

**CONTRIBUTION TO THE KNOWLEDGE OF THE PROSERIATA  
(PLATYHELMINTHES: SERIATA) FROM THE GULF OF ELAT (RED SEA):  
GENUS *MINONA* MARCUS, 1946**

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**ABSTRACT**

Three new species of the genus *Minona* Marcus, 1946 from Elat (Red Sea) are described: *M. ileanae* n. sp., *M. pseudoileanae* n. sp., and *M. albicincta* n. sp. They differ in details of the copulatory organ, size and shape of the accessory organ, pigmentation, and karyotype. The three species present a rare morphological feature for the genus *Minona* (i.e., fusion of male and accessory organ pores). Due to the presence of this synapomorphy, a sister-species relationship is proposed for them.

**INTRODUCTION**

The genus *Minona* Marcus, 1946 is one of the largest of the family Monocelididae, with more than 20 species described. It is cosmopolitan in distribution, and particularly varied in species in tropical waters. The genus has not been reported thus far for the Red Sea. Research at the H. Steinitz Marine Biological Laboratory of Elat resulted in the finding of three new species. They are described here.

**METHODS**

Specimens were collected in sandy habitats by scooping the superficial layer of sediment. Extraction of the animals from the sediment was done with  $MgCl_2$  decantation (Martens, 1984). Preservation techniques routinely adopted for Proseriata were used (see Martens et al., 1989). For microscopical analysis, specimens were fixed in Bouin's fluid, embedded in 56 °C Paraplast, and serial sagittal sections were cut at 2–4  $\mu m$ , stained with Heidenhain's stain (see Westheide and Purschke, 1988), and mounted in Euparal.

Karyological techniques were as described by Curini-Galletti et al. (1989). Relative lengths (r.l. = length of chromosome  $\times$  100/total length of haploid genome) and centromeric indices (c.i. = length of short arm  $\times$  100/length of entire chromosome) were obtained from measurements of camera lucida drawings of 5–10 metaphase plates for each species. Ideograms are based on karyometrical data presented in the karyotype formula: haploid

genome absolute length in  $\mu\text{m}$ , nombre fondamental (NF), relative length and centromeric index of each chromosome, and chromosome nomenclature in parentheses (m = metacentric; sm = submetacentric). The fundamental number is derived according to Matthey (1949), and chromosome nomenclature follows Levan et al. (1964).

Holotypes are stored in the collections of the Queensland Museum (S. Brisbane, Queensland, Australia). Paratypes are in the author's collection, Dipartimento di Zoologia e Antropologia Biologica, Sassari (ZS).

### SPECIES DESCRIPTIONS

#### *Minona ileanae* n. sp.

(Figs 1, 4A)

#### MATERIAL EXAMINED

*Holotype*: Red Sea, Elat, North Beach, medium to coarse terrigenous sand, about 30 cm deep. April 1988; sagittally sectioned: G 211681.

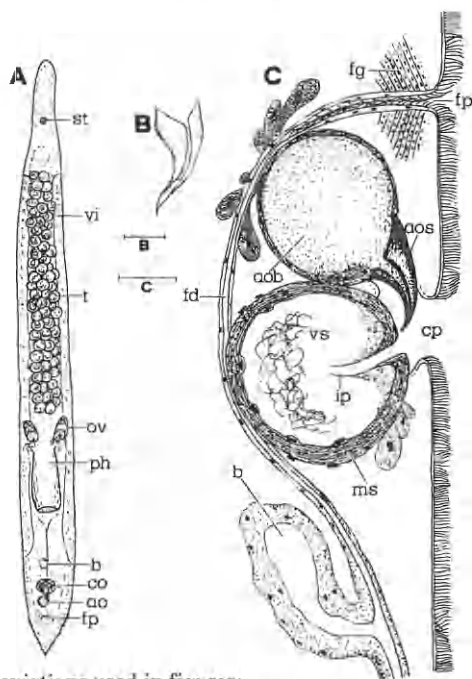


Fig. 1. *Minona ileanae* n. sp. A: general organization of a live animal; B: accessory organ stylet (from a karyological slide); C: reconstruction of the genital organs from sagittal sections. Scale bars = 10  $\mu\text{m}$ .

#### Abbreviations used in figures:

ao: accessory organ; aob: accessory organ bulb; aos: accessory organ stylet; b: hursa; cm: circular muscles; co: copulatory bulb; cp: common pore; fd: female duct; fg: female glands; fp: female pore; ip: inverted penis; m: mouth; ms: muscle coating; od: oviduct; ov: ovaria; ph: pharynx; phg: pharyngeal glands; pi: pigment band; rh: rhabdoid; st: statocyst; t: testes; vd: vaginal duct; vi: vitellaria; vp: vaginal pore; vs: seminal vesicle.

*Paratypes*: same data as holotype, four specimens sagittally sectioned: ZC 9–12.

*Other material*: several specimens studied alive, two specimens studied karyologically.

#### DESCRIPTION

Animals slender, up to 2 mm long in sectioned material. Without pigment or pigmented eyespots. Anterior end rounded, provided with oily droplets. Posterior end with adhesive glands. The epidermis, with depressed nuclei, is ciliated except at the caudal tip. Cilia (3–4  $\mu\text{m}$  in length) are slightly longer ventrally than dorsally. The epidermis is provided with elongated rhabdoids (up to 15  $\mu\text{m}$  long in sections), particularly evident in the cephalic and caudal region. Pharynx in the distal third of the body, comparatively narrow and elongate. It is ciliated (cilia length: about 1.5  $\mu\text{m}$ ) except at its luminal proximal fourth and its most distal tip, where pharyngeal glands discharge. The esophagus, with high and nucleated epithelium, is about 1/5 of the total length of the pharynx.

*Male genital organs*: 60 to 70 testes irregularly arranged in front of the pharynx. The copulatory bulb, of the simplex type, is ovoid (27 to 42  $\mu\text{m}$  wide and 33 to 38  $\mu\text{m}$  high in sections). It consists of a seminal vesicle, lined by a nucleated epithelium, which becomes high and glandular distally, with nuclei outside the bulb. The bulb is surrounded by a thin coat of muscles (3–4  $\mu\text{m}$  thick). The most distal portion of the bulb is inverted, forming an eversible penis, about 15  $\mu\text{m}$  long, with thin and nonciliated walls. The male copulatory organ protrudes into the common (male + prostatoid) antrum, forming a short and obtuse penis papilla. The antrum is large and nonciliated; it opens to the outside through a broad pore, easily appreciable in semi-squashed living specimens.

The accessory ('prostatoid') organ is located close behind the copulatory bulb. The bulb of the accessory organ is nearly spherical (27–37  $\mu\text{m}$  in diameter), filled with a granular secretion produced by glands whose nuclei lie outside the bulb. It is surrounded by a very thin coat of muscles, up to 1.5  $\mu\text{m}$  thick. The stylet is nib-shaped, with a broad base; it is  $31.1 \pm 1.1$   $\mu\text{m}$  long in two strongly squashed specimens that were used for karyology, and about 22  $\mu\text{m}$  in a sectioned specimen. The stylet protrudes into the wide common (male + prostatoid) antrum.

*Female genital organs*: two ovaries lie in front of the pharynx. Vitellaria extend from the level of the first testes to just in front of the copulatory organ. Posterior to the pharynx, the two oviducts fuse to form the common female duct. Caudal to the fusion, the duct widens dorsally into a rather small roundish bursa, lined by a thick, nucleate epithelium. Posterior to the bursa, the common female duct runs over the male organ and opens caudally through the female pore. At least the caudalmost portion of the duct is ciliated. Numerous glands are present around the female pore.

#### KARYOLOGY

With 3 chromosomes in its haploid set. Karyotype formula:  $9.2 \pm 0.4$   $\mu\text{m}$ ; NF = 6; chromosome I:  $35.97 \pm 2.24$ ;  $41.95 \pm 2.97$  (m); chromosome II:  $34.21 \pm 1.67$ ;  $25.69 \pm 4.20$  (sm); chromosome III:  $29.81 \pm 2.02$ ;  $32.03 \pm 1.58$  (sm).

## REMARKS

*M. ileanae* n. sp. is one of the few *Minona* species known with male and accessory organ pores fused into a common pore. Among them, *M. bistylifera* Karling, Mack-Fira, and Dorjes, 1972 is immediately recognizable from the new species due to the presence of an intrapenial stylet. *M. gemella* Ax and Sopott-Ehlers, 1985 (see Curini-Galletti, 1991, for an integrated description) has two accessory prostatoid organs, one (rostral) in front and one (caudal) behind the copulatory organ. *M. secta* Sopott-Ehlers and Ax, 1985 is provided with a vagina (absent in the new species), and has fewer (18) testes. Furthermore, this species has a larger (60–70  $\mu\text{m}$ ) copulatory bulb, overlain by a much thicker muscle coat. *M. bermudensis* Ax and Sopott-Ehlers, 1985 (see Curini-Galletti, 1991, for an integrated description) has a very large squarish bursa, quite obvious in semi-squashed living specimens, and a copulatory bulb about three times the size of the accessory organ bulb (see Ax and Sopott-Ehlers, 1985: 378, fig. 4D).

Among the species where the presence of an accessory organ pore could not be ascertained, *M. hastata* Martens and Curini-Galletti, 1989 has fewer testes (8), and a much longer (about 70  $\mu\text{m}$  long in not-strongly-squashed whole mounts) and slender stylet. Furthermore, the bulb of the accessory organ is surrounded by a much thicker muscle coat (see Martens and Curini-Galletti, 1989: 181, figs. C,D). *M. indonesiana* Martens and Curini-Galletti, 1989 has small, banana-shaped rhabdoids, and a differently shaped accessory stylet (see Martens and Curini-Galletti, 1989: 182, fig. 4D). Furthermore, this species lacks a bursa.

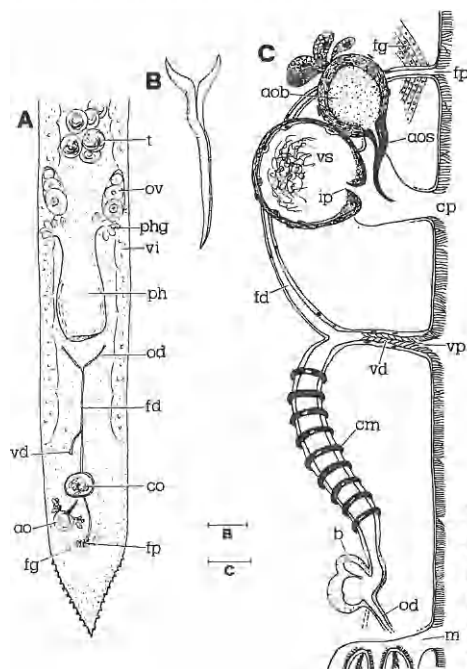


Fig. 2. *Minona pseudoileanae* n. sp. A: general organization of the posterior region of the body from a live animal; B: accessory organ stylet (from a karyological slide); C: reconstruction of the genital organs from sagittal sections. Scale bars = 10  $\mu\text{m}$ .

### ETYMOLOGY

The species is named for Prof. Ilana Puccinelli (Pisa) in recognition of her meticulous work on the cytotaxonomy of Platyhelminthes over a period of thirty-five years, and in gratitude for her encouragement of my own work.

***Minona pseudoileanae* n. sp.**  
(Figs 2,4C)

### MATERIAL EXAMINED

*Holotype*: Red Sea, Elat, North Beach, medium to coarse terrigenous sand, about 30 cm deep. April 1988; whole mount: G 211682.

*Paratypes*: same data as holotype, two specimens sagittally sectioned on one slide: ZC 13; one whole mount: ZC 14.

*Other material*: several specimens studied alive, four specimens studied karyologically.

### DESCRIPTION

Animals very similar to the previous species in general shape, size, ciliation, general topography of internal organs, and structure of the pharynx. Epidermis with small, comma-shaped rhabdoids, about 5  $\mu$ m long.

*Male genital organs*: About 70 testes irregularly arranged in front of the pharynx. The copulatory bulb, of the simplex type, is small and nearly globular (27  $\mu$ m wide, 25  $\mu$ m high in sections). It consists of a spherical seminal vesicle, lined with a nucleated epithelium. Distally, the lining epithelium is high and glandular. The bulb is surrounded by an extremely thin coat of muscles (about 1.5  $\mu$ m thick). The distalmost portion of the bulb is inverted, forming an extremely short eversible penis. The bulb protrudes into the common (male + prostatoid) antrum. This antrum is very large and nonciliated, and opens to the outside through a rather narrow pore.

The accessory organ bulb is globular (20  $\mu$ m wide; 23  $\mu$ m high), surrounded by a muscle coat about 3  $\mu$ m thick; it is located postero-lateral to the male bulb. The stylet is  $49.6 \pm 2.2$   $\mu$ m long in strongly squashed karyological slides, and about 45  $\mu$ m in a sectioned specimen; it is narrow, elongated, and nearly tubular in shape. The stylet protrudes into the common (male + prostatoid) antrum.

*Female genital organs*: Ovaries lie in front of the pharynx. The two oviducts fuse just behind the pharynx into a small bursa of the resorbiens type, from which the common female duct originates. The latter opens to the exterior in front of the copulatory bulb through a vagina. The portion of the female duct between the bursa and the vaginal duct is swollen, and lined with numerous circular muscle fibers. The vaginal duct is lined with a swollen, nucleate, and ciliated epithelium. The vaginal pore is closer to the common (male + prostatoid) pore than to the mouth. Posterior to the connection with the vaginal duct, the female duct becomes less swollen and muscular, and opens behind the common pore into the female pore; it is surrounded by female glands.

### KARYOLOGY

With 2 chromosomes in its haploid set. Karyotype formula:  $7.3 \pm 0.6 \mu\text{m}$ ;  $\text{NF} = 4$ ; chromosome I:  $54.30 \pm 1.10$ ;  $44.54 \pm 2.70$  (m); chromosome II:  $45.70 \pm 1.10$ ;  $46.05 \pm 1.58$  (m).

### REMARKS

*M. pseudoileanae* n. sp. occurs syntopically with *M. ileanae*, and the two species are indistinguishable at low magnification. However, the different shape of the stylet (nib-shaped with a broad basis in *M. ileanae*, tubular with a narrow basis in *M. pseudoileanae*) allows discrimination in gently squashed living specimens. The two species are easily recognized in sectioned material, as *M. pseudoileanae* is provided with an external vagina, has a smaller, less muscular copulatory bulb, and a shorter inverted penis. Furthermore, its accessory organ is smaller and more muscular. The chromosome number is also different.

Among the species with combined male + accessory organ pore and a vagina, *M. secta* has a copulatory bulb that is larger and much more muscular than that of *M. pseudoileanae*, and is provided also with a longer inverted penis. The prostatoid stylet of *M. secta* is nib-shaped (see Sopott-Ehlers and Ax, 1985: 339, fig. 5 D,E), as in most *Minona* species; it is tube-shaped in *M. pseudoileanae*. In *M. gemella* the vaginal pore is fused with the anterior accessory organ pore (see Curini-Galletti, 1991), which is absent in the new species. The presence of a vagina could not be ascertained in

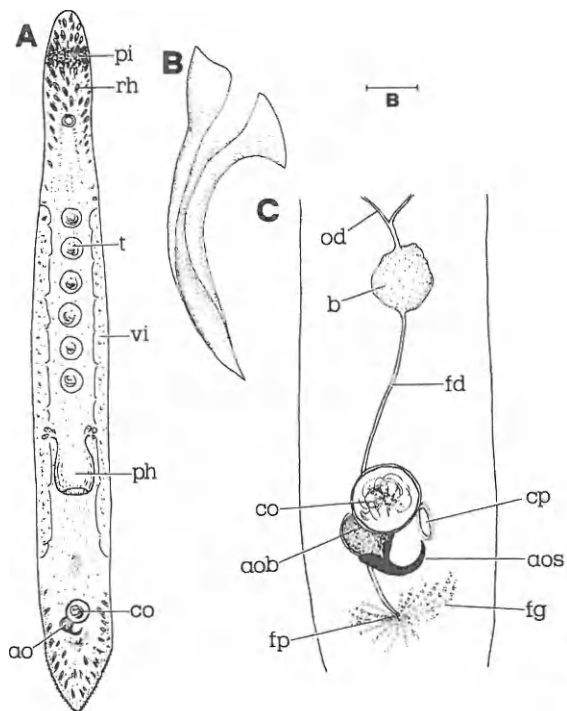


Fig. 3. *Minona albicincta* n. sp. A: general organization of a live animal; B: accessory organ stylet (from a karyological slide); C: general organization of the genital organs from live animals. Scale bar =  $10 \mu\text{m}$ .

*M. bermudensis* (see Ax and Sopott-Ehlers, 1985, and Curini-Galletti, 1991). However, this species has a characteristic broad squarish bursa, and a copulatory bulb about thrice the size of the accessory organ bulb.

#### ETYMOLOGY

The name reflects the close similarity among the new species and *M. ileanae*.

***Minona albicincta* n. sp.**  
(Figs 3,4B)

#### MATERIAL EXAMINED

*Holotype*: Red Sea, Elat, between the H. Steinitz Marine Biological Laboratory and the Underwater Observatory, about 2 m deep in a sea grass meadow; fine sand. April 1988; whole mount: G 211683.

*Paratype*: same data as holotype, whole mount: ZC 15.

*Other material*: two specimens observed alive and used for karyology.

#### DESCRIPTION

Animals slender in shape, agile, up to about 2 mm long in semi-squashed conditions. With a narrow but conspicuous pigment band (white in reflected light, black in transmitted light) close to the anterior tip of the body. Epidermis provided with very large rhabdoids, which are particularly numerous in the anterior and the posterior tips. Numerous, very small rhabdoids also present all over the body, sometimes arranged in longitudinal rows. The short pharynx is situated in the posterior half of the body.

*Male genital organs*: 5 to 7 testes are arranged in a median row in front of the pharynx. The copulatory bulb, of the simplex type, is globular (70–80  $\mu\text{m}$  wide in semi-squashed conditions), very weakly muscular, and with glandular tissue around the penis tip. The male pore is fused with the accessory organ pore to a wide, slit-like, common pore.

The accessory organ is located just behind the copulatory bulb. The accessory organ bulb is weakly muscular and distinctly smaller than the copulatory bulb. The stylet is large ( $62.2 \pm 3.3 \mu\text{m}$  long in strongly squashed karyological slides;  $44.2 \pm 1.9 \mu\text{m}$  in lactophenol whole mounts). The stylet has a broad basis, which abruptly narrows into a tube-like funnel, slightly tapering to the pointed tip.

*Female genital organs*: Vitellaria extend from in front of the first testes to just anterior to the copulatory bulb. Posterior to the pharynx, the two oviducts fuse to form the common female duct. In front of the copulatory organ, the female duct forms a small roundish bursa. No vagina has been observed. The female duct opens to the outside just behind the accessory organ through the female pore, which is surrounded by female glands.

#### KARYOLOGY

With 3 chromosomes in its haploid set. Karyotype formula:  $11.3 \pm 1.0 \mu\text{m}$ ; NF = 6; chromosome I:  $41.67 \pm 2.39$ ;  $46.29 \pm 0.91$  (m); chromosome II:  $30.57 \pm 1.58$ ;  $46.58 \pm 1.09$  (m); chromosome III:  $27.73 \pm 2.03$ ;  $35.19 \pm 3.47$  (sm).

## REMARKS

*M. albicincta* n. sp. is immediately distinguishable from congeneric species in the Gulf of Aqaba/Elat due to its pigmentation, size, and morphology of stylet, and rhabdoids. This set of characters also allows immediate distinction among the *Minona* species with common (male + accessory organ) pore.

No other *Minona* known to date has a subapical transverse pigment band, white in reflected light. Only *M. peteraxi* Karling, 1978 is pigmented anterior to the statocyst. In this species, however, the pigment band is black and longitudinal, leading to two rostral pigmented eye spots.

The stylet of *M. albicincta* is unusually large. Only *M. gigantea* Ax and Armonies, 1990, *M. baltica* Karling and Kinnander, 1953, and *M. hastata* Martens and Curini-Galletti, 1991 have stylets of comparable size. The two former species, however, are comparatively very large, and thus the stylet is proportionally much smaller than in the new species. Both of these species have distinct male and accessory organ pores. *M. hastata*, on the contrary, is a small-bodied species, and the relative proportion of the stylet is similar to the new species. The stylet of *M. hastata*, however, is even larger (about 70  $\mu\text{m}$  long in not-strongly-squashed whole mounts), characteristically needle-like and with a narrow base.

## ETYMOLOGY

The name refers to the species' peculiar pigmentation (L: *albus*, white; *cinctus*, girdled).

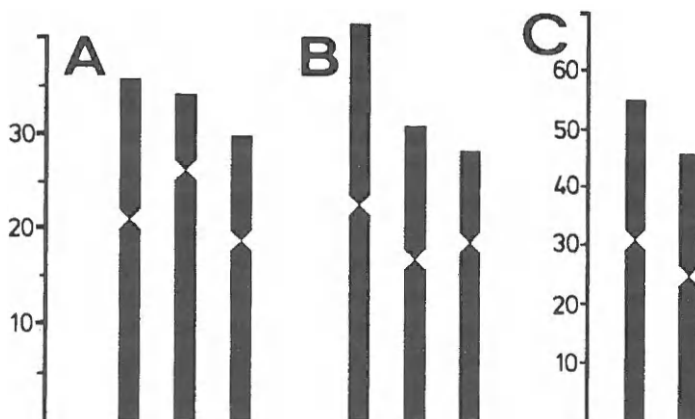


Fig. 4. Ideograms. A: *Minona ileanae* n. sp.; B: *M. albicincta* n. sp.; C: *M. pseudoileanae* n. sp.



## DISCUSSION

The condition with four distinct genital openings (vagina, male pore, accessory organ pore, female pore) is considered to be the plesiomorphic character within the genus *Minona* (Ax and Sopott-Ehlers, 1985; Curini-Galletti, 1991). Rare cases of fusion among adjoining pores are known: they involve accessory organ and female pores (*M. trigonopora* Ax, 1956), or, as in the three new species described here, male and accessory organ pores.

Species sharing with the new species such an apomorphic condition range from Hawaii (*M. bistylifera*), the Pacific coast of U.S.A. (Washington) (*M. secta*), and the Caribbean (*M. gemella*, *M. bermudensis*). No other synapomorphy for all these species is known, and the possibility that independent events of pore fusion have happened cannot be ruled out. However, the presence of such a rare apomorphic condition in all the species found in the Gulf of Aqaba/Elat suggests, on basis of parsimony, that they descend from a common ancestor showing this state. Sympatry of related species is often caused by allopatric speciation and subsequent dispersal into the sister species' habitat. The system formed by the twin gulfs of Aqaba/Elat and Suez, isolated from the Red Sea during glacials (see Por, 1989), forms a plausible scenario for isolation, speciation, and subsequent contact among species.

The new species markedly differ in the size of stylet, which is small in *M. ileanae*, medium in *M. pseudoileanae*, and large in *M. albicincta*. Although specific studies are lacking, the accessory prostatoid organ is plausibly connected with reproduction. The marked difference among the three species might be a means to avoid interspecific copulations, at least between the strictly syntopic pair *M. ileanae*–*M. pseudoileanae*, thus acting as reinforcement (i.e., a prezygotic barrier).

The three species are further distinguished by their karyotypes. The basic karyotype for the genus *Minona* is as shown by *M. ileanae* (see Litvaitis et al. (1996), and Curini-Galletti and Martens, 1990, for a general discussion on karyological evolution of the Monocelididae). *M. albicincta* has a derived karyotype, with different relative lengths, possibly the result of a translocation involving chromosomes I and II. *M. pseudoileanae* has a derived chromosome number ( $n = 2$ ), due to a Robertsonian mechanism of fusion. In this case too, the marked difference between the syntopic species pair *M. ileanae*–*M. pseudoileanae* might reflect hybrid avoidance (i.e., a postzygotic barrier). It is worth noting that species of the genus *Pseudomonocelis* Meixner, 1943 copulate indiscriminately in the laboratory, at least among congeneric sibling species, and that various degrees of postzygotic barriers exist among them (Curini-Galletti; in prep.).

The presence of pigment in only one species of the group (*M. albicincta*) is possibly related to its ethology. Platyhelminth species which actively emerge from the sediment are more pigmented than strictly mesopsammic species (Armonies, 1989). A comparable pigmentation is found in species belonging to monocelidid genera related to *Minona* (*Monocelis* Ehrenberg, 1831, and *Pseudomonocelis* Meixner, 1943) (Schockaert and Martens, 1987; Martens and Curini-Galletti, 1989; Curini-Galletti and Cannon, 1995). The capacity to store pigments in a distinct cephalic band might thus be an underlying synapomorphy for the Monocelidinae Midelburg, 1908, expressed only in

species exposed to more photic conditions.

The finding of three sympatric *Minona* species in a very limited stretch of coast in the Gulf of Elat bears some implications for the biodiversity of the area. The genus *Minona* is cosmopolitan. Relatively few species are known from boreal and temperate areas, usually occurring singly in any given area, whereas higher diversity is attained in some tropical areas. However, this might not be a general trend. The maximum number of sympatric species (five) has been found around Magueyes (Puerto Rico, Caribbean) (Curini-Galletti, 1991). On the contrary, in tropical Australia no more than two sympatric species have been found, and no obvious increase in diversity of this genus has been observed from Tasmania to the Great Barrier Reef (Curini-Galletti and Cannon, 1996). The Northern Red Sea appears thus to be an area of rather high diversity for the group, as well as for other Proseriata genera (Curini-Galletti and Martens, 1991, 1992; Martens and Curini-Galletti, 1993).

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