinae*. Spermatophores are characteristic of the Paludicola and are not known from the Maricola (Sluys 1989*a*).

I have suggested that the presence of resorptive vesicles is a derived feature, linking the Terricola and the Paludicola (Sluys 1989*a*). The term resorptive vesicle has been coined for (a) the ventral, vacuolated part of vitellarian follicles, close to the point of communication with the oviducts, and (b) for vesicles which are presumably altered vitellarian follicles (cf. Sluys 1989*c*). In these resorptive vesicles degeneration and resorption of excess sperm takes place. Resorptive vesicles are absent in marine triclads (Sluys 1989*a*), while I have observed very distinct vesicles in *O. mexicana* (Fig. 13). In *O. josephinae* and *R. evelinae* resorptive vesicles are absent; unfortunately, the preparations of *B. thetisae* and *M. sarawakana* did not allow a proper assessment of the presence or absence of this feature. It is here proposed that presence of resorptive vesicles in *O. mexicana* at least precludes inclusion of the Dimarcusidae in the Maricola and it is suggested that absence of these vesicles in the other members of the family results from retention of the primitive character state.

According to all previous workers *R. evelinae* should be classified among the freshwater planarians (Marcus 1946; Ball, 1974*a*; Kawakatsu & Mitchell 1984) and therefore it is appropriate to examine here whether or not the Dimarcusidae belongs phylogenetically to the infraorder Paludicola. Under the phylogenetic hypothesis proposed by Sluys (1989*a*) the Dimarcusidae cannot belong to the monophylum Paludicola, as presently defined, because members of the family lack at least two of the three apomorphies of the infraorder.

Members of the Dimarcusidae have the primitive type of subepidermal musculature and lack the extra outer layer of longitudinal fibres, which was postulated as a synapomorphy for the freshwater planarians.

Furthermore, the probursal condition, another apomorphy for the Paludicola, obviously does not apply to the Dimarcusids.

The third presumed apomorphy for the Paludicola, the spermatophore, may also apply to the Dimarcusidae (see above), so that perhaps this character should be used at a higher level of universality (see below) than proposed by Sluys (1989*a*).

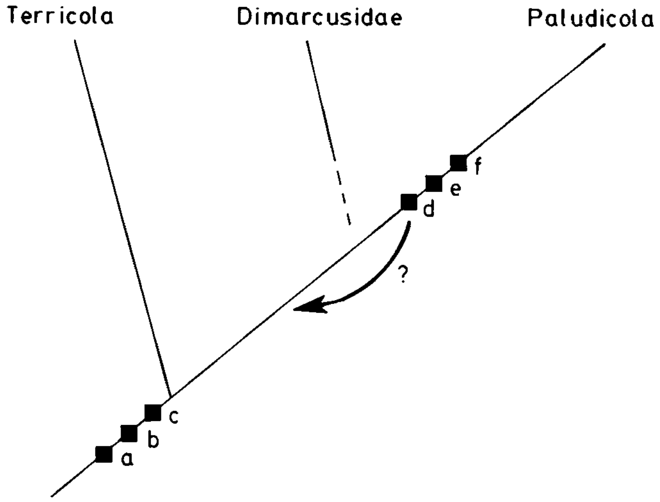


Fig. 15. Phyletic position of the Dimarcusidae. Postulated apomorphous characters: a, absence of adhesive papillae; b, resorptive vesicle; c, reduction number of longitudinal nerve cords; d, spermatophore; e, probursal condition; f, extra, outer longitudinal muscle layer (for further explanation, see text).

The Dimarcusidae does not belong to the monophylum Terricola because every representative of the family lacks the presumed synapomorphies of the land planarians, viz. creeping sole, complex pharynx musculature and diploneuran nervous system (Sluys 1989*a*).

Conclusion

Above, arguments were presented for not including the Dimarcusidae in any of the triclad infraorders as diagnosed by Sluys (1989*a*). The Dimarcusidae represents a separate phylogenetic lineage, characterized by the presumed apomorphous characters 1, 2 and 3 (Fig. 14). Thus, the remaining question to be answered is that of the phyletic position of the Dimarcusidae in relation to the infraorders Maricola, Terricola and Paludicola.

It is here proposed that the Dimarcusidae is more closely related to freshwater and the land planarians than to the Maricola because the family shares at least two of the hypothesized apomorphies of the Paludicola + Terricola, viz. loss of adhesive papillae (Fig. 15, character a), and presence of resorptive vesicles (Fig. 15, character b; see above); concerning the Dimarcusidae I have not been able to obtain information on the third presumed synapomorphy, the reduction of the number of longitudinal nerve cords (Fig. 15, character c).

The data set at hand suggests little else than a sistergroup relationship between the Paludicola and the Dimarcusidae, although this presumed affinity remains poorly supported by apomorphous characters. It may be that one of the postulated apomorphies of the Paludicola, sperm transfer by means of a spermatophore, also applies to the Dimarcusidae. In the above it was pointed out that Benazzi (1975) observed a spermatophore in *O. josephinae,* but that such a structure was absent in the newly sectioned animals. Spermatophores are not known from the other species in the Dimarcusidae. It may be that sperm transfer through spermatophores is a feature that is already expressed in some members of the Dimarcusidae and therefore should be used at a higher level of universality in the phylogenetic analysis. In that case presence of a spermatophore could be used to support the postulated sistergroup relationship between the Paludicola and the Dimarcusidae, as suggested in Fig. 15. However, it is evident that present information is too scant for formally proposing the spermatophore as supporting evidence for a sistergroup relationship between the Dimarcusidae and the Paludicola.

Biogeography

The distributional range of the Dimarcusidae covers a large part of the globe and is mainly concentrated around the Pacific Ocean. Biogeographic tracks between the Indo-West Pacific and Central, and South America are by no means uncommon as is amply illustrated in the works of Croizat (cf. Croizat 1958: 82–88; see also Thorne 1972). However, it is much more likely that the biogeographic track of the Dimarcusidae covers at least the entire former Gondwanaland and that absence of Dimarcusids in Africa, for example, is the result of lack of information instead of true absence of species in that region. Under the vicariance paradigm the present data points on the map already suggest at least a Gondwanian range for ancestral Dimarcusids, which appears to be supported by the area cladogram of the Tricladida (Fig. 17).

With respect to the Maricola, I have shown that the major groups of species had already evolved on Pangea (Sluys 1989*b*). A comprehensive biogeographic analysis of the Terricola is presently unavailable. But a preliminary analysis of several genera (Sluys unpublished) showed that also in the Terricola biogeographic tracks cover large parts of the globe, thus suggesting at least a Pangean origin of the infraorder. Ball (1974*a*) suggested that the Dugesiidae had a Gondwanian origin and that present disjunct distributions within the family are best explained by the breakup of Gondwanaland (see also Ball & Fernando 1969). According to Ball (1974*a*: 350, 351) the family Planariidae is restricted to Holarctis and part of Orientalis, while of the Dendrocoelidae the Kenkiinae occur in Central and East Asia and in North America, and the Dendrocoelinae have a holarctic range. This implies that the ancestral range of the monophylum Planariidae plus Dendrocoelidae comprised at least Laurasia.

From the above it can be deduced that under the phylogenetic hypothesis proposed in this study ancestral Dimarcusids at least ranged over Gondwanaland, or had already differentiated on Pangea (Fig. 17).

Discussion

Phylogeny

In Fig. 14 autapomorphous characters for individual species have not been indicated because I have been unable to distinguish such characters. In this study individual species can only be characterized by a constellation of features, a situation frequently encountered in phylogenetic analyses on the species level. That a species can be diagnosed as different from other species is generally sufficient evidence for its evolutionary reality, in which convicton we may be strengthened by the finding of apomorphies.

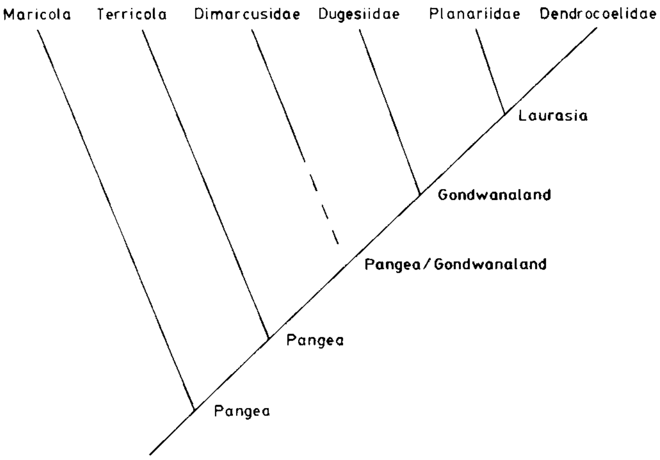


Fig. 17. Area cladogram of the Tricladida, with postulated ancestral areas at the nodes.

The present analysis omits *Eviella hynesae*, a species frequently mentioned in connection with the Dimarcusids (cf. Ball 1977*a*; Gourbault 1978; Kawakatsu & Chapman 1983), since it does not share the apomorphous characters 1, 2 and 3 in Fig. 14 with the Dimarcusidae.

Eviella hynesae, however, shares two hypothesized apomorphies of a more restrictive group within the Dimarcusidae, viz. the fusion of testicular follicles and loss of the copulatory bursa (Fig. 14, characters 7, 8). However, in the above it was already noted that testes tubes also occur in several paludicolans, so that this

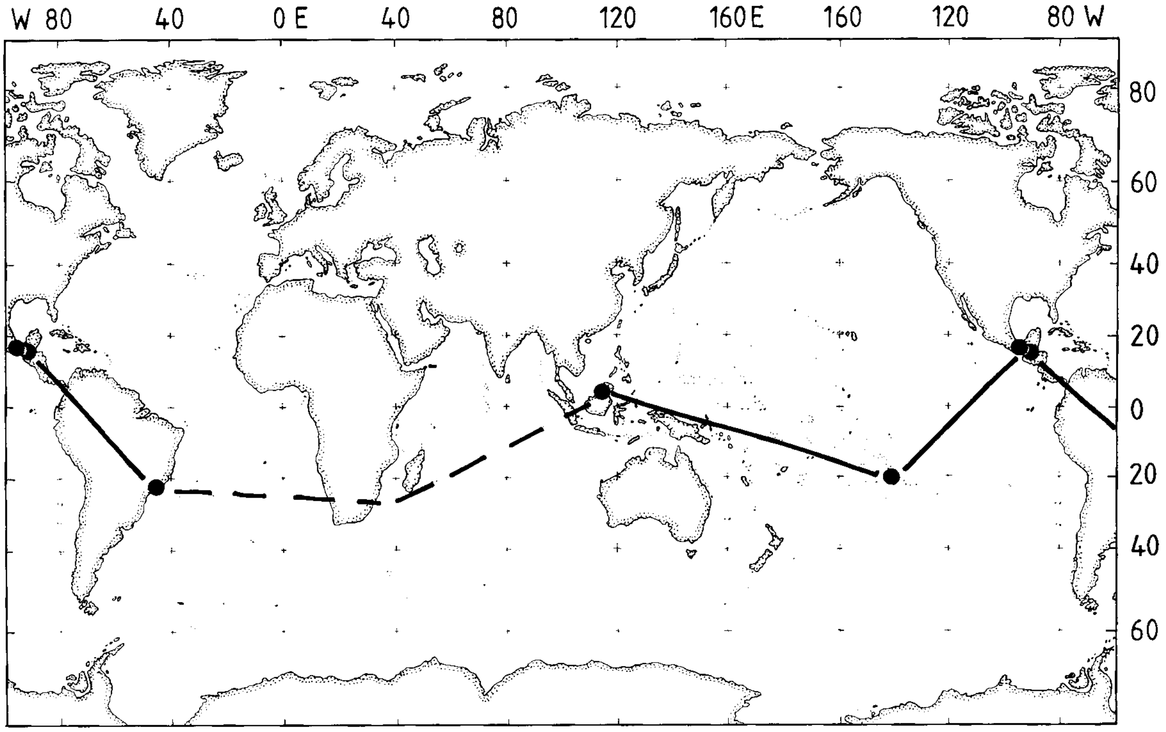


Fig. 16. Biogeographic track of the Dimarcusidae.

character on its own is a poor indicator of phylogenetic affinity. Presence of testes tubes in *E. hynesae*, *Spathula foeni* and *Romankenkius bilineatus* (see above) is consistent with suggestions that *E. hynesae* may be closely related to the dugesiid genera *Spathula*, *Reynoldsonia* and *Romankenkius* (Ball 1977*a*; de Vries & Sluys in press). With respect to the loss of the copulatory bursa it is also important to assess the correct level of universality because in several lineages within the Tricladida the primary copulatory bursa has been lost. Loss of the primary bursa may only be used as a derived character within lineages. Therefore, absence of a bursa cannot be used to link *Eviella* with the Dimarcusidae because there is evidence that these taxa belong to different lineages.

In view of the differences observed in ciliation of the bursal canal/female genital duct within the Dimarcusidae one may be inclined to question the postulated homologous relationship between these structures, as implied by the use of character 2 (Fig. 14). It may indeed well be true that the anterior section of the bursal canal/female genital duct forms part of the atrium in *R. evelinae*, *O. mexicana* and *O. josephinae* and therefore could better be indicated as the female atrium. However, I am not convinced that this difference in ciliation necessarily denies the homology of the bursal canal/female genital duct in the five members of the Dimarcusidae. The positional criterion and aspects of the histology of the bursal canal/female genital duct—apart from the ciliation—suggest little else than a homologous relationship. Furthermore, it may well be that the entire bursal canal/female genital duct is formed by an outgrowth of the atrium, so that in the various species these structures have a similar ontogenetic origin. Evidently, future studies on the post-embryonic development may test the assumption of the present study that the bursal canal/female genital duct in Dimarcusids share a homologous relationship.

Within the Dimarcusidae the occurrence of testes tubes (character 8, Fig. 14) parallels the distribution of testes which extend from dorsal to ventral body surface, both features being confined to *R. evelinae* and *O. josephinae*. Outgroup comparison, combined with the algorithm of Maddison *et al.* (1984), indicates that ventral testes probably represent the ancestral condition for the Dimarcusidae. It is not difficult to imagine that dorso-ventral testes evolve from a ventral, ancestral condition and thus could represent a derived character state within the Dimarcusidae, supporting the proposed unique common ancestry of the genera *Rhodax* and *Opisthobursa*. However, it may be that the formation of testes tubes is functionally linked to rather large testicular follicles, which occupy most of the space between dorsal and ventral body surface. Therefore, I have refrained from using dorso-ventral testes as a supporting character in the phylogenetic analysis.

There is one apomorphic characteristic that has arisen in parallel in *R. evelinae* and *B. thetisae*, viz. the position of the mouth in the middle of the pharyngeal pocket. I have earlier (Sluys 1989*b*) concluded that in the Proseriata, Maricola, Terricola and Paludicola there are species in which the position of the mouth differs from the ancestral condition for the Seriata, i.e. the mouth being located at the posterior end of the pharyngeal cavity. Very similar derived conditions, e.g. the mouth in the middle of the pharyngeal cavity, have independently evolved in only distantly related taxa, one such group being the Dimarcusidae.

Previous classifications

Already Marcus (1946) discussed at length the taxonomic affinities of *Rhodax*. He placed the genus in the Planariidae of the infraorder Paludicola. Ball (1974*a*), however, placed *Rhodax* in the newly erected paludicolan family Dugesiidae, but noted that the species also seems to have affinities with the Maricola. Mitchell & Kawakatsu (1972) suggested that *Rhodax* is a marine relict and relatively closely related to *O. mexicana*, which they also considered to be descended from marine ancestors; Ball (1977*a*) expressed a similar opinion. Nonetheless, Kawakatsu & Chapman (1983) and Kawakatsu & Mitchell (1984) still classify *Rhodax* with the Dugesiidae, a taxonomic decision which almost certainly is incorrect because the eyes of *R. evelinae* do not correspond with the dugesiid type. Kawakatsu & Chapman (1983) and Kawakatsu & Mitchell (1984) assigned the genus *Opisthobursa* to the maricolan family Procerodidae, into which Gourbault (1978) had also placed, albeit provisionally, the genus *Ballíania*. Kawakatsu & Chapman (1983) tentatively assigned *Mitchellia* to the Procerodidae.

Discussions on the inclusion of Dimarcusids in either the Maricola or the Paludicola are rendered superfluous under the phylogenetic hypothesis proposed in the present study. The Dimarcusidae is more closely related to the Paludicola than to the Maricola and cannot be incorporated in either of these infraorders.

Taxonomic implication

For almost 100 years systematists have recognized within the Tricladida three major groupings, for which they have generally used Hallez's (1890) ecological names Maricola, Terricola and Paludicola. The taxonomic rank of these groups has shifted between that of suborder and infraorder. The taxonomy of the Seriata as presently understood suggests that these three major groups within the Tricladida are best considered as infraorders and that Hallez's names may still be used as taxonomic labels, irrespective of their previous ecological connotation.

The present study indicates that there is a fourth major lineage within the Tricladida which should be given the same categorical rank as the three lineages previously recognized. I propose to use the name Cavernicola, which has ecological roots but no such connotation, for the lineage from which at present we only know the genera *Rhodax*, *Opisthobursa*, *Balliania* and *Mitchellia* of the family Dimarcusidae.

Acknowledgements

I am very grateful to Professor M. Benazzi (Pisa) for entrusting to me preserved type specimens of *Opisthobursa josephinae* and for his permission to section some of the animals. Preparations of *Balliania thetisae* were made available through the courtesy of Dr N. Gourbault (Museum National d'Histoire Naturelle, Paris). Dr M. Machida (National Science

Museum, Tokyo) kindly arranged the loan of a paratype of *Mitchellia sarawakana*. Professor I. R. Ball (Memorial University, St. John's, Newfoundland) made available to me Marcus' material of *Rhodax evelinae*, preparations of *Opisthobursa mexicana* which he had on loan from the United States National Museum, Washington, and a paratype of *Eviella hynesae* from his private collection. Additional type material of *Rhodax evelinae* was obtained through a loan from the Royal Ontario Museum, Toronto. Mr L. van der Laan (Institute of Taxonomic Zoology, Amsterdam) is thanked for printing the photographs. Dr E. J. de Vries is thanked for her comments on a draft of the manuscript.

Abbreviations used in the figures

agl	adhesive glands
as	adhesive slit
bc	copulatory bursa
bs	bursal canal
cod	common oviduct
cvd	common vas deferens
di	diverticulum
ed	ejaculatory duct
ep	epithelium
in	intestine
fgd	female genital duct
fsv	false seminal vesicle
gl	gland
go	gonopore
lu	lumen
nug	nucleus of gland cell
od	oviduct
pb	penis bulb
pg	penis gland
ph	pharynx
pp	penis papilla
sa	sensory area
se	secretion
sg	shell gland
so	sensory organ
sv	seminal vesicle
te	testes
vd	vas deferens
ves	resorptive vesicle
vi	vitellarium
vnc	ventral nerve cord

References

Ax, P. 1984. *Das Phylogenetische System*. Fisher, Stuttgart.

Ball, I. R. 1974*a*. A contribution to the phylogeny and biogeography of the freshwater triclads (Platyhelminthes: Turbellaria). In *Biology of the turbellaria* (eds N. W. Riser & M. P. Morse): 339–401. McGraw-Hill, New York.

Ball, I. R. 1974*b*. A new genus and species of freshwater planarian from Australia (Platyhelminthes: Turbellaria).—*J. Zool., Lond. 174:* 149–158.

Ball, I. R. 1977*a*. A new and primitive retrobursal planarian from Australian fresh waters (Platyhelminthes, Turbellaria, Tricladida).—*Bijdr. Dierk. 47:* 149–155.

Ball, I. R. 1977*b*. A monograph of the genus *Spathula* (Platyhelminthes: Turbellaria: Tricladida).—*Aust. J. Zool., Suppl. Ser. No. 47:* 1–43.

Ball, I. R. & Fernando, C. H. 1969. Freshwater triclads (Platyhelminthes, Turbellaria) and continental drift.—*Nature 221:* 1143–1144.

Ball, I. R., Gourbault, N. & Kenk, R. 1981. The planarians (Turbellaria) of temporary waters in eastern North America. *Life Sci. Contr. R. O. M. 127:* 1–27.

Ball, I. R. & Tran, T. V.-H. 1979. New freshwater triclads from Tasmania (Platyhelminthes, Turbellaria).—*Bijdr. Dierk. 49:* 153–161.

Benazzi, M. 1972. Notizie preliminari sulle planarie raccolte nella seconda spedizione in Messico.—*Acc. Naz. dei Lincei, Ser. VIII, 52:* 403–405.

Benazzi, M. 1973. Priority of the name *Opisthobursa mexicana* Benazzi, 1972 over *Dimarcus villalobosi* Mitchell and Kawakatsu, 1972.—*Acc. Naz. dei Lincei, Ser. VIII, 54:* 133–134.

Benazzi, M. 1975. *Opisthobursa josephinae*, a new troglobitic planarian from Chiapas, Mexico.—*Acc. Naz. dei Lincei, Ser. VIII, 59:* 533–536.

Benazzi, M. & Giannini, E. 1973. A remarkable cave planarian: *Opisthobursa mexicana* Benazzi, 1972.—*Acc. Naz. dei Lincei, Quaderno, No. 171, Subterranean Fauna of Mexico, part II:* 47–54.

Croizat, L. 1958. *Panbiogeography*. Published by the author, Caracas.

Gourbault, N. 1978. Une nouvelle planaire primitive d'origine marine, Turbellarie Triclade de Polynésie.—*Cah. Biol. mar. 19:* 23–36.

Hallez, P. 1890. Catalogue des Turbellariés (Rhabdocoelides, Triclades et Polyclades) du Nord de la France et de la Côte Boulonnaise.—*Revue Biol. Nord de la France 2:* 1–179.

Hennig, W. 1966. *Phylogenetic systematics*. University of Illinois Press, Urbana.

Kaburaki, T. 1918. Freshwater triclads from the basin of the Inle Lake.—*Rec. Indian Mus. 14:* 187–194.

Karling, T. G. 1940. Zur Morphologie und Systematik der Alloeocoela cumulata und Rhabdocoela lecithophora (Turbellaria).—*Acta zool. fenn. 26:* 1–260.

Kawakatsu, M. & Chapman, P. 1983. *Mitchellia sarawakana* gen. et sp. nov. (Turbellaria, Tricladida, Maricola), a new freshwater planarian from Water Polo Cave, Sarawak, East Malaysia.—*J. Speleol. Soc. Japan 8:* 21–34.

Kawakatsu, M. & Mitchell, R. W. 1983. Record of a cave-adapted planarian (Turbellaria, Tricladida, Maricola) from Guatemala.—*Annotnes. zool. jap. 56:* 291–298.

Kawakatsu, M. & Mitchell, R. W. 1984. A list of retrobursal triclads inhabiting freshwaters and aquatic probursal triclads regarded as marine relicts, with corrective remarks on our 1984 paper published in *Zoological Science*, Tokyo.—*Occ. Publ., Biol. Lab. Fuji Women's Coll., Sapporo No. 11:* 1–8.

Kawakatsu, M., Oki, I., Tamura, S. & Yamayoshi, T. 1985. Reexamination of freshwater planarians found in tanks of tropical fishes in Japan, with a description of a new species, *Dugesia austroasiatica* sp. nov. (Turbellaria; Tricladida; Paludicola).—*Bull. biogeogr. Soc. Japan 40:* 1–19.

Kenk, R. 1978. The planarians (Turbellaria, Tricladida, Paludicola) of Lake Ohrid in Macedonia.—*Smithson. Contr. Zool. 280:* I–IV + 1–56.

Maddison, W. P., Donoghue, M. J. & Maddison, D. R. 1984. Outgroup analysis and parsimony.—*Syst. Zool. 33:* 83–103.

Marcus, E. 1946. Sôbre Turbellaria brasileiros.—*Bol. Fac. Fil. Ciênc. Letr., Univ. São Paulo, Zool. 11:* 5–187.

Mitchell, R. W. & Kawakatsu, M. 1972. A new family, genus and species of cave-adapted planarian from Mexico (Turbellaria, Tricladida, Maricola).—*Occ. Pap. Mus. Texas Tech. Univ. 8:* 1–16.

Saether, O. 1983. The canalized evolutionary potential: inconsistencies in phylogenetic reasoning.—*Syst. Zool. 32:* 343–359.

Sluys, R. 1989*a*. Phylogenetic relationships of the triclads (Platyhelminthes, Seriata, Tricladida).—*Bijdr. Dierk. 59:* 3–25.

Sluys, R. 1989*b*. *A monograph of the marine triclads*. A. A. Balkema, Rotterdam, Brookfield.

Sluys, R. 1989*c*. Sperm resorption in triclads (Platyhelminthes, Tricladida).—*Invert. Reprod. Dev. 15:* 89–95.

Thorne, R. F. 1972. Major disjunctions in the geographic ranges of seed plants.—*Q. Rev. Biol. 47:* 365–411.

Vries, E. J. de & Sluys, R. In press. Phylogenetic relationships of the genus *Dugesia* (Platyhelminthes, Tricladida, Paludicola). *J. Zool., Lond.*

Watrous, L. E. & Wheeler, Q. D. 1981. The out-group comparison method of character analysis.—*Syst. Zool. 30:* 1–11.

Wiley, E. O. 1981. *Phylogenetics. The theory and practice of phylogenetic systematics*. Wiley, New York.