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The morphology, arrangement, and ultrastructure of a new type of microtrich sensilla in marine isopods (Crustacea, Isopoda)

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Abstract

Background: Microtrich sensilla are a special type of cuticular structures found on the external surface of amphipod and isopod crustaceans. These cuticular microstructures, being important for systematic and phylogenetic studies, display a wide diversity of shapes and distribution among different taxa. Here, the microstructure of the cuticular surface of 11 marine isopod species was investigated by scanning electron microscopy.

Results: On the dorsal and lateral sides of all the taxa examined, only one distinct type of microtrich sensillum was found in the regular rows. While this structure is very similar to that of the Ib microtrich type (*sensu* Crustaceana 13:100–106, 1988), however, it is distinct from the previous type, in which it has an elongate cuticular ring or specialized collar encircling the shaft base of the microtrichs. The findings indicate that different taxa of marine isopods may show a new type of microtrich sensilla differing in morphology and arrangement.

Conclusions: The findings indicate that different taxa of marine isopods may show a new type of microtrich sensilla differing in morphology and arrangement.

Keywords: Isopoda; Marine; Cuticular surface; Scanning electron microscopy

Background

Examination of cuticular surface features of crustaceans, especially Peracarida, has been conducted for many years. Fish (1972) described the setae of the aquatic isopod *Eurydice pulchra* Leach, 1815 on the basis of size as either macrotrichs or microtrichs. Powell and Halcrow (1982) described the surface microstructures of several marine littoral and terrestrial isopods using SEM, and Oshel and Steele (1988) and Oshel et al. (1988) described and discussed the setae and microtrich sensilla of some amphipods. In most cases, these studies were conducted with an emphasis on only one type of cuticular structure, *viz.*, tricorn setae (Holdich and Lincoln 1974; Schmalfuss 1978), sensory spines (Brandt 1988), pores (Halcrow and Bousfield 1987; Khalaji-Pirbalouty and Sari 2004, 2006; Khalaji-Pirbalouty and Sari 2006), or microtrichs (Oshel et al. 1988; Platvoet 1985; Steele 1991; Olyslager and Williams 1993). Recently, Zimmer et al. (2009) described variation of five different kinds of cuticular structures for hyallelid amphipods, including 30 types of setae, four types of microtrich, and three types of pores. Of those, microtrich sensilla were found only on aquatic crustaceans, e.g., gammaridean amphipods and marine isopods; there is no report of these structures in terrestrial isopods. Fish (1972) used the term microtrich to refer to setae less than 10 μm in length. Later, Oshel et al. (1988) defined microtrichs as setae less than 25 μm in length with specialized sockets, and they divided microtrichs into two types, I and II, based on the socket morphology. Type I has a socket bearing a bowl-shaped, shallow depression, with a dome on one edge and is formed by 3 to 4 epidermal cells. Microtrich type II has a simple and circular socket. Type I microtrichs were further subdivided into three types (Ia, Ib, and Ic) on the basis of the setal morphology: Ia with a terminal pore directed to one side of the seta, Ib with long filaments radiating from a hood that may be one to two or more times as long as the setal shaft, and

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Ic a short and plumose setae whose filaments are either restricted to the apical end of the setal shaft or originate from the distal third of the shaft (see Oshel et al. 1988, pages 102 and 103, Figures two (a,b), three (a,b), and four). Microtrich sensilla type II are defined as short, longitudinally compressed seta with a bifurcate tip. Type II microtrichs also were called slide-line organ or flattened microtrichs on gammaridian amphipods by Platvoet (1985) and Platvoet et al. (2007). More recently, Kaim-Malka (2010) introduced the term 'unispathes' (along with spatheform organ for whole set of structures) instead of previous terms and despite the fact that spatheform is a common name for plants of the genus *Arisaema*. These structures have been found in many amphipods and also in some marine isopods (e.g., Platvoet 1985; Laverack and Barrientos 1985; Halcrow and Bousfield 1987; Oshel et al. 1988; Olyslager and Williams 1993; Kaim-Malka 2010). This paper describes the morphology and arrangement of a new kind of Ib microtrich sensilla *sensu* (Oshel et al. 1988) on marine isopods.

Methods

Specimens for this study were collected primarily from intertidal and subtidal habitats along the Iranian coastline of the Persian Gulf. Collecting techniques included sieving sand and washing algae and sea grass, as well as direct capture. *Excirolana* sp., *Dynamenella granulata* (Javed and Ahmed 1988), *Sphaeroma khalijfarsi* (Khalaji-Pirbalouty and Wägele 2010), *Sphaeroma walkeri* (Stebbing 1905), *Sphaeromopsis sarii* Khalaji-Pirbalouty and Wägele 2010), *Cymodoce* sp., *Lanocira* sp., and *Atarbolana* sp. were collected from the beach of the Persian Gulf. In addition, specimens of *Excirolana orientalis* (Dana 1853), *Sphaeromopsis amathitis* (Holdich and Jones 1973), and *Sphaeromopsis mourei* Loyola e Silva 1960 were obtained from Copenhagen Museum and the Museum of Tropical Queensland. Of these, *Excirolana* sp., *E. orientalis*, *Lanocira* sp., and *Atarbolana* sp. belong to the family Cirolanidae, and *D. granulata*, *S. khalijfarsi*, *S. walkeri*, *Cymodoce* sp., *S. sarii*, *S. amathitis*, and *S. mourei* belong to the family Sphaeromatidae. Scanning electron microscopy (SEM) specimens were washed in chilled 1% sodium acetate solution for 10 min and then cleaned using an ultrasonic cleaner to remove the attached sediment and debris from the cuticle. After the specimens were dehydrated in an ethanol series and after a final 100% ethanol, they were transferred to 100% hexamethyldisilazane (HMDS) through a three-graded series of ethanol-HMDS mixtures (100% E 2:1, 1:1, 1:2, 100% HMDS) in a fume hood. For the final step, the specimens were immersed in a HMDS container for 15 to 60 min, depending on their size. The samples were then mounted on stubs using double-sided carbon tapes before being coated with gold in a sputter coater to 40-nm thickness. The SEM micrographs were taken using a Hitachi S-2460 N SEM (Tokyo, Japan).

Results

On the cuticular surface of all the examined specimens, only one distinct type of structure was found in regular rows on the dorsal and lateral sides of the specimens. These structures are very similar to type Ib microtrichs (*sensu* Oshel et al. 1988) by having a socket with a shallow depression, a long shaft with a branch of relatively long filaments radiating from the distal part. In contrast to previously described Ib microtrichs, the socket here has an elongate cuticular ring or specialized collar that encircles the shaft base. The shape of the socket, the polygons or knobs on the adjacent cuticular depressions, the collars, shafts, and the distal filaments all show variation among the examined taxa (Table 1). Furthermore, the arrangement and number of rows of these cuticular structures are variable among different genera or among species of the genus. However, dorsal and lateral groups

Table 1 Description of microtrich structures on the cuticular surface of some representatives of marine isopods

Species	Description	NF
Excirolana orientalis	Cuticular depressions are furnished with three to four knobs arranged in a semi-circular row; collar short, oval, anterior margin shorter than posterior one; shaft short with a tuft of long filaments (Figures 2B and 3B).	≈30
Excirolana sp.	Cuticular depressions are furnished with three to five knobs arranged in a semi-circular row; collar short, anterior margin shorter than posterior one; shaft short with a tuft of long filaments (Figures 2A and 3A).	≈30
Lanocira sp.	Simple cuticular depression; collar very short, oval; shaft very short with a tuft of long filaments (Figure 2C).	≈12
Atarbolana sp.	Collar very short, round; shaft long.	≈5
Sphaeromopsis sarii	Shallow, flower-shaped cuticular depression; collar spool-shaped, large; shaft large with a tuft of long filaments (Figures 2D,E and 3D).	≈8
S. amathitis	Shallow, flower-shaped cuticular depression; collar spool-shaped, large; shaft large with a tuft of long filaments (Figure 3E).	10 to 12
S. mourei	Shallow, flower-shaped cuticular depression; collar spool-shaped, large; shaft large with a tuft of long filaments (Figure 3F).	6-8
Sphaeroma khalijfarsi	Collar short, shaft long with long filaments (Figures 2F and 4A).	≈8
S. walkeri	Collar short; shaft long with long filaments (Figure 4B).	4 to 5
Cymodoce sp.	Collar medium, shaft very long with very long filaments (Figures 2G and 4E).	5
Dynamenella granulata	Collar medium, shaft short with long filaments (Figure 4C).	5

NF, number of distal filaments.

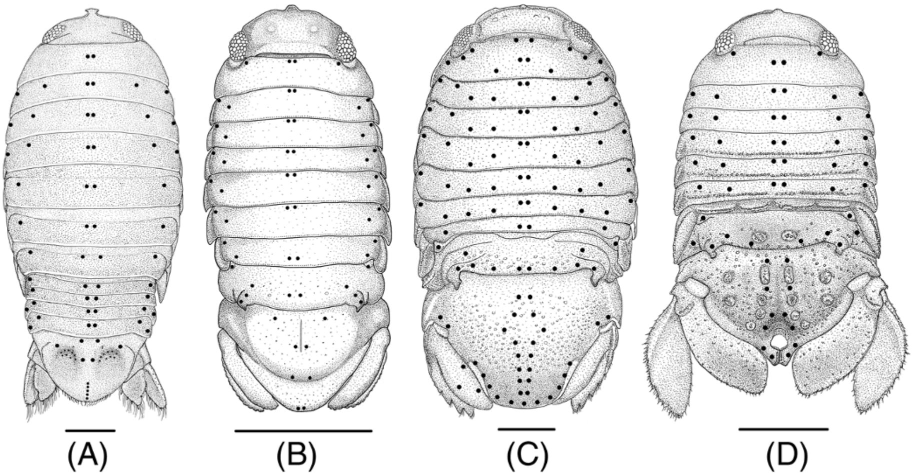

Figure 1 Dorsal view of the species (black circles indicate the position of the microtrichs). (A) *Excirolana* sp. (from Persian Gulf). **(B)** *Sphaeromopsis sarii*. **(C)** *Sphaeroma khalijfarsi*. **(D)** *Dynamenella granulata*. Scale bars = 1 mm.

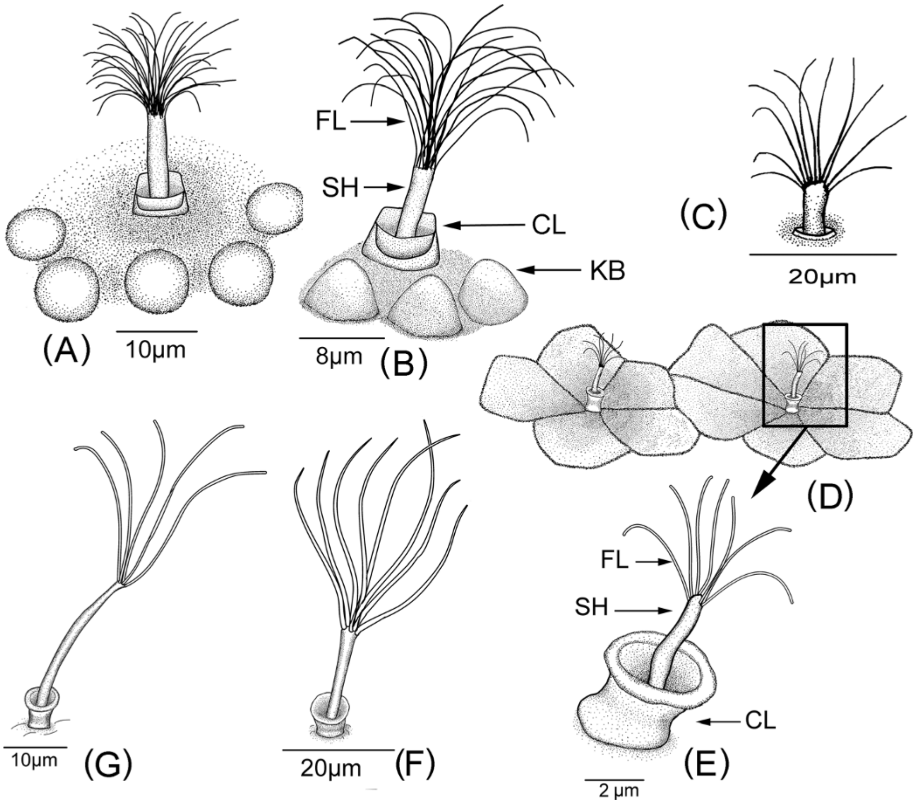

Figure 2 Schematic drawings of the microtrich structures. (A) *Excirolana* sp., **(B)** *Excirolana orientalis* (the cuticular depressions are furnished with three to five knobs arranged in a semi-circular row; in both species, each microtrich has a short collar, elongate shaft, and a tuft of long filaments), **(C)** *Lanocira* sp. (with very short collar and shaft, long filaments), **(D and E)** *Sphaeromopsis sarii* (shallow flower-shaped cuticular depressions on mid-dorsal surface of pereonite 1; collar spool-shaped, large; shaft large with a tuft of a few long filaments); **(F)** *Sphaeroma khalijfarsi* (with short collar, long shaft and filaments); **(G)** *Cymodoce* sp. (with medium collar, very long shaft and filaments); CL, collar; SH, shaft; FL, filament; KB, knob.

of these structures show near perfect symmetry. For example, *E. orientalis* Dana 1853 and *Excirolana* sp. from the Persian Gulf have two rows of these structures very close to each other on the mid-dorsal surface, one row on each dorsolateral side and one row on each lateral side (Figure 1A). On the pleotelson, there are two pairs of dorsal structures, a lateral pair, a dorsal row posteriorly, and several smaller structures on two depressions of the pleotelson (Figure 1A). The specimens of both species of *Excirolana* (from the Persian Gulf, New Guinea, and Philippines) have a microtrich that is encircled by a short collar, bearing an elongate shaft and a tuft of long filaments (approximately 30). In both species of *Excirolana*, the cuticular depressions are furnished with three to five knobs arranged in a semi-circular row (Figures 2A,B and 3A,B). *Lanocira* sp. (Figure 2C) has a microtrich that is encircled by a very short oval collar, bearing a small shaft and a tuft of long filaments (approximately 12), and microtrichs of *Atarbolana* sp. possess a very short and round collar with an elongate shaft and approximately five distal filaments (Figure 3C). In all examined species of the genus *Sphaeromopsis*, microtrichs are located in six rows: two close rows on mid-dorsal surface and two rows on each lateral side (Figure 1B), and each structure consists of a shallow flower-shaped cuticular depression (Figure 2D) and elongated spool-shaped cuticular collar (Figures 2E,D and 3D). In *S. sarii* (Figures 2E and 3D) and *S. mourei* (Figure 3F), each shaft has a tuft of 6 to 8 filaments on the

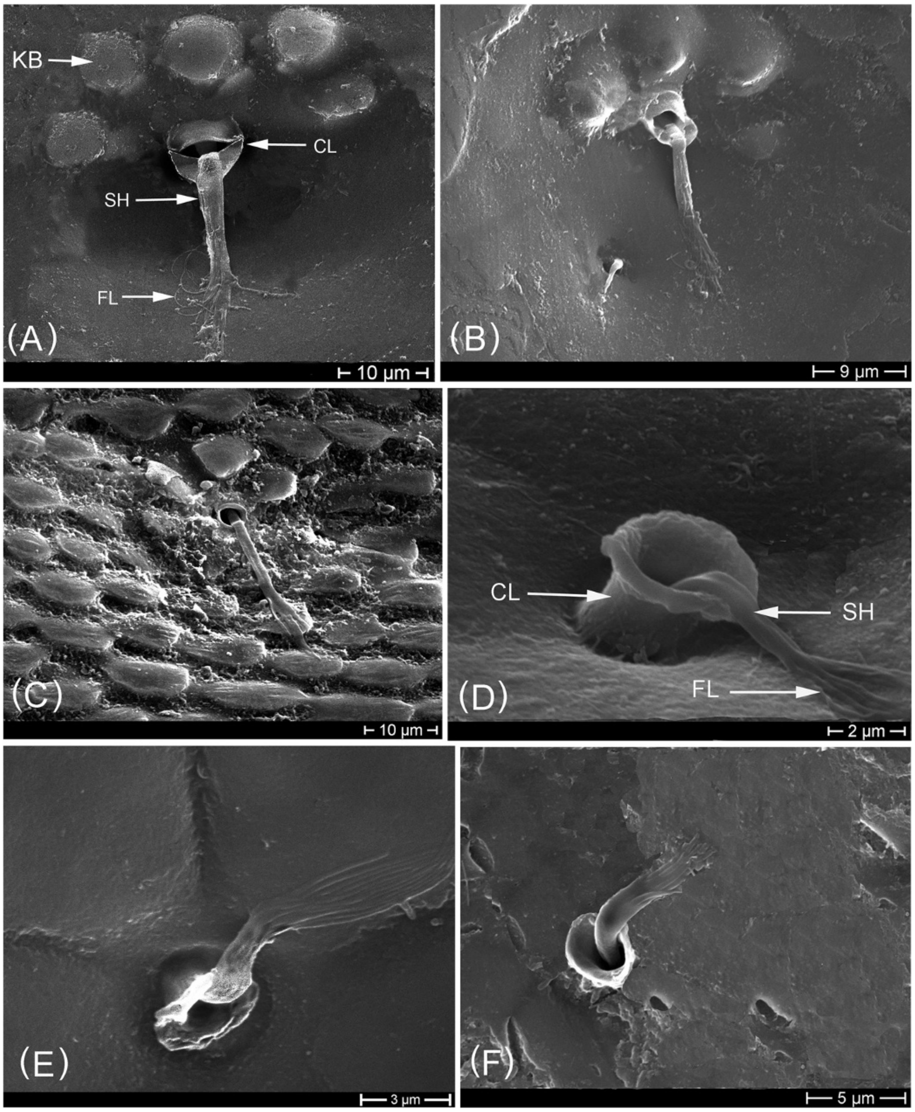

Figure 3 Scanning electron micrographs of the microtrich structures of *Excirolana*, *Atarbolana*, and *Sphaeromopsis* species. **(A)** *Excirolana* sp. from the Persian Gulf. **(B)** *Excirolana orientalis* (from Nagada, New Guinea), **(C)** *Atarbolana* sp. from the Persian Gulf, Iran. **(D)** *Sphaeromopsis sarii*. **(E)** *Sphaeromopsis amathitis*. **(F)** *Sphaeromopsis mourei*. CL, collar; SH, shaft; FL, filament; KB, knob.

distal part, and *S. amathitis* 10 to 12 filaments (Figure 3E). *D. granulata* Javed and Ahmed 1988 similar to the genus *Sphaeromopsis* has also six rows of microtrichs (Figure 1D). However, on *D. granulata*, the number of distal filaments is less (approximately five filaments), and the shaft is shorter (Figure 4C).

In contrast to the previous taxa, on *S. khalijfarsi* Khalaji-Pirbalouty and Wägele 2010, microtrichs are located in ten rows (rather than six rows), and there are two rows on the mid-dorsal surface of the pleotelson and one row on each marginal surface (Figure 1C). Each microtrich bears a short cuticular collar, an elongate shaft and a tuft of approximately eight long distal filaments (Figures 2F and 4A). Similarly, on *S. walkeri* Stebbing 1905, each structure has a short collar, a long shaft, and a few distal filaments (Figure 4B). However, on *Cymodoce* sp., each structure possesses a longer collar, very long shaft, and long distal filaments (Figures 2G and 4D).

Discussion

The cuticular microstructures of a few aquatic species of crustaceans like amphipods and isopods have been studied previously by SEM (e.g., Schmalfuss 1978; Meyer-Rochow 1980; Powell and Halcrow 1982; Holdich 1984; Laverack and Barrientos 1985; Platvoet 1985; Oshel et al. 1988; Olyslager and Williams 1993; Halcrow and Bousfield 1987; Read and Williams 1991; Khalaji-Pirbalouty and Sari 2006; Zimmer et al. 2009; Kaim-Malka 2010). The majority of these studies investigated the cuticular surface of Amphipoda, and only few attempts have been conducted to study the microscopic structures on the tegument surface of aquatic isopods (e.g., Wägele 1993; Escobar et al. 2002; Brandt 1988; Kaim-Malka 2010). Different types of cuticular structures such as scales, setae, microtrichs, setules, pores, or denticles were observed in marine isopods. Of these, microtrich sensilla type II were found only in a few marine isopods. However, microtrich sensilla type II were observed in several amphipods (Platvoet 1985; Oshel et al. 1988; Zimmer et al. 2009; Kaim-Malka 2010). Recently, Kaim-Malka (2010) found similar kinds of microtrich sensilla type II in *Eurydice truncata* (Norman 1868) and *Natatolana borealis* (Lilljeborg 1851) and named them as 'unispathes'. The structures reported here are very similar to type Ib microtrichs (*sensu* Oshel et al. 1988) reported for Amphipoda. However, the type Ib microtrichs reported here differ from those observed by previous authors for the majority of amphipods and also some isopods, because they have a cuticular collar that arises from the cuticular surface in the basal part of the seta. Moreover, the type Ib microtrichs reported here are

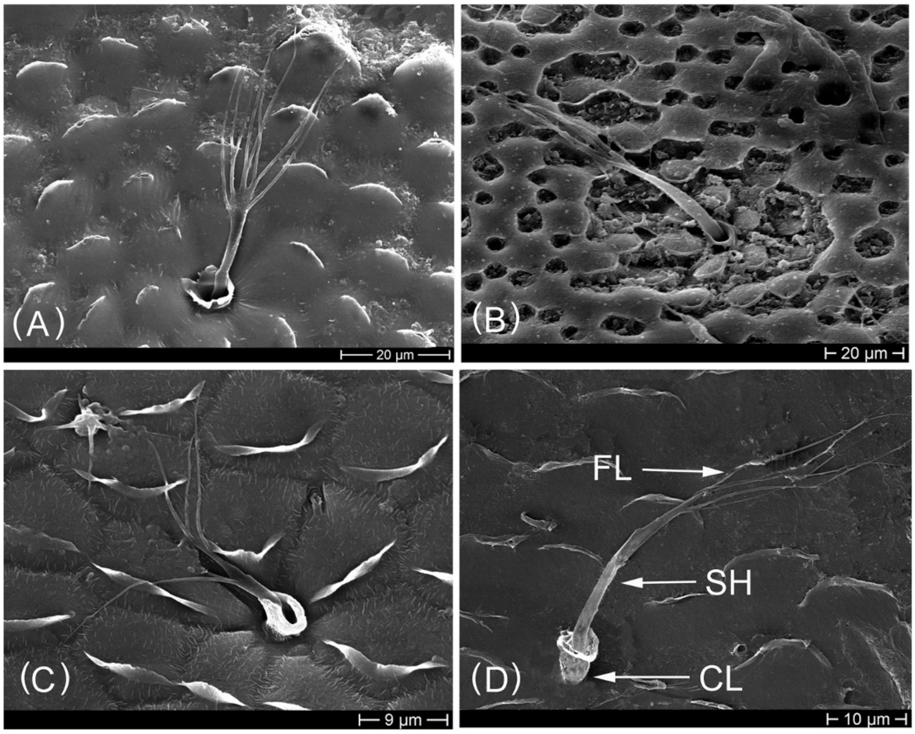

Figure 4 Scanning electron micrographs of the microtrich structures of *Sphaeroma*, *Dynamenella*, and *Cymodoce* species. (A) *Sphaeroma khalijfarsi* (with short collar and shaft, long filaments). **(B)** *Sphaeroma walkeri* (with short collar, long shaft, long filaments). **(C)** *Dynamenella granulata* (with short collar and shaft, long filaments). **(D)** *Cymodoce* sp. (with medium collar, very long shaft and filaments); CL, collar; SH, shaft; FL, filament.

arranged in regular and symmetrical rows on the body surface. Microtrich sensilla type II were not found in any species in this study. Kaim-Malka (2010) reported microtrich sensilla type II in the marine isopods *E. truncata* Norman 1868 and *N. borealis* Lilljeborg 1851, both species are found in marine subtidal environments. *E. truncata* is restricted to depths of 50 to 200 m (Schotte 2012), and *N. borealis* is found more broadly at 5 to 1,478 m (Keable and Bruce 1997; Johansen and Brattegard 1998). These data suggest that microtrich sensilla type II may characterize swimming species. In addition, Kaim-Malka (2010) stated that this structure may be less well developed or completely absent among species less able to swim and living in intertidal habitats. The type Ib microtrichs reported here are well developed on the dorsal and lateral sides of the examined marine species.

A comparison of these microtrichs shows that the number of distal filaments has correlation with living habit on different habitats. The result suggests that swimming species (e.g., *Excirolana* and *Lanocira*) have microtrichs with high number of distal filaments (15 to 30 filaments), whereas cirolanid isopods living in low algal turfs over rocky shore, e.g., *Atarbolana* sp., have microtrichs bearing few distal filaments (approximately five). In less-able swimming sphaeromatid isopods examined here, this structure has clearly low number of distal filaments. For example, in *S. walkeri*, *S. khalijfarsi*, *D. granulata* (all living in burrows or beneath stones in intertidal habitats), and *Cymodoce* sp. (live amongst algal and seagrass beds in subtidal habitats), the microtrichs have approximately four to eight distal filaments. Whereas, in *S. sarii*, *S. mourei*, and *S. amathitis* (mainly occur and swim in intertidal tide pools and partly on low algal turfs), this structure has approximately 8 to 12 filaments. In conclusion, the number of distal filaments on these structures has close correlation with the habitat structure. Swimming species in water column have more filaments than low swimmer species and species that living in burrows and beneath stones in intertidal habitats. According to Halcrow and Bousfield (1987), Watling (1989), Halcrow and Powell (1992), Khalaji-Pirbalouty and Sari (2006), Zimmer et al. (2009), and Kaim-Malka (2010), the kind and number of cuticular structures represent diagnostic traits for the identification of crustaceans, e.g., copepods and amphipods, as well as isopods, both on genus and species level. Therefore, the arrangement, diversity, and morphology of these elements constitute important tools for taxonomic analyses among genera of marine isopods or among species within a genus. For example, the arrangement and number of rows of these cuticular structures, their collar shape, shaft, and the number of filaments are commonly similar in two examined species of the genus *Excirolana* and three species of *Sphaeromopsis.* However, the shape of the cuticular collar, length of shaft, and the number of filaments are different between the examined species.

There are a variety of sensory receptors that transmit information to the central nervous system of crustaceans. Among the most obvious of these sensory structures are the different types of setae and sensilla that cover various regions of the body. A mechanosensory function is suggested by several authors for these kinds of cuticular structures (Fish 1972; Rider 1978; Bush and Laverack 1982; Platvoet 1985; Brandt 1988; Wägele 1993; Escobar et al. 2002). In addition, according to Brandt (1988), Laverack (1989), Felgenhauer (1992), and Escobar et al. (2002), sensory movable setae without a terminal pore have a mechanosensory function. As the cuticular structure reported here has no terminal pore on the apical part and also due to their arrangement and distribution over the body, a mechanosensory function can be supposed. These structures may provide the animals with information about the direction and possibly velocity and changes of water currents and hydrodynamic pressure. However, further investigations are necessary to clarify in greater detail the function of these structures in marine isopods.

Conclusions

This study suggests that the new type of microtrich sensilla reported here differ from those observed by previous authors for amphipod and isopod crustaceans, in which they have a cuticular collar that arises from the cuticular surface in the basal part of the seta. They are arranged in regular and symmetrical rows on the body surface. Notably, this structure with a relatively small number of distal filaments was observed in species with lower swimming ability.

Competing interests

The author declares that he has no competing interests.

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