



# The genus *Minona* (Platyhelminthes, Seriata) in eastern Australia

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Four new species of the genus *Minona* Marcus, 1946 from eastern Australia are described. *Minona proculvaginata* sp. n., *M. australis* sp. n. and *M. concharum* sp. n. have four genital pores, and differ in details of the copulatory organs. The epidermis of these three species is unciliated in a ventral area around the male pore. Based on this synapomorphic feature, they are sister species. The karyotype of *M. proculvaginata* and *M. concharum* is described. *Minona heronensis* sp. n. is distinct from congeneric species on the basis of the size of its accessory stylet and copulatory organ, pore indices and karyotype. It is compared to numerous similar Indo-Pacific species. Additional data on the anatomy and karyology of *M. beaglei* Martens & Curini-Galletti, 1989 from N. Australia, are given. One specimen from Heron Island is tentatively attributed to *M. dolichovesicula* Tajika, 1982 a species widely distributed in the Indo-Pacific. Its karyotype is given. © 1997 The Norwegian Academy of Science and Letters. All rights reserved

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## Introduction

The genus *Minona* Marcus, 1946 (Platyhelminthes, Proseriata) is characterized by prepharyngeal ovaries, prepenial bursa, a copulatory bulb of the simplex type, and by the presence of an accessory 'prostatoid' organ, provided with a stylet, posterior to the copulatory bulb (see Karling, 1966). It is one of the largest genera in the family Monocelididae, with more than 20 species described. Assessment of phylogenetic relationships among these species have scarcely been attempted.

The genus is cosmopolitan in distribution, and is particularly speciose in tropical waters [e.g. Galapagos (Ax & Ax, 1977); the Caribbean (Curini-Galletti, 1991; Ax & Sopott-Ehlers, 1985; Karling, 1978) and Sulawesi (Martens & Curini-Galletti, 1989)]. One species only, *Minona beaglei* Martens & Curini-Galletti, 1989 has been reported so far from Australian waters.

We undertook a study of mesopsammic Proseriata to further our knowledge of eastern Australia interstitial fauna. This research yielded five species of the genus *Minona*, four of which are new to science. They are described and discussed here. Furthermore, additional

data on the morphology and karyology of *M. beaglei*, whose original description was based on observations on living specimens only, are given.

## Material and methods

Specimens were collected in sandy habitats by scooping up the superficial layer of sediment. Extraction of the animals from the sediment was with MgCl<sub>2</sub> decantation (Martens, 1984). Preservation techniques routinely adopted for Proseriata were used (see Martens *et al.*, 1989). For microscopical analysis specimens were fixed in warm Bouin's fluid (about 50°C), embedded in 56°C Paraplast and serial sagittal sections were cut at 2–4 µm, stained with Mayer's haematoxylin and eosin and mounted in Depex or Canada Balsam.

Karyological techniques were as described by Curini-Galletti *et al.* (1989). Relative lengths (r.l. = length of chromosome × 100/total length of haploid genome) and centromeric indices (c.i. = length of short arm × 100/length of entire chromosome) were obtained from measurements of camera lucida drawings of 5–10 metaphase plates for each species. Idiograms are based on karyometrical data presented in the karyotype formula: haploid genome absolute length in µm, fundamental number, relative length and centromeric index of each chromosome; chromosome nomenclature between parentheses (m = metacentric; sm = submetacentric; st = subtelocentric; t = acrocentric). The fundamental number is derived according to Matthey (1949) and chromosome nomenclature follows Levan *et al.* (1964). Histological material is stored in the collections of the Queensland Museum.

**Abbreviations used in figures:**

ao	accessory ('prostatoïd') organ
aob	accessory organ bulb
aop	accessory organ pore
aos	accessory organ stylet
b	bursa
co	copulatory organ
fd	female duct
fg	female glands
fp	female pore
gid	genito-intestinal duct
gl	gut lumen
ia	inner antrum
ip	inverted penis
m	mouth
ma	male antrum
mp	male pore
ms	muscle sheath
oa	outer antrum
od	oviduct
ov	ovary
pg	prostatoïd glands
ph	pharynx
phg	pharyngeal glands
pp	penis papilla
st	statocyst
t	testes
vd	vaginal duct
vg	vaginal glands
vi	vitellaria
vp	vaginal pore
vs	seminal vesicle

3 mm in length in sectioned material. Without pigment or pigmented eyespots. Anterior end rounded, provided with numerous oily droplets and small elongate rhabdites. Posterior end rounded, with adhesive glands. Epidermis with depressed nuclei. It is ciliated apart from the caudalmost area. This caudal non ciliated area is markedly more extended dorsally than ventrally. In addition, a rather large, subquadrangular ventral area in front of the the male pore is devoid of cilia. Cilia (about 5  $\mu\text{m}$  long) are slightly longer ventrally than dorsally. The elongate rhabdites range from 10–12  $\mu\text{m}$  long. Large glands, whose necks pierce the epithelium, are present caudally. They are about 30  $\mu\text{m}$  long, ovoid, with a flat nucleated lining and a granular content. Pharynx nearly median. It is comparatively short and broad. Its external and luminal epithelia are ciliated; its most distal tip, where pharyngeal glands discharge, is non ciliated. The pharyngeal glands are well developed, particularly on the ventral side. The proximal fourth of the luminal epithelium is non ciliated; the oesophageal portion is very small (about 1/10 of the whole length of the pharynx).

**Male genital organs.** About 100 testes irregularly arranged in front of the pharynx. The copulatory bulb, of the simplex type, is ovoid, dorso-ventrally elongated (about 80  $\mu\text{m}$  long and 55  $\mu\text{m}$  wide in sections). It consists of a seminal vesicle, lined by an epithelium which becomes high and presumably glandular distally, with nuclei both inside and outside the bulb. It is surrounded by a thin muscle coat (with inner circular and outer longitudinal muscles). The penis papilla is small, obtuse, and protrudes into the antrum. The most distal portion of the bulb is inverted, forming a rather short eversible penis, with a very thick coat of muscles (mostly circular muscles). The antrum is divided by a fold into two portions: a non ciliated inner antrum, and a smaller, ciliated outer antrum, the latter opening to the outside through the male pore. The two portions of the antrum are connected through a small pore. No retractor muscles have been observed.

**Family MONOCELIDIDAE**

***Minona proculvaginata* sp. n. (Figs 1, 6A, Fig. 7A, G)**

**Material examined.** Holotype: Tasmania, Fossil Island, intertidal in coarse sand/gravel (October 1993), sagittally sectioned: G211684 (in two slides). Paratypes: same data as holotype: 22 specimens sagittally sectioned (G211685-706); three whole mounts (in two slides: G211707-8). Other material: numerous specimens observed alive; five studied karyologically.

**Etymology.** The specific epithet refers to the distance of the vagina from the other genital openings (from lat. *procul*: far away).

**Description.** Animals slender, large for the group, up to

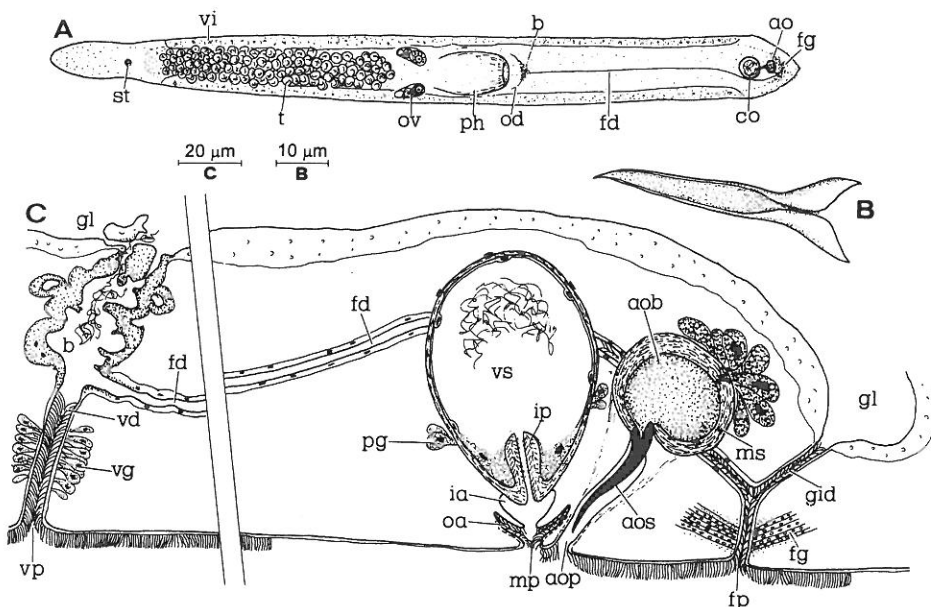


Fig. 1. *Minona proculvaginata* sp. n. A. General organisation of a live animal; B. Stylet; C. Reconstruction of the genital organs from sagittal sections.

*Accessory ('prostatoid') organ.* It is located immediately behind the copulatory bulb. The bulb of the accessory organ is globose (about 40  $\mu\text{m}$  in diameter), and surrounded by a thick muscle coat. It is filled with a granular secretion produced by numerous cells whose nuclei lie outside the bulb and whose necks pierce the muscular layers. The stylet is very elongated and about 50  $\mu\text{m}$  long in squashed specimens used for karyology (Fig. 1B, Fig. 7G). The stylet protrudes into a narrow non ciliated antrum, which opens to the outside in close proximity, albeit independently, of the male pore. The narrow area between the male and accessory organ pores is ciliated.

*Female genital organs.* Ovaries lie in front of the pharynx. Vitellaria stretch from the level of the first testes to just in front of the copulatory organ. The two oviducts fuse just behind the pharynx. At the place of fusion, a small bursa of the resorbiens type is present. It is lined with a high, infranucleate epithelium, and consists of several interconnected vacuoles containing sperm. The gut lining overlying the bursa is thin and incomplete in a few areas, and sperm have been observed in canals connecting the bursa to the gut lumen. This bursa is connected to the outside through a straight vaginal duct, whose ciliated epithelium is surrounded by numerous glands. The vaginal pore is located very close to the mouth. The common female duct originates from the posterior portion of the bursa. It is lined with an infranucleate epithelium, and is ciliated in its distal part. The duct runs postero-laterally to the male bulb and the accessory organ and opens to the outside through the female pore that is located just behind the accessory organ pore; the female pore is surrounded by female glands. A ciliated genito-intestinal duct is present.

*Karyology.* With 3 chromosomes in its haploid set (Fig. 6A, Fig. 7A). Karyotype formula:  $8.2 \pm 0.5 \mu\text{m}$ ; NF = 6; Chrom. 1:  $39.06 \pm 1.56$ ;  $46.58 \pm 0.88$  (m); Chrom. 2:  $33.68 \pm 1.96$ ;  $42.91 \pm 1.77$  (m); Chrom. 3:  $27.23 \pm 1.84$ ;  $46.75 \pm 2.22$  (m).

*Remarks.* *Minona proculvaginata* sp. n. is clearly distinct from congeneric species, being the only known *Minona* with the distance between vagina and male pore markedly longer than the distance between mouth and vagina. Furthermore, no other species is known to present a subquadrangular patch of epidermis devoid of cilia ventrally in front of the male pore (see also remarks on the two following species, and general discussion).

Only *Minona baltica* Karling & Kinnander, 1953 and *Minona gigantea* Ax & Armonies, 1990 have an accessory organ stylet of comparable size. In addition to the differences mentioned above, *M. baltica* lacks a vagina, while in *M. gigantea* the bursa stretches over a long section of the germo-vitelloduct, and the vagina opens posterior to the bursa. Furthermore, in the two last mentioned species the accessory organ pore opens far behind the male pore.

### *Minona australis* sp. n. (Figs 2, 7F)

*Material examined.* Holotype: Tasmania, Fossil Island, intertidal in coarse sand/gravel (October 1993), sagittally sectioned: G211709. Other material: one specimen studied alive, same data as holotype.

*Etymology.* The specific epithet is coined after the geographical area of finding.

*Description.* Animals slender, about 2 mm long in sectioned material. Without pigment or pigmented eyespots. Anterior end provided with few rhabdites. Posterior end rounded, with numerous rhabdites and adhesive glands. In the brain the central tissue (neuropile) is lined with a thin membrane and surrounded by numerous nuclei and, anteriorly, by the statocyst. Epithelium with depressed nuclei, ciliated apart from the caudal area. The caudal non ciliated area is markedly more extended dorsally than ventrally. A subquadrangular patch devoid of cilia is present ventrally between the vagina and the female pore. Cilia (about 5  $\mu\text{m}$  long) are slightly longer ventrally than dorsally. The rhabdites are about 7  $\mu\text{m}$  long. Large glands (about 20  $\mu\text{m}$  long), similar in structure to those present in *M. proculvaginata*, are present caudally. The tubular, rather short pharynx is located in the posterior part of the body. Its luminal epithelium is non ciliated in a very small proximal area. The oesophageal portion is much reduced, and is about 1/7 of the length of the whole pharynx.

*Male genital organs.* Numerous testes in front of the pharynx. The copulatory bulb, of the simplex type, is ovoid and dorso-ventrally elongated (about 85  $\mu\text{m}$  long and 60  $\mu\text{m}$  wide in sections). It consists of a seminal vesicle, lined by an epithelium which becomes high and glandular in a small distal area. The bulb is surrounded by a thick muscle coat (up to 11  $\mu\text{m}$  in diameter), with inner circular and outer longitudinal muscles. The distal portion of the bulb is inverted, forming an eversible penis, which has a thick muscle coat with mostly circular muscles. The epithelium covering the distal tip of the bulb is high and somewhat vacuolar. The antrum is small and non ciliated, and opens to the outside through the male pore. No retractor muscles have been observed.

*Accessory ('prostatoid') organ.* It is found close behind the copulatory bulb. The globular accessory organ bulb is filled with a granular secretion, measures 35–40  $\mu\text{m}$  in diameter, and is surrounded by a rather thin muscle coat. The extremely elongated stylet is about 31  $\mu\text{m}$  long in the sectioned specimen (Fig. 2B, Fig. 7F). It protrudes into an antrum, which opens to the outside through its own pore nearly halfway between the male and the female pores. The distal part of the antrum is ciliated.

*Female genital organs.* Ovaries lie in front of the pharynx. The two oviducts fuse behind the pharynx into the common oviduct. In front of the copulatory bulb, this duct widens into a bursa of the resorbiens type. It is lined by a high, irregular, infranucleate and vacuolated epithelium. The bursa is connected to the outside through a slightly posteriorly oriented vaginal duct, which originates ventrally from the posterior part of the bursa,

and is ciliated in its distal half. The vaginal pore is located very close to the male pore.

The common female duct originates from the posterior part of the bursa close to the emergence of the vaginal duct. It has a non ciliated infranucleate lining. The female duct runs posteriorly laterally to the male bulb and the accessory organ and opens to the outside through the ciliated female pore that is located posterior to the accessory organ pore. The female pore is surrounded by female glands.

*Remarks.* The two specimens of *M. australis* sp. n. have been found in the same samples wherein *M. proculvaginata* was abundant. Both species are large and unpigmented, and they cannot be discriminated at low magnification. In semi-squashed conditions, they are easily recognized due to the position of the bursa, which is much closer to the copulatory bulb in *M. australis*. Furthermore, the vagina opens very close to the male pore in *M. australis*, whereas it is adjacent to the mouth in *M. proculvaginata*. The fine anatomy revealed further differences in the morphology of the copulatory bulb (strongly muscular in *M. australis*, while the muscle layers surrounding the bulb of *M. proculvaginata* are very thin), of the male antrum and in details of ciliation.

Among the unpigmented species described with four genital openings, only *Minona dolichovesicula* Tajika, 1982 and *Minona paulmartensi* Curini-Galletti, 1991 have both the accessory organ pore and the vaginal pore close to the male pore. However, *M. dolichovesicula* has an extremely long copulatory bulb that is longitudinally oriented (see below). *M. paulmartensi* is a minute subtidal species from the Caribbean, with a much smaller bulb and stylet (Curini-Galletti, 1991). New information on the anatomy of *Minona beaglei* Martens & Curini-Galletti, 1989 revealed that this species is provided with a vagina which opens close to the male pore. However, the bursa of *M. beaglei* is highly characteristic (see below) and quite unlike the small bursa resorbiens of *M. australis*. Furthermore, none of the above mentioned species has the ventral bare patch of epidermis in the genital pore area found in *M. australis*.

***Minona concharum* sp. n.** (Figs 3, 6B, Fig. 7B, I)

*Material examined.* Holotype: Queensland, Caloundra, Shelly Beach; high intertidal pools; very coarse sediment, consisting predominantly of small shells and shell fragments (August, September 1993); sagittally sectioned: G211710. Paratypes: same data as holotype: four specimens sagittally sectioned (G211711-14); seven whole mounts (in five slides: G211715-19); four specimens embedded (in one block: G211720). Other material: several specimens observed alive; four studied karyologically.

*Etymology.* The specific epithet (from latin *concha*: shell) refers to the species' habitat, a shelly sediment in Shelly Beach.

*Description.* Embedded specimens about 1.5 mm long. Living animals massive and stocky. Without pigment or pigmented eyespots. Anterior end obtuse, provided with oily droplets and numerous needle-shaped rhabdites. The area in front of the statocyst is comparatively short. In sections, frontal glands are evident. Posterior end rounded, with very numerous rhabdites and adhesive glands. Epithelium with depressed nuclei and ciliated apart from the posterior region. Caudal non ciliated area similar to that of the two previous species. Ventrally, a wide non ciliated area is present posterior to the vagina, joining the caudal non ciliated area. Some specimens, however, presented a small ciliated patch around the female pore (Fig. 3C). Cilia (about 3.5–5 µm long) are slightly longer ventrally than dorsally. The extremely elongate rhabdites range between 10–12 µm long. Large eosinophilous glands are present all over the body. Brain as in the previous species. The short and broad tubular pharynx is located in the second half of the body. It is ciliated except for the distal tip, where pharyngeal glands discharge. Pharyngeal cilia are shorter than those on the body. The luminal epithelium of the pharynx is non ciliated in its most proximal region, and the short oesophageal portion is located in its proximal sixth.

*Male genital organs.* 70–80 testes are irregularly arranged in front of the pharynx. The ovoid copulatory bulb is of the simplex type and dorso-ventrally very elongate (about 86 µm long and 41 µm wide). It consists of a seminal vesicle lined by an epithelium which becomes high and glandular distally, with some of the nuclei lying lateral to the bulb. It is surrounded by a very thin muscle

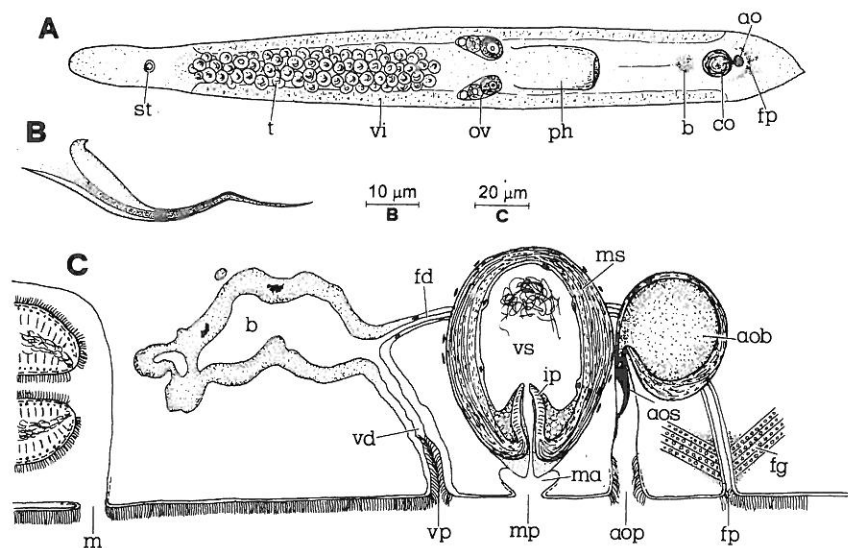


Fig. 2. *Minona australis* sp. n. A. General organisation of a live animal; B. Stylet; C. Reconstruction of the genital organs from sagittal sections.

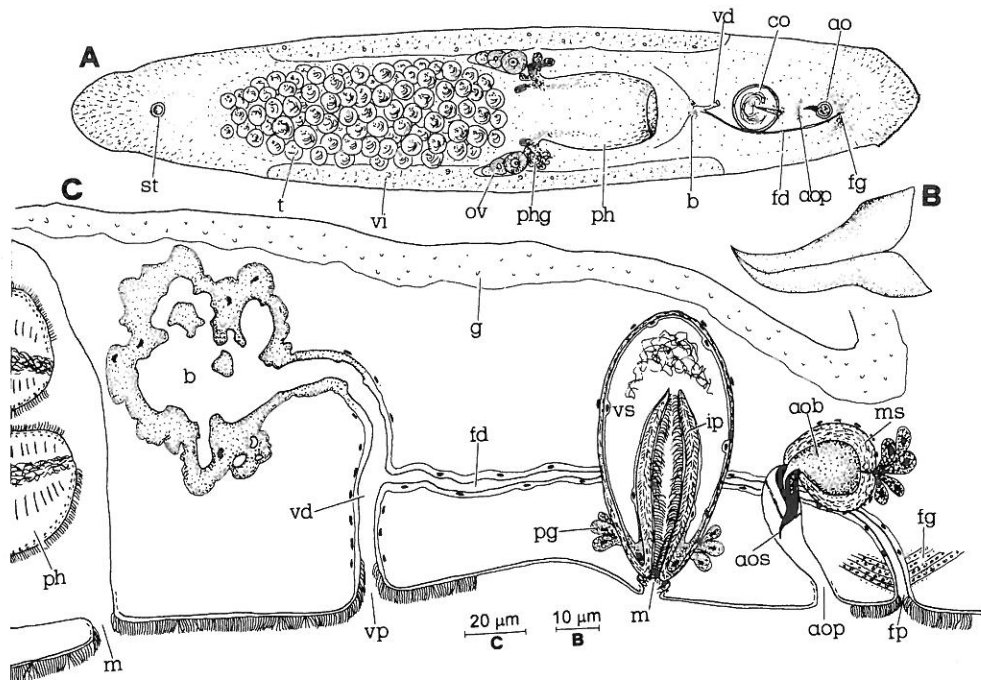


Fig. 3. *Minona concharum* sp. n. A. General organisation of a live animal; B. Stylet; C. Reconstruction of the genital organs from sagittal sections.

coat. The distal portion of the bulb is inverted, forming a relatively enormous eversible penis, easily appreciable in semi-squashed living specimens. The penis has a thick muscle (with apparently mostly circular muscles) coat, and its luminal epithelium is ciliated. The ciliated and small antrum opens to the outside through the male pore. No retractor muscles have been observed. Penial and antral cilia are shorter than body ciliation.

**Accessory ('prostatoid') organ.** It is found close behind the copulatory bulb. The bulb of the accessory organ is globose (30 to 35  $\mu\text{m}$  in diameter), and surrounded by a thick coat of muscles. It is filled with a granular secretion produced by cells whose nuclei lie outside the bulb. The nib shaped stylet is about 45  $\mu\text{m}$  long in squashed karyological slides, and comparatively broad (Fig. 3B, Fig. 7I). A thin membrane, pierced by the stylet tip, separates the accessory organ from a non ciliated antrum, which opens to the outside through its own pore, located close to the female pore.

**Female genital organs.** Ovaries in front of the pharynx. Vitellaria stretch from the level of the first testes to just in front of the copulatory organ. The two oviducts join posterior to the pharynx and form a bursa of the resorbiens type. It is lined by a high, irregular, infranucleate and vacuolated epithelium. The bursa continues posteriorly as a ventrally curved duct, lined with a non ciliated epithelium with depressed nuclei, which is probably the proximal part of the vaginal duct. The distal portion of the vaginal duct is straight and non ciliated apart from its most distal portion. The vaginal pore is located halfway between the mouth and the male pore.

The common female duct originates perpendicularly from the vaginal duct, from which it differs by being lined with an infranucleate epithelium. The female duct runs postero-laterally to the male bulb and the accessory organ and opens to the outside through the female pore posterior

to the accessory organ pore. The female pore is surrounded by female glands.

**Karyology.** With 3 chromosomes in its haploid set (Fig. 6B, Fig. 7B). Karyotype formula:  $7.7 \pm 0.8 \mu\text{m}$ ; NF = 6; Chrom. 1:  $38.82 \pm 0.70$ ;  $46.64 \pm 1.46$  (m); Chrom. 2:  $33.72 \pm 0.74$ ;  $36.93 \pm 3.32$  (sm); Chrom. 3:  $27.46 \pm 0.67$ ;  $41.41 \pm 2.01$  (m).

**Remarks.** *Minona concharum* sp. n. shares with the two previous species the presence of a ventral non ciliated area around the genital pores. The position of the vagina allows immediate distinction among the three species: it is close to the male pore in *M. australis*, close to the mouth in *M. proculvaginata*, and nearly halfway between these two pores in *M. concharum*. This latter species is further distinguished by the enormous relative size of the eversible penis, approaching 2/3 the length of the whole bulb.

Among the unpigmented *Minona* species with distinctly elongate copulatory bulb with a ratio length/width approaching or surpassing 2:1, *M. dolichovesicula* has a much more elongated bulb that is longitudinally oriented. *Minona trigonopora* Ax, 1956 has the accessory organ pore fused with the female pore. *Minona bistylifera* Karling, Mack-Fira & Dörjes, 1972 has the copulatory bulb provided with a stylet. *Minona cornupenis* Karling, 1966 has the accessory organ pore very close to the male pore, and its bulb musculature is more developed. *Minona paulmartensi* has much fewer testes (eight), a shorter stylet, a bursa apparently not of the resorbiens type, and a different karyotype (see Curini-Galletti, 1991).

#### *Minona heronensis* sp. n. (Figs 4, 6C, Fig. 7D, L)

**Material examined.** Holotype: Queensland, Heron Is., Shark Bay, low intertidal 'Otoplana zone'; coarse sand and shell fragments (October

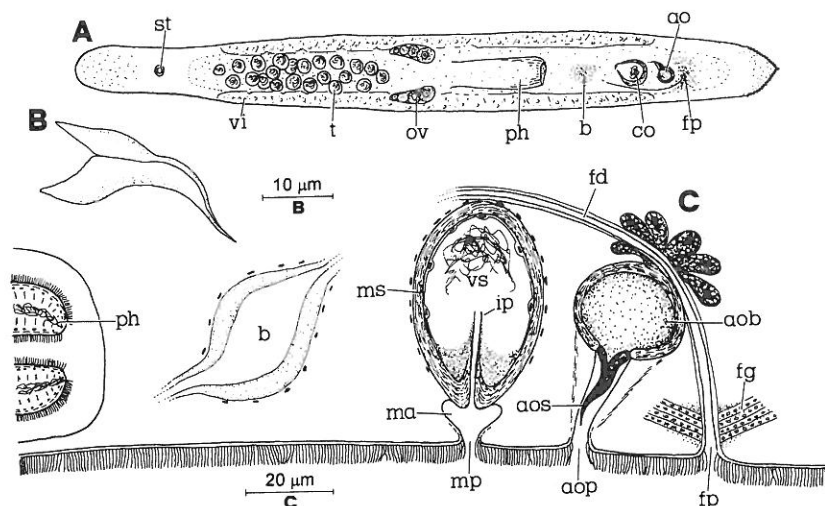


Fig. 4. *Minona heronensis* sp. n. A. General organisation of a live animal; B. Stylet; C. Reconstruction of the genital organs from sagittal sections and observations on living specimens.

1993), sagittally sectioned: G211721. Paratypes: same data as holotype: two specimens sagittally sectioned (G211722-23); two whole mounts (G211724-25). Other material: four specimens observed alive and used for karyology.

*Etymology.* The species is named after the type locality.

*Description.* Animals slender, comparatively minute (sectioned specimens about 1 mm long). Without pigment or pigmented eyespots. Anterior end provided with a few oily droplets. The epithelium, with depressed nuclei, is ciliated apart from the most caudal region, where adhesive glands are present. Cilia are about 4 µm long. The elongate pharynx is situated in the posterior half of the body. The pharynx is ciliated apart from the distal tip, where pharyngeal glands discharge. Pharyngeal cilia are about 2 µm long. The luminal epithelium of the pharynx is proximally non ciliated; the oesophageal portion is located in its proximal third.

*Male genital organs.* 15–30 testes are irregularly arranged in front of the pharynx. The copulatory bulb, of the simplex type, is dorso-ventrally elongated (about 50 µm high and 30 µm wide). It consists of an ovoid seminal vesicle, lined with an epithelium which is proximally thin, and becomes high and glandular distally. The distal portion of the bulb is inverted, forming a long eversible penis with thin and non ciliated walls. The bulb is surrounded by a muscle coat (ranging 2 to 5 µm in diameter, with inner circular and outer longitudinal muscles). The antrum is small, apparently non ciliated, and opens to the outside through the male pore. No retractor muscles have been observed.

*Accessory ('prostatoid') organ.* It is found close behind the copulatory bulb. The bulb of the accessory organ is globose (about 20 µm in diameter), filled with a granular secretion that is produced by glands whose nuclei lie outside the bulb itself. The bulb is surrounded by a muscle coat that is 2–4 µm thick. The nib shaped stylet is about 35 µm long in a squashed specimen used for karyology, slender and slightly curved (Fig. 4B, Fig. 7L). From sectioned material no clear information on its pore could be obtained. In living animals, however, a distinct

accessory organ pore, located almost halfway between the male pore and the female pore, could be observed.

*Female genital organs.* Ovaries in front of the pharynx. Vitellaria stretch from the level of the first testes to the copulatory bulb. A roundish bursa is present in front of the copulatory bulb. In sections, the bursa appears as a differentiation of the common oviduct, lined with an epithelium with depressed nuclei. Neither in sections, nor in living animals a vagina could be detected. The common female duct runs over the copulatory bulb and the accessory organ to the female pore, which is surrounded by female glands.

*Karyology.* With 3 chromosomes in its haploid set (Fig. 6C, Fig. 7D). Karyotype formula:  $8.6 \pm 0.2 \mu\text{m}$ ; NF = 6; Chrom. 1:  $37.54 \pm 0.78$ ;  $42.10 \pm 2.54$  (m); Chrom. 2:  $33.29 \pm 0.73$ ;  $35.38 \pm 2.14$  (sm); Chrom. 3:  $29.16 \pm 1.30$ ;  $41.94 \pm 1.14$  (m).

*Remarks.* *Minona heronensis* sp. n. belongs to the group of species which lack a permanent vagina. Among the numerous species of this group, *M. bistylifera* is immediately recognizable due to the presence of an intrapenial stylet. *Minona obscura* Karling, 1966 is distinguished by the presence of parenchymal pigment and pigmented eye spots. *Minona baltica* has a very large copulatory bulb (145 µm in diameter) and an equally large stylet of the accessory organ (42 µm). Similarly, *M. hastata* Martens & Curini-Galletti, 1989 has an extremely long stylet (about 70 µm). Among the other species, *M. fernandinensis* Ax & Ax, 1977 has a roundish bulb which is wider than high (about 70 × 50 µm), a slightly larger stylet (35–38 µm), and more numerous tests (80–90) than *M. heronensis*. *Minona bermudensis* Ax & Sopott-Ehlers, 1985 has a characteristic long and broad squarish bursa in front of the copulatory bulb, numerous testes (60), male and accessory organ pores fused together, and Chroms. 2 and 3 submetacentric with low indices (cf Ax & Sopott-Ehlers, 1985; Curini-Galletti, 1991). *Minona indonesiana*

Martens & Curini-Galletti, 1989 lacks a prepenial bursa and has a large atrium between the copulatory bulb and the accessory organ. Furthermore, the proximal part of its stylet is characteristically curved (see Martens & Curini-Galletti, 1989, Fig. D, p. 182).

***Minona cf dolichovesicula* (Tajika, 1982)** (Fig. 6E, Fig. 7E, H)

*Material examined.* One specimen from Heron Island, Queensland, off South Reef, about 16 m deep in silty medium sand (October 1993); used for karyology.

*Description.* Limited observations were performed on a semi-squashed orcein-stained specimen prior to its strong squashing for karyological studies. Minute *Minona* species with an extremely long, muscular copulatory organ (about 150  $\mu\text{m}$  long in squeezed condition), provided with an inverted penis 50  $\mu\text{m}$  long. Accessory organ stylet about 22  $\mu\text{m}$  long in squashed condition (Fig. 7H). The stain obscured the female genital system.

*Karyology.* With 2 chromosomes in its haploid set (Fig. 6E, Fig. 7E). Karyotype formula:  $5.3 \pm 0.1 \mu\text{m}$ ; NF = 3; Chrom. 1:  $59.85 \pm 0.23$ ;  $18.25 \pm 1.08$  (st); Chrom. 2:  $40.14 \pm 0.23$ ;  $41.17 \pm 0.69$  (m).

*Remarks.* *Minona* specimens from the Pacific Ocean with an extremely long, longitudinal copulatory bulb have been usually classified as *M. dolichovesicula*, originally described from N. Japan (Tajika, 1982). Populations, however, strongly differ in the length of the bulb (N. Japan: 120  $\mu\text{m}$ ; S. Juan Archipel, Washington, USA: 85  $\mu\text{m}$  (both from sections); Alaska: 170  $\mu\text{m}$  (in squashed conditions)), and in the length of the accessory organ stylet (around 48  $\mu\text{m}$ , 20  $\mu\text{m}$ , and 30  $\mu\text{m}$  respectively) (Tajika, 1982; Sopott-Ehlers & Ax, 1985; Ax & Armonies, 1990). The Australian specimen, with a long copulatory bulb and a small stylet, adds further variability. Although comparisons between fixed and squashed material deserve caution, the morphological 'variability', the unusually large distribution and latitudinal range for the group, as well as differences in ecology and habitat among the various populations suggest that a species-complex might exist at present under the name *M. dolichovesicula*.

***Minona beaglei* (Martens & Curini-Galletti, 1989)** (Figs 5, 6D, Fig. 7C)

*Material examined.* Holotype: Northern Territory, Darwin, Fannie Bay, medium to coarse sand, in a tidal creek (October 1987); whole mount (Zoological collection of Limburgs Universitair Centrum, Diepenbeek, Belgium, no 116). Other material: nine specimens, same data as the holotype: seven sagittally sectioned (G211726-211732); two studied karyologically.

*Description.* For the general shape and organisation, see the original description (Martens & Curini-Galletti, 1989), based on observations from living semi-squashed specimens. Only new information from sections is presented here. Epithelium with depressed nuclei; homogeneously ciliated apart from the most caudal

region. Cilia are 4–5  $\mu\text{m}$  long. Pharynx similar in anatomy to that of *M. heronensis* sp. n.

*Male genital organs.* The copulatory bulb, of the simplex type, is very elongated dorso-ventrally (about 22–30  $\mu\text{m}$  wide and 40–43  $\mu\text{m}$  high). It consists of an ovoid seminal vesicle, lined with an epithelium which distally becomes high and presumably glandular, with nuclei mostly outside the bulb. The seminal vesicle is surrounded by a thin muscle coat, which is less than 1  $\mu\text{m}$  thick proximally and up to 4  $\mu\text{m}$  distally, where most muscles are circular. The penis papilla is minute and pointed. The distal portion of the bulb is inverted, forming a long eversible penis (about 20  $\mu\text{m}$  long in the everted condition), which has rather thick, muscular, and probably non ciliated walls. The antrum is small, opening to the outside through the male pore. Neither ciliation nor antral musculature have been observed.

*Accessory ('prostatoid') organ.* The bulb of the accessory organ is ovoid (about 30  $\mu\text{m}$  high and 25  $\mu\text{m}$  wide) and filled with a granular secretion. The bulb is surrounded by a muscle coat 3–5  $\mu\text{m}$  thick. The stylet is nib shaped, about 25  $\mu\text{m}$  long in squashed conditions. The accessory organ pore opens nearly halfway between the male pore and the female pore.

*Female genital organs.* The two oviducts fuse into the common oviduct posterior to the pharynx. The common oviduct forms a very large bursa in front of the male bulb. This bursa stretches along a considerable portion of the oviduct and consists of numerous vacuoles, increasing in size posteriorly. Most of the vacuoles are more or less isolated from each other, and have a direct connection with the oviduct. The most anterior ones are lined with a vacuolated epithelium, and are presumably of the resorbiens type. The posteriormost ones are very large and are lined with a thin, membrane-like, epithelium. The infranucleate and non ciliated common female duct originates basally from the posteriormost vacuole. Shortly after its emergence, the female duct joins the nearly straight vaginal duct, which has depressed nuclei, and is ciliated in its distal half. The vaginal duct opens to the outside through a vaginal pore, located very close to the male pore. The common female duct runs posteriorly over the male bulb and the accessory organ and opens to the outside through the female pore, which is surrounded by female glands.

*Karyology.* With 3 chromosomes in its haploid set (Fig. 6D, Fig. 7C). Karyotype formula:  $9.3 \pm 0.2 \mu\text{m}$ ; NF = 6; Chrom. 1:  $36.05 \pm 1.73$ ;  $37.94 \pm 4.80$  (m); Chrom. 2:  $32.77 \pm 3.31$ ;  $25.08 \pm 3.91$  (sm); Chrom. 3:  $31.18 \pm 2.08$ ;  $35.34 \pm 4.69$  (sm).

*Remarks.* *Minona beaglei* was originally described without a vagina (cf Martens & Curini-Galletti, 1989). However, a well developed vaginal duct with a vagina has been found in all sectioned specimens. The close proximity of the vaginal duct to the male bulb probably prevented its

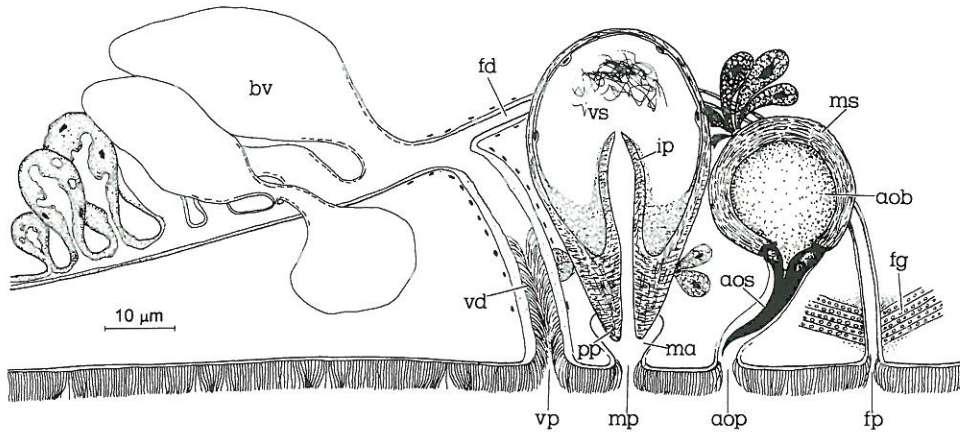


Fig. 5. *Minona beaglei* Martens & Curini-Galletti, 1989. A. Stylet; B. Reconstruction of the genital organs from sagittal sections.

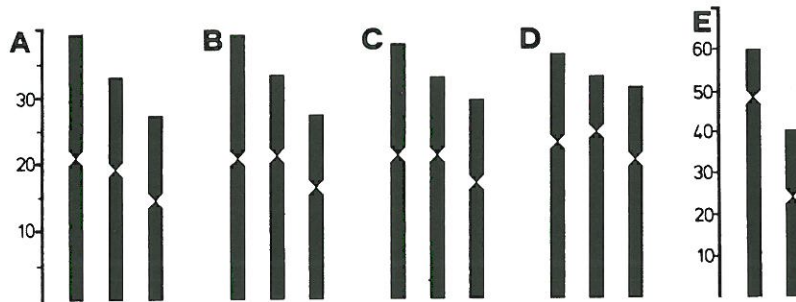


Fig. 6. Idiograms representing the haploid sets of *M. proculvaginata* sp. n. (A); *M. concharum* sp. n. (B); *M. heronensis* sp. n. (C); *M. beaglei* (D); *M. cf. dolichovesicula* (E).

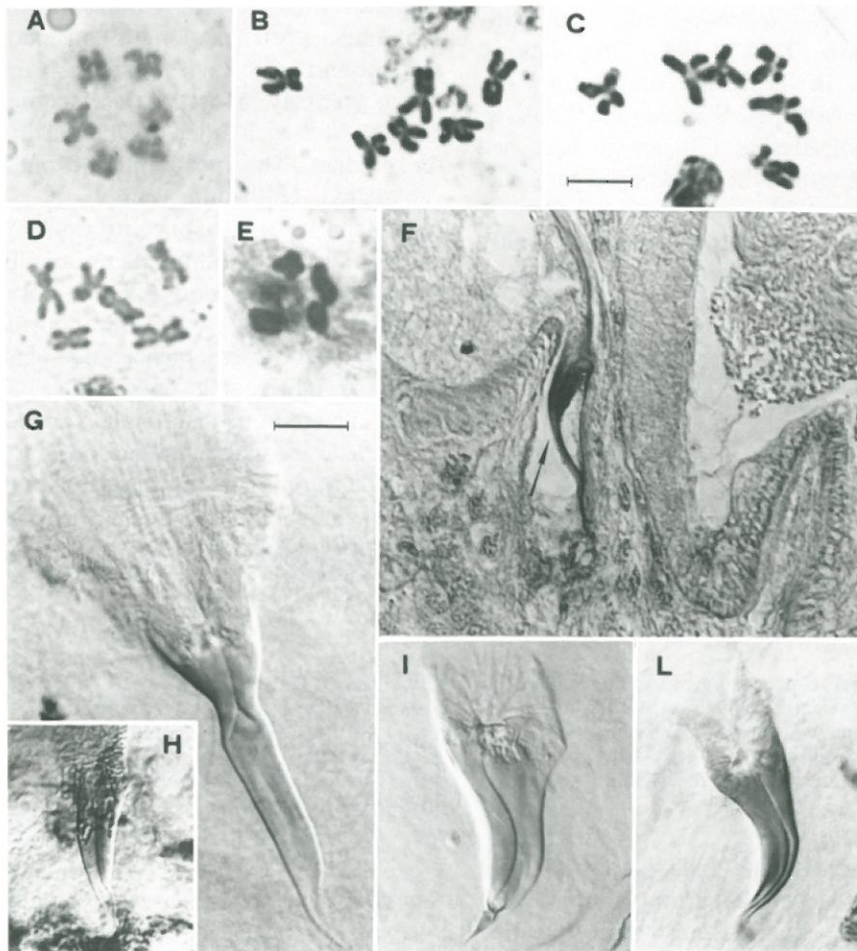


Fig. 7. A-E. Spermatogonial mitoses. A. *M. proculvaginata* sp. n.; B. *M. concharum* sp. n.; C. *M. beaglei*; D. *M. heronensis* sp. n.; E. *M. cf. dolichovesicula*; F. Sagittal section of *M. australis* sp. n. (arrow points to the stylet). G-L. Stylets (from karyological slides). G. *M. proculvaginata* sp. n.; H. *M. cf. dolichovesicula*; I. *M. concharum* sp. n.; L. *M. heronensis* sp. n. Scale bars: A-E: 5 µm; F-L: 10 µm.



observation in living animals. The extraordinary bursa of the species, stretching over a large tract of the female duct and consisting of more or less isolated large vacuoles, which are easily appreciable in living specimens, allows immediate specific recognition. Only *M. gigantea* has a somewhat comparable bursa, though proportionally much smaller and wholly of the resorbiens type. Furthermore, it has the vagina nearly halfway between the mouth and the male pore, and a much larger stylet.

The further locality given by Martens & Curini-Galletti (1989) (Fitzroy Is., N.E. Queensland) in the original description could not be confirmed and is possibly the result of sediment contamination in the laboratory.

## Discussion

Three of the new species (*M. proculvaginata*, *M. australis* and *M. concharum*) present a non ciliated area ventrally around the genital pores. This bare patch varies somewhat in extent in the three species, being most developed in *M. concharum*, where it may join the caudal non ciliated area present in all Monocelididae. No other *Minona* species is known to have a similar non ciliated patch, nor is it present in other genera with a simplex copulatory bulb (*Monocelis* Ehrenberg, 1831; *Pseudomonocelis* Meixner, 1943). This feature is thus a synapomorphy for the three species.

Non ciliated areas, though different in pattern and position, are present in other Proseriata (e.g. Otoplanidae and in the genus *Archimonocelis* Meixner, 1938; cf. *A. glabrodorsata* Martens & Curini-Galletti, 1993); however, the situation present in the three species is exceptional for the Monocelididae. All three species share the same habitat (high intertidal in very coarse sediment), and the pattern of ventral ciliation might be correlated with high energy stress. However, no adhesive glands have been observed in the non ciliated area. It is worth noting that the bare patch roughly corresponds to the area that is overlapped among mates during copulation, and a reproductive involvement cannot be excluded.

The three species share two additional features, which might prove to be further synapomorphies: i) a very short, non ciliated luminal portion of the pharynx provided with intraepithelial nuclei ('oesophagous'), not longer than 1/6 of the whole length of the pharynx itself. Currently known species of the genus *Minona* and related genera with a simplex copulatory bulb show a much more developed oesophageal portion, ranging 1/3–1/4 of the total length of the pharynx. ii) the extension of the caudal non ciliated area. In congeners and in the outgroup (see above) the non ciliated area is very small, and rather symmetrical on the ventral and the dorsal side (see, for example, Schockaert & Martens, 1987, p. 106, Fig. 2). In the three new species the non ciliated area is much larger and markedly asymmetrical, extending much more dorsally than ventrally.

The three sister species are very similar in habitus (being large and rather torpid), habitat (see above), and occur in neighbouring biogeographical areas (S. Australia and eastern Australia Overlap zone). Within this monophyletic group it is interesting to note how variable some of the characters are which have been used for phylogenetic

reconstruction in the monocelidids, i.e. relative position of the vagina, shape of bulb and stylet, length of eversible penis, and relative position of the accessory organ pore.

Ax & Sopott-Ehlers (1985) recognize within the genus *Minona* a monophylum based on the lack of vagina. *M. heronensis* would then be a member of this large group of species, which is mostly widespread in the tropics. However, the grouping might be homoplasous, since reduction of the vagina might have happened independently in many cases. Caution is also suggested by the situation found in the closely related genus *Monocelis*, where recognized sister-species may differ in the presence or absence of the vagina (cf. Curini-Galletti & Cannon, in press).

Similarly, no unequivocal synapomorphic features have been found for *M. beaglei* and *M. dolichovesicula*, and their phyletic position cannot be assessed at present.

As a whole, *Minona* species in Australia appear to be restricted in distribution to each of the recognized Australian biogeographical areas: northern Australia (*M. beaglei*); Barrier Reef province (*M. heronensis*; *M. cf. dolichovesicula*); eastern Australia Overlap area (*M. concharum*); southern Australia (*M. proculvaginata*; *M. australis*). This pattern of endemic narrow-range species has also been found in the genus *Monocelis* in Australia (Curini-Galletti & Cannon, in press).

Finally, Australian *Minona* species appear to be in general rare and/or with very restricted habitat. Only in Tasmania one species (*M. proculvaginata*) abounds, characterizing intertidal coarse sediments. Comparable habitats in the N. Pacific (Alaska) and along the northern coasts of both sides of the Atlantic are occupied by two similarly large *Minona* species, *M. gigantea* and *M. baltica*. These three species form a good example of very similar, albeit unrelated, vicariant species in cold-water boreal/austral environments.

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