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ORIGINAL PAPER

Diversity and potential correlations to the function of Collembola cuticle structures

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Abstract Collembola (springtails) are soil arthropods, representing the most widespread hexapod group worldwide. Being skin-breathing animals, Collembola evolved special cuticular patterns, which are robust and antiadhesive allowing cuticular respiration under humid conditions in the soil environment. Details about function and formation of these unique cuticle characters are still unknown. Here we demonstrate that a high diversity of cuticular structures exists and that the different observed structural patterns of Collembola cuticles might go along with specific adaptations to life in soil. We examined the cuticle structures of 40 different species using scanning electron microscopy and compared the cuticle patterns of the different species with information about their preferred habitat. In addition, we compare the results with current systematic concepts, showing that certain cuticle structures are typical for different collembolan groups.

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Introduction

The cuticle structures of Collembola (springtails) have gained interest because of unique antiadhesive characteristics caused by special nanoscaled patterns (Noble-Nesbitt 1963a).

Representing about 8,000 species (Bellinger et al. 1996–2012) Collembola are the most widespread and abundant terrestrial arthropods worldwide. According to recent literature, Collembola belong to a monophyletic or paraphyletic group of entognathous hexapods that is or includes the sister group of insects (Davis et al. 2010; Giribet and Edgecombe 2012; Grimaldi 2010; Jenner 2010; Letsch et al. 2010).

Collembola are divided in different life forms with respect to their dispersal in the soil layer (Christiansen 1964; Eisenbeis and Wichard 1985; Gisin 1943). Species living on the surface of the soil or in the vegetation are called epedaphic. They are comparatively large, possessing bristles, scales, pigment patterns, long extremities and antennae. In contrast, species living in the deep soil, referred to as euedaphic, are relatively small with short extremities, a cylindrical body ("worm-shaped") and mostly lacking pigments or eyes. A third life form is called hemiedaphic. These species are inhabitants of the coarse structures of the soil surface (e.-g., moss and litter layer).

Collembola, with some exceptions, are skin-breathing arthropods (Davies 1927; Zinkler 1966).

Few species of the Symphypleona order possess an additional tracheal respiratory system (Davies 1927; Eisenbeis and Wichard 1985). *Allacma fusca*, of the

Sminthuridae family, is one of the species investigated in this study possessing tracheae. As described by Eisenbeis and Wichard (1985) this species changes its respiratory system during aging. While the tracheal system is expanding, the skin structures are reduced.

Nevertheless, the cuticle, especially its surface features, plays an important role for respiration concerning all species. The specific properties of the collembolan cuticle are a result of comb-like structures first described by Hale and Smith (1966). These structures mainly consist of primary granules in a form of triangles which are connected by ridges-forming hexagons (Fig. 1). Varieties of these structures include rhombic patterns, secondary granules (Lawrence and Massoud 1973) and also irregular patterns. These complex structures afford a non-wetting character of the cuticle, which is important for the respiration in temporarily rain-flooded habitats by keeping air in the cavities of the structures (Helbig et al. 2011; Noble-Nesbitt 1963a).

More studies on cuticular structures of Collembola are provided by early publications of Noble-Nesbitt (1963b); Krzysztofowicz et al. (1972); Dallai (1974); Ghiradella and Radigan (1974); Eisenbeis (1976) and Rosciszewska (1985). These studies partly also present SEM (scanning electron microscopy) images of Collembola cuticle structures.

Water-repellent, antimicrobial and self-cleaning surfaces are key targets of current research on biofunctional materials. However, very little is known about the diversity

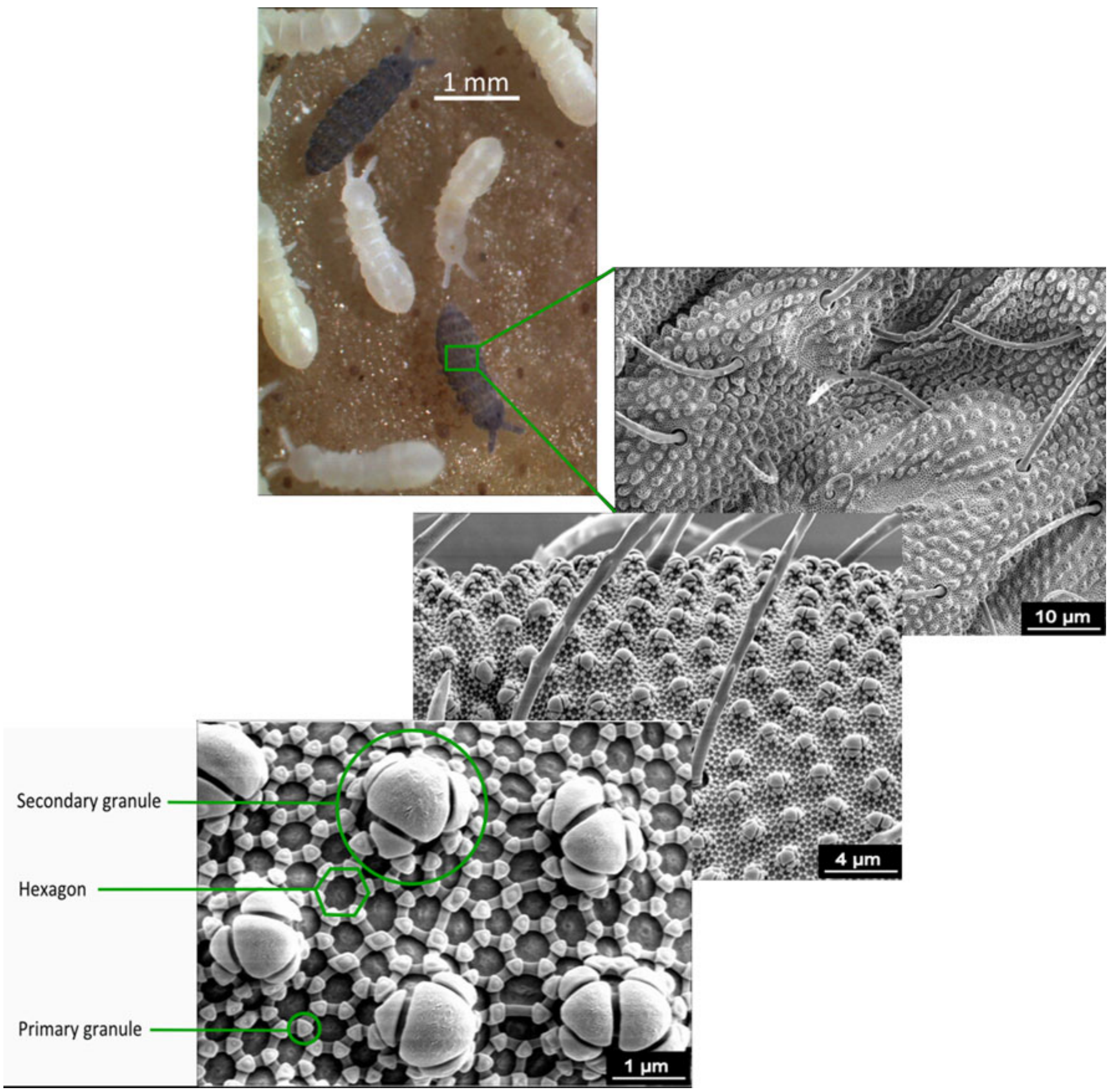

Fig. 1 Collembolan cuticles are composed of unique nanoscaled structures forming a protective, robust and antiadhesive surface. The basic comb structure is formed by primary granules which are connected by ridges-forming *hexagons*. This basic comb structure can also be of *rhombic shape* formed by primary granules which are quadrangles. Some species possess secondary granules as an additional hierarchical layer. Hairs are present in almost all species. (Species: *C. denticulata* (pigmented), *O. stachianus* (non-pigmented))

of cuticle structures of Collembola and the correlations between structure and properties of the cuticle.

To contribute to a better knowledge of the collembolan cuticle patterns, we analyzed the cuticle of 40 different species with SEM giving results of which revealed a high diversity of cuticle structures. Beyond that, the presented study aims at comparing the different cuticle structures of Collembola with their life forms to unravel correlations between cuticle patterns and the preferential habitat.

Materials and methods

Animals

The cuticle structures of 40 different species were analyzed in our studies (Table 1). Most species were obtained from the collection of the Senckenberg Museum of Natural History Görlitz, Germany (preserved in 70 % Ethanol). Three of the examined species (*Orthonychiurus stachianus*, *Ceratophysella denticulata*, *Sinella tenebricosa*) were cultivated to obtain fresh cuticles. These species were collected from the tropical greenhouse at the Dresden Botanical Garden. For cultivation, petri dishes with a substrate of gypsum (Goto 1960) and chromite powder (20:1) were used. Black chromite powder allows easy observation of non-pigmented species. The substrate was regularly wetted to maintain humid conditions in the dishes, which were kept at 21 °C. The cultivated species were fed with yeast.

A total of 16 different families of all four collembolan orders [Entomobryomorpha, Poduromorpha, Symphypleona, Neelipleona (Bellinger et al. 1996–2012)] were examined to cover a wide range of species and habitats.

Information about the species habitat were taken from literature (Bretfeld 1999; Dunger 1961; Zimdars and Dunger 1994; Fjellberg 1998, 2007; Pomorski 1998; Potapov 2001; Thibaud et al. 2004).The Senckenberg Museum of Natural History Görlitz provided most information on the life forms of Collembola based on data from the museums Collembola and literature collection. All observed species in the current study were collected by scientists of this institute. Therefore, a corresponding life form to each species could be provided. However, a detailed and comprehensive literature about the vertical distribution of collembolan species does not exist yet.

Scanning electron microscopy and sample preparation

The investigations of the cuticle structures were carried out using a high-resolution scanning electron microscope (Zeiss, Supra 40VP).

Due to chemical treatments for fixation, major parts of the surfaces were covered with a layer or particles, most probably sugars and proteins which were washed out by the fixative and adhered on the cuticle. The fixation and conservation agent used was modified Törne mixture containing 50 % isopropanol with 3 % glacial acetic acid and 0.3 % formalin (Dunger and Fiedler 1997; Törne 1965). To ensure that cuticle microstructures were not significantly affected, chemical-treated samples were compared with fresh samples of the mentioned species above (*O. stachianus*, *C. denticulata*, *S. tenebricosa*). The comparison gave evidence that the structures of treated species were not affected in their shape. The best and simplest method to ensure removal of particles from the cuticle was an ultrasonic treatment (Badalin Sonorex SUPER RK 100). Specimens were placed in 0.5 ml Eppendorf tubes filled with 70 % ethanol and treated for 5–10 min. Subsequently, the specimens were air-dried and placed on aluminum stubs covered with carbon tabs. Prior to examination in the SEM, the examples were coated with a 15-nm gold layer (Emitech K 550 Sputter coater).

Air-drying of ethanol-conserved specimens usually leads to shrinking and deformation effects of structures, especially in arthropods that have a less sclerotized cuticle. To compare the impact of these effects, samples previously were dried with the critical point method. Air- and critical point-dried samples led to same results. Accordingly, all species were air-dried before examination.

Results

Cuticle patterns of different collembolan orders

The scanning electron microscopy studies revealed that a hierarchical structure of nanoscopic interconnected granules (primary granules), which form a basic hexagonal or rhombic comb-like pattern, are characteristic for the cuticle of Collembola species. 22 of the 40 investigated species were found to possess microscopic, papillose granules (secondary granules) which form an additional hierarchical layer (Fig. 1).

The results of the examination of the cuticular structures are summarized in Table 1. Characteristic patterns for species of the orders Entomobryomorpha and Poduromorpha could be identified.

Most species of Entomobryomorpha are epedaphic living on the soil surface. They do not show any secondary granules (Fig. 2a–a″). The surface structures consist of hexagonal or rhombic comb-like patterns. According to our results, the rhombic patterns are typical for species of the Isotomidae family (Fig. 2b–b″) belonging to Entomobryomorpha. These species are also hemiedaphic.

Table 1 The investigated species incl. family and order, their ecology and main cuticle structures are combined

Order	Family	Species	Ecology	Reference	Main comb structure	Secondary granules
Entomobryomorpha	Entomobryidae	*Entomobrya corticalis* (Nicolet, 1842)	Epedaphic	Fjellberg (2007)	Hexagonal	No
	Entomobryidae	*Entomobrya muscorum* (Nicolet, 1842)	Epedaphic	Fjellberg (2007)	Hexagonal	No
	Entomobryidae	*S. tenebricosa* (Folsom, 1902)	Epedaphic	Fjellberg (2007)	Hexagonal	No
	Entomobryidae	*Lepidocyrtus paradoxus* (Uzel, 1890)	Epedaphic	Fjellberg (2007)	Hexagonal	No
	Entomobryidae	*Orchesella flavescens* (Bourlet, 1839)	Epedaphic	Fjellberg (2007)	Hexagonal	No
	Entomobryidae	*Seira domestica* (Nicolet, 1842)	Epedaphic	No literature	Hexagonal	No
	Tomoceridae	*Pogonognathellus flavescens* (Tullberg, 1871)	Epedaphic	Fjellberg (2007)	Hexagonal	No
	Tomoceridae	*Tomocerus minor* (Lubbock, 1862)	Epedaphic	Fjellberg (2007)	Hexagonal	No
	Oncopoduridae	*Oncopodura crassicornis* (Shoebotham, 1911)	Epedaphic	Fjellberg (2007)	Hexagonal	No
	Isotomidae	*Hydroisotoma schaefferi* (Krausbauer, 1898)	Hemiedaphic	Potapov (2001)	Hexagonal	No
	Isotomidae	*Desoria violacea* (Tullberg, 1876)	Hemiedaphic	Potapov (2001)	Rhombic	No
	Isotomidae	*Folsomia candida* (Willem, 1902)	Hemiedaphic	Potapov (2001)	Rhombic	No
	Isotomidae	*Folsomia quadrioculata* (Tullberg, 1871)	Hemiedaphic	Potapov (2001)	Rhombic	No
	Isotomidae	*Anurophorus coiffaiti* (Cassagnau & Delamare, 1955)	Hemiedaphic	Potapov (2001)	Rhombic	No
	Isotomidae	*Isotoma viridis* (Bourlet, 1839)	Hemiedaphic	Potapov (2001)	Rhombic	No
Poduromorpha	Onychiuridae	*Supraphorura furcifera* (Börner, 1901)	Euedaphic	Pomorski (1998)	Hexagonal/ rhombic	Yes
	Onychiuridae	*O. stachianus* (Bagnall, 1939)	Euedaphic	Pomorski (1998)	Hexagonal/ rhombic	Yes
	Onychiuridae	*Kalaphorura heterodoxa* (Gisin, 1964)	Hemiedaphic	No literature	Irregular	Yes
	Onychiuridae	*Kalaphorura paradoxa* (Schäffer, 1900)	Hemiedaphic	Pomorski (1998)	Irregular	Yes
	Onychiuridae	*Tetrodontophora bielanensis* (Waga 1842)	Hemiedaphic	Dunger (1961)	Rhombic	Yes
	Tullbergiidae	*Stenaphorura quadrispina* (Börner, 1901)	Euedaphic	Thibaud et al. (2004)	Hexagonal	Yes
	Tullbergiidae	*Mesaphorura macrochaeta* (Rusek, 1976)	Euedaphic	Thibaud et al. (2004)	Hexagonal	Yes
	Tullbergiidae	*Tullbergia callipygos* (Börner, 1902)	Euedaphic	Thibaud et al. (2004)	Hexagonal	Yes
	Neanuridae	*Neanura muscorum* (Templeton, 1836)	Hemiedaphic	Fjellberg (1998)	Hexagonal	Yes
	Neanuridae	*Deutonura conjuncta* (Stach, 1926)	Hemiedaphic	No literature	Hexagonal	Yes
	Neanuridae	*Friesa mirabilis* (Tullberg, 1871)	Hemiedaphic	Fjellberg (1998)	Hexagonal	Yes
	Hypogastruridae	*C. denticulata* (Bagnall, 1941)	Hemiedaphic	Thibaud et al. (2004)	Hexagonal	Yes
	Hypogasturidae	*Ceratophysella scotica* (Carpenter & Evans 1899)	Hemiedaphic	Thibaud et al. (2004)	Hexagonal	Yes
	Brachystomellidae	*Brachystomella parvula* (Schäffer, 1896)	Hemiedaphic	Fjellberg (1998)	Hexagonal	Yes
	Poduridae	*Podura aquatica* (Linneus, 1758)	Hemiedaphic	Fjellberg (1998)	Rhombic	Yes

Table 1 continued

Order	Family	Species	Ecology	Reference	Main comb structure	Secondary granules
Symphypleona	Bourletiellidae	*Borletiella hortensis* (Fritch, 1863)	Epedaphic	Bretfeld (1999)	Hexagonal/ rhombic	No
	Bourletiellidae	*Heterosminthurus insigris* (Reuter, 1876)	Epedaphic	Bretfeld (1999)	Hexagonal	No
	Sminthuridae	*A. fusca* (Linnaeus, 1758)	Hemiedaphic	Bretfeld (1999)	Irregular	No
	Sminthuridae	*L. lubbocki* (Tullberg, 1872)	Hemiedaphic	Bretfeld (1999)	Irregular	Yes
	Dicyrtomidae	*Dicyrtomina ornata* (Nicolet, 1842)	Hemiedaphic	Bretfeld (1999)	Hexagonal/ rhombic	Yes
	Dicyrtomidae	*Dicyrtoma fusca* (Lubbock, 1873)	Hemiedaphic	Bretfeld (1999)	Hexagonal/ rhombic	Yes
	Arrhopalitidae	*Arrhopalites pygmaeus* (Wantzel, 1860)	Hemiedaphic	Bretfeld (1999)	Hexagonal/ rhombic	Yes
	Arrhopalitidae	*Arrhopalites caecus* (Tullberg, 1871)	Hemiedaphic	Bretfeld (1999)	Hexagonal/ rhombic	Yes
	Katiannidae	*Sminthurinus aureus* (Lubbock, 1962)	Hemiedaphic	Bretfeld (1999)	Hexagonal/ rhombic	Yes
Neelipleona	Neelidae	*Megalothorax minimus* (Willem, 1900)	Euedaphic	Bretfeld (1999)	Hexagonal	Yes

The ecology of Collembola comprises three life forms: epedaphic (living on top of the soil), euedaphic (living in the soil) and hemiedaphic (living in the coarse structure of the soil). The main patterns of comb-like structures are hexagonal, rhombic or irregular. Some species show a mixture of hexagonal and rhombic structures. About half of the examined species possess secondary granules

In contrast to Entomobryomorpha including Isotomidae, the Poduromorpha show secondary granules (Fig. 2c–c″). Some of these species permanently live in the soil, thus being euedaphic.

Some species of Poduromorpha live only in the coarse structure of the soil, but still show secondary granules.

The only investigated species of the order Neelipleona shows patterns similar to Poduromorpha, which consist of secondary granules with hexagonal basic patterns (Table 1). This species lives also euedaphically.

The investigated examples of the order Symphypleona do not show just one typical surface structure, but exhibit various different cuticle patterns, partly with secondary granules (Table 1; Fig. 2d–d″).

Allacma fusca possessing an additional tracheal respiratory system shows an irregular cuticle pattern (Table 1). *Lipothrix lubbocki* belonging to the same family (Sminthuridae) also shows irregularities concerning the surface structures (Table 1; Fig. 6d).

Diversity of cuticle structures

An overview of the high diversity of collembolan cuticle structures is provided by Figs. 3, 4, 5 and 6. Each figure provides examples of different species belonging to the presented order or family.

The hexagonal patterns of the investigated Entomobryomorpha depicted in Fig. 3 are similar in size and shape and do not show great variability.

The investigated Isotomidae possess a high diversity in their rhombic structures (Fig. 4). As shown by the micrographs, the shapes of the primary granules can differ in size and form. Some patterns appear irregular.

In Poduromorpha, size and shape of the secondary granules vary widely among species (Fig. 5). The basic patterns are hexagonal or rhombic and may also be irregular (Fig. 5a, e).

Figure 6 provides examples of cuticle structures of Symphypleona, which are also highly diverse. Basic patterns are rhombic with aberrant primary granules, hexagonal or irregular. The partly existing secondary granules are of different size and shape.

Size proportions of cuticle structures between species

The structures of each examined species were measured and data are summarized in Table 2. The measurements were done using the free java image processing software ImageJ 1.43u. The table presents an overview about species size, diameter of the comb-like rhombic or hexagonal pattern, side length of primary granules, distance between adjacent secondary granules and their height.

The patterns of Entomobryomorpha partly vary in size giving hexagonal diameters of 700–1,200 nm. The primary granules range from 160 to 320 nm which is similar to the primary granules size of Poduromorpha and Symphypleona varying from 160 to 300 nm. Comb diameters of Poduromorpha with 470–840 nm are smaller in size compared to

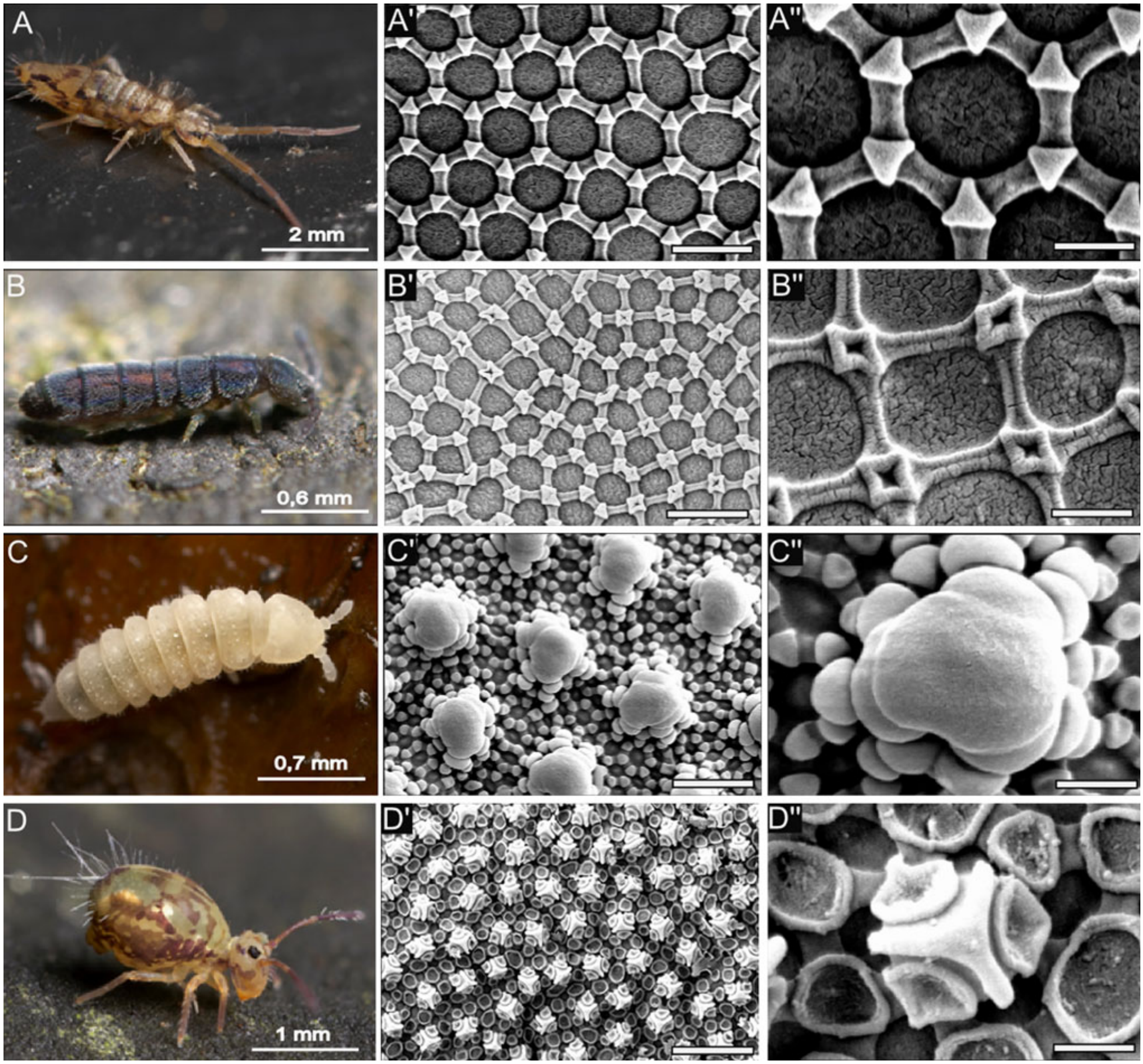

Fig. 2 Cuticle patterns of different life forms and different orders of Collembola. Habitus, respective morphological characteristics and cuticle patterns of representatives of different life forms of Collembola are given. Habitus images were taken from http://www.collembola.org with kind permission of Brian Valentine. **a**–**a**″ Habitus and *hexagonal* structures of epedaphic Entomobryomorpha species. **a** *Entomobrya intermedia*, **a**′, **a**″ *P. flavescens.* **b**–**b**″ Habitus and rhombic structures of hemiedaphic Isotomidae (Entomobryomorpha). **b** *Vertagopus arboreus*, **b**′, **b**″ *I. viridis.* **c**–**c**″ Habitus and secondary granules with basic hexagonal structures of euedaphic Poduromorpha. **c** *Kalaphorura burmeisteri*, **c**′, **c**″ *S. quadrispina.* **d**–**d**″ Habitus and cuticle of hemiedaphic Symphypleona with secondary granules and aberrant basic patterns. **d** *D. ornata*, **d**′, **d**″ *A. pygmaeus*. *Scale bars* **a**′–**d**′ = 2 μm, **a**″–**d**″ = 500 nm

Entomobryomorpha. Species of Symphypleona show diameters of 470–1,200 nm which presents a high variability.

Secondary granule distances are of 1,300–6,800 nm, the secondary granule height ranges from 430 to 5,000 nm.

The structure sizes are mostly correlated with the variation of species size. Smaller species present smaller structures and vice versa.

Discussion

Diversity of collembolan cuticle structures

In this study, we explored the differences of cuticle patterns among Collembola demonstrating a high diversity of structures between the species. The differences of cuticle patterns featuring the Collembola were already mentioned

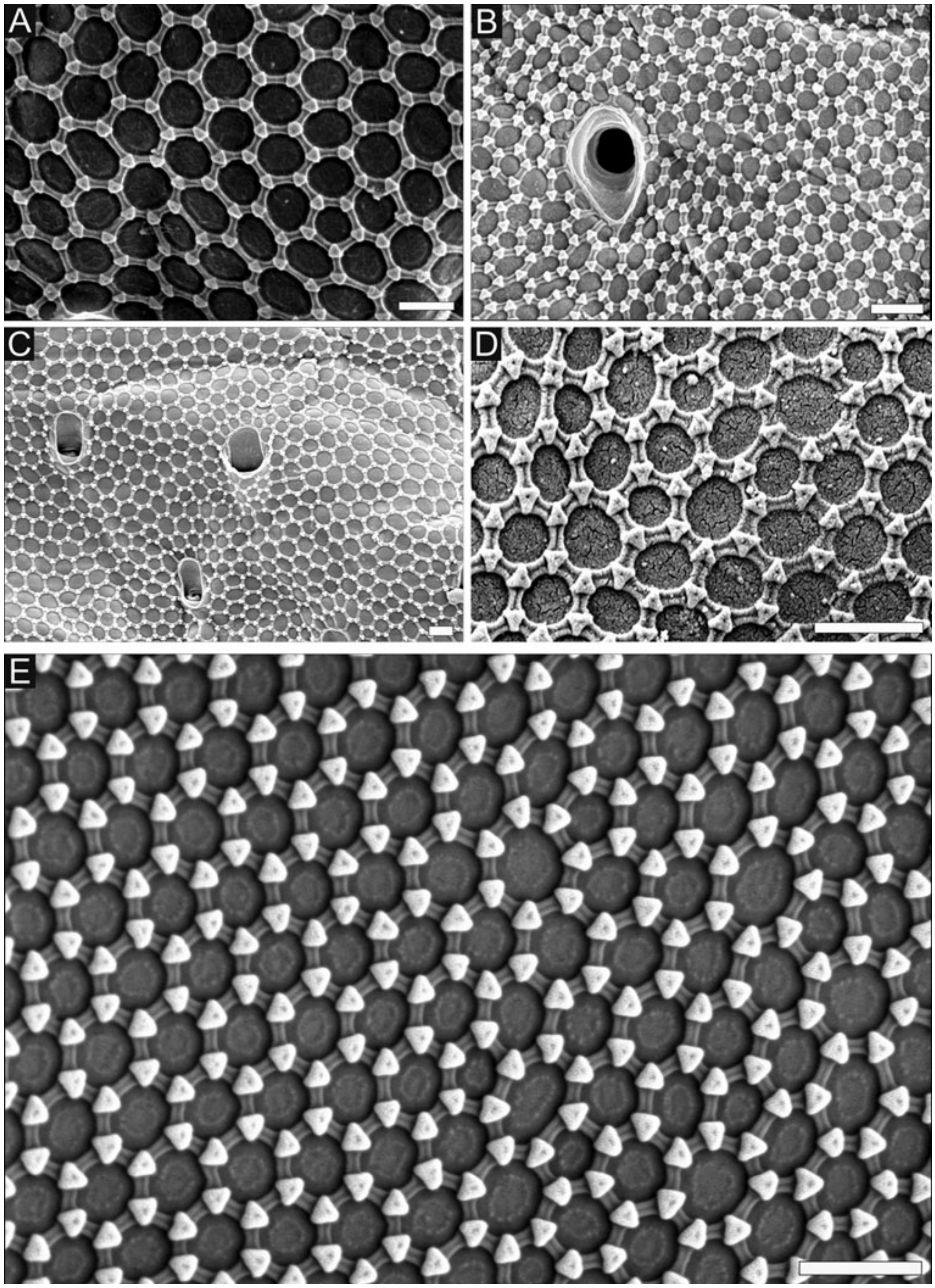

Fig. 3 Cuticle structures of different Entomobryomorpha species. All species show *hexagonal* structures lacking secondary granules (see Fig. 1). The patterns are of different size depending on the species. Images **b** and **c** show openings which represent setal sockets. **a** *E. corticalis*, **b** *E. muscorum*, **c** *L. paradoxus*, **d** *O. flavescens*, **e** *S. tenebricosa*. *Scale bars* = 1 μm

for some species by Lawrence and Massoud (1973) and Lawrence (1975).

The obtained results show that there are similarities regarding the pattern structures within the different orders and families. Based on the high diversity of cuticle structures, groups representing patterns of similar characteristics such as rhombic and hexagonal patterns with and without secondary granules were formed (Table 1).

Taking these groups into account, the cuticular patterns can be attributed to taxonomical groups that will be discussed in detail in the following section to explore the correlation between the cuticular surface features and the habitat of certain Collembola species.

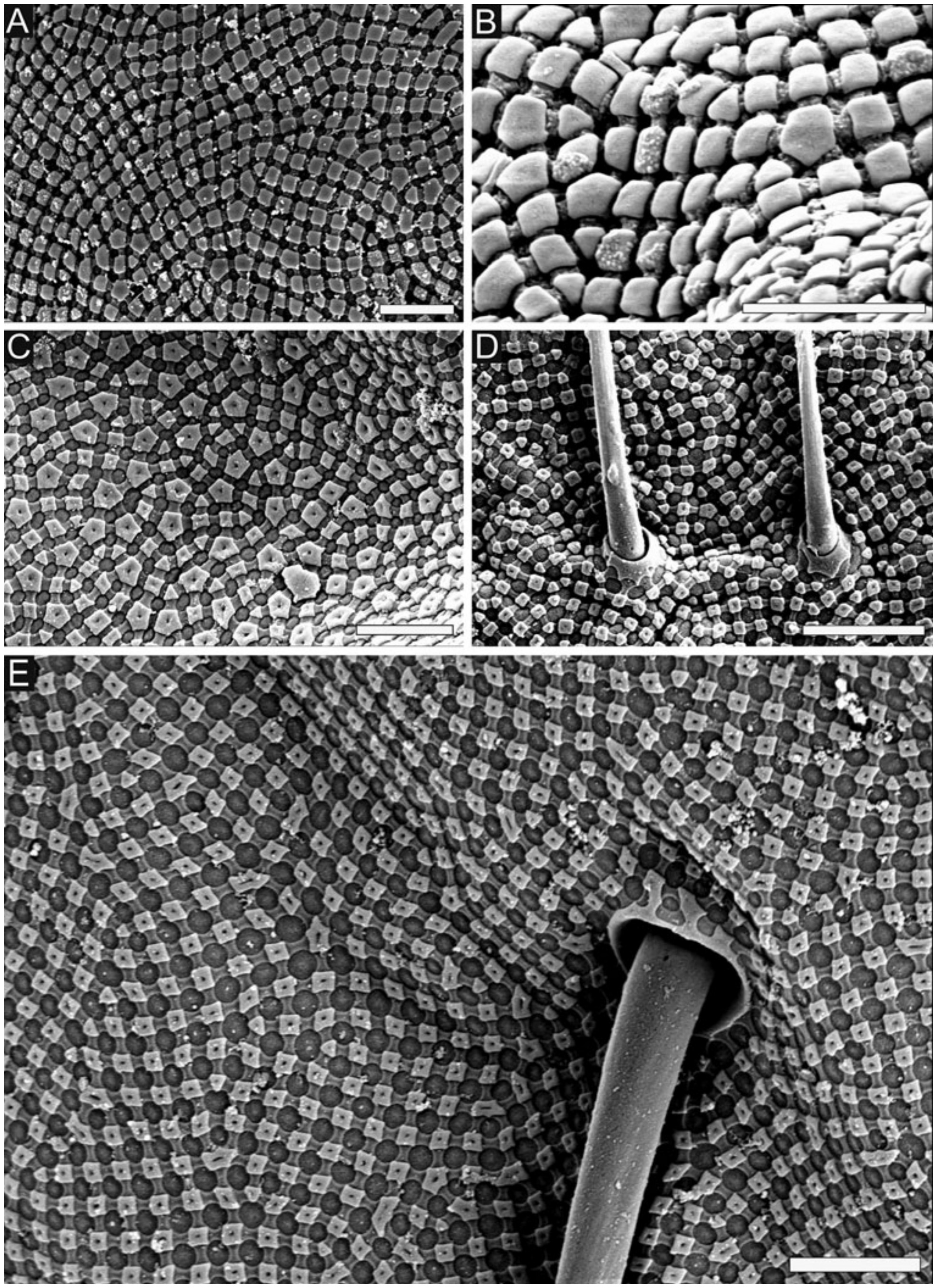

Fig. 4 Cuticle patterns of Isotomidae (Entomobryomorpha) with *typical rhombic* structures that are diverse with respect to the shape of primary granules. Secondary granules are absent (see Fig. 1). **a**, **b** *A. coiffaiti*, **c**, **d** *F. quadrioculata*, **e** *D. violacea*. *Scale bars* = 2 μm

Taxonomical aspects concerning cuticle patterns

In our studies, we found that secondary granules are a typical cuticle structure for the order Poduromorpha. In contrast, the order Entomobryomorpha does not show secondary granules. The order Symphypleona exhibiting great variability of cuticle patterns does not show a characteristic structure. Here species partly show secondary granules.

Concerning primary granules hexagonal or rhombic comb-like patterns are found in all orders.

The Isotomidae displaying rhombic patterns without secondary granules presents a typical structure for this family within the Entomobryomorpha.

Systematic significance of the cuticle patterns in Collembola were already considered by Lawrence and Massoud (1973) and Dallai (1977). These authors described characteristic cuticle patterns for different collembolan

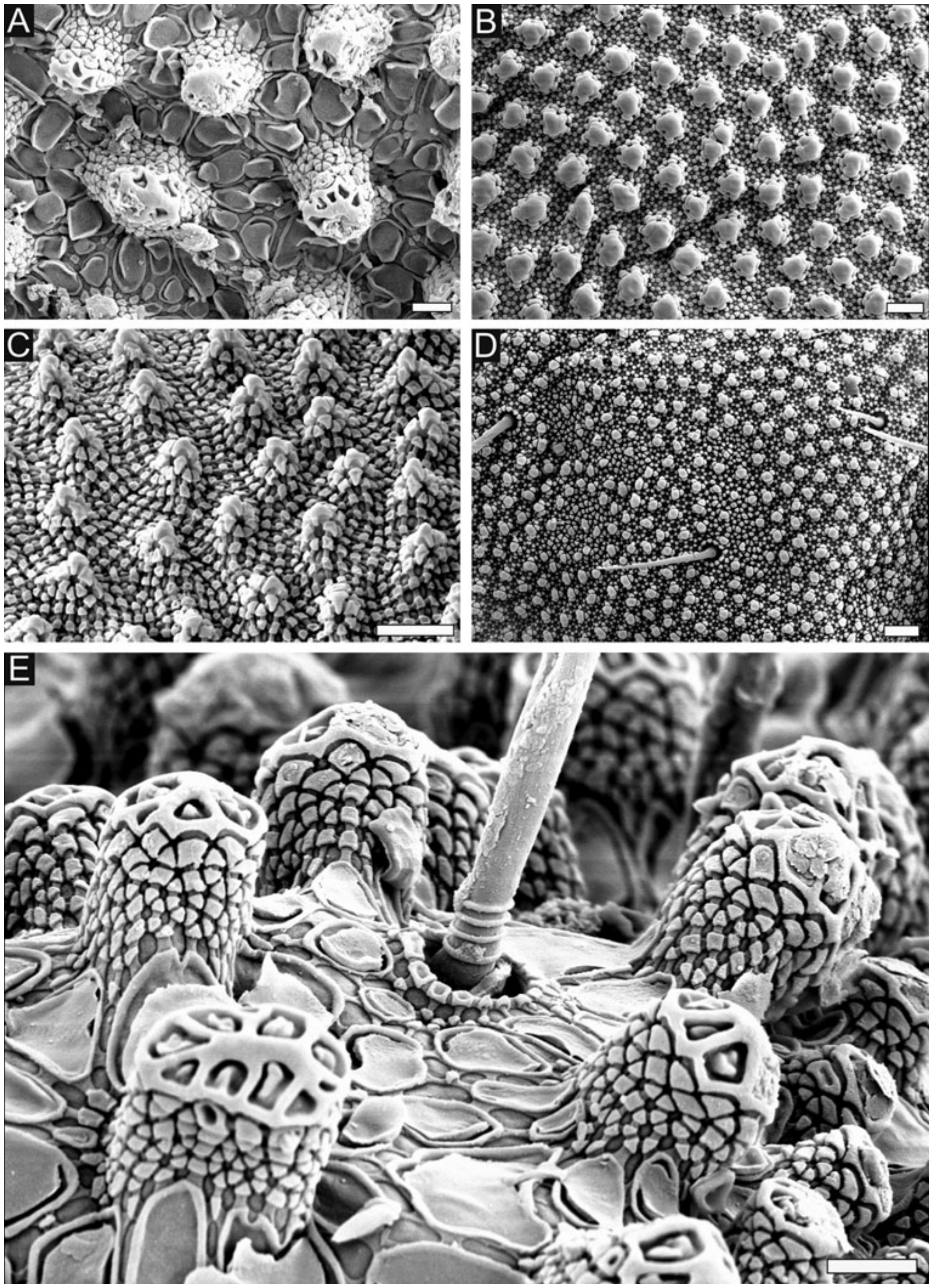

Fig. 5 Cuticle structures of Poduromorpha species all showing secondary granules of different shape and size. The basic cuticle structures are *hexagonal, rhombic or irregular* (see Fig. 1). **a** *K. heterodoxa*, **b** *S. quadrispina*, **c** *P. aquatica*, **d** *M. macrochaeta*, **e** *K. paradoxa.* Species of the Genus *Kalaphorura* (Images **a** and **e**) show partly alternative cuticle structures. Secondary granules are columnar shaped. Primary granules are only visible on the secondary columnar-like granules. Other parts of the cuticle surface show *circular structures*. *Scale bars* = 2 µm

families and genera concerning Entomobryomorpha and Symphypleona.

The above described taxonomical aspects are based on morphological characteristics not concerning a phylogeny which could proof this classification. Currently a broad scale phylogeny of Collembola, which could give a deeper insight into species relationships, is not available. Most investigations on Collembola systematics are based on morphological characters, while phylogenetic studies within Collembola based on molecular data are still in the beginning (D'Haese 2002; Deharveng 2004; Xiong et al. 2008).

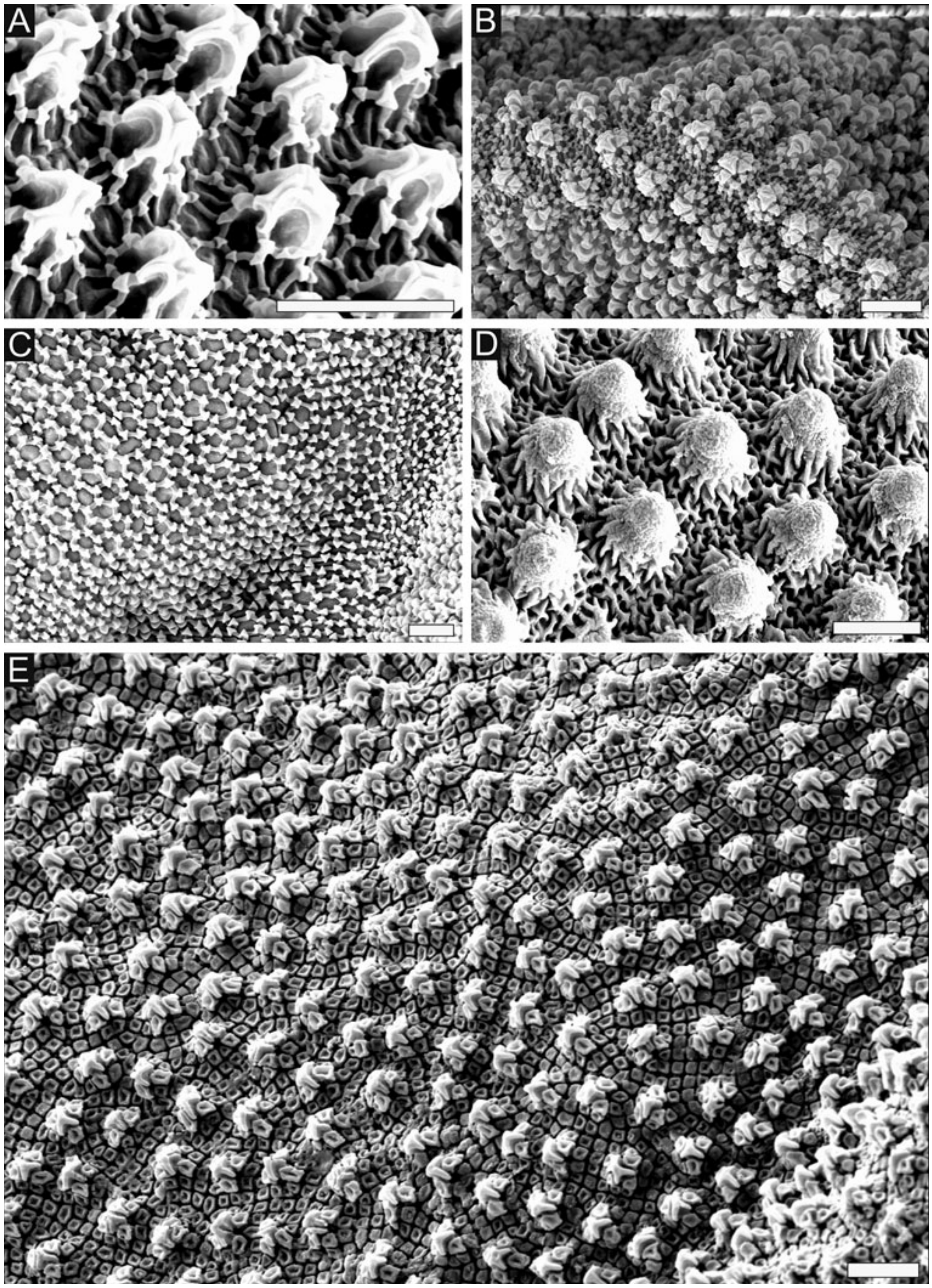

Fig. 6 Different cuticle patterns of Symphypleona species that partly show secondary granules of different shape. Basic patterns are *hexagonal, rhombic* with aberrant primary granules or irregular (see Fig. 1). **a** *D. ornata*, **b** *D. fusca*, **c** *S. aureus*, **d** *L. lubbocki*, **e** *B. hortensis*. *Scale bars* = 2 µm

Correlation between cuticle structures and ecology

Lawrence and Massoud (1973) and Dallai (1977) had already proposed such a correlation based on an adaptation of the springtails to various habitats. However, the authors did not specify their assumptions nor referred to certain particular cuticle patterns and their ecological function.

Therefore, we tried to clarify this specific aspect, which is decisive for a future application of collembolan cuticle structures in materials science and a translation into functional surfaces.

Secondary granules can be interpreted as a protection against abrasion. Soil particles mainly scratch the tips of the secondary granules protecting the basic structures

Table 2 All examined species including their cuticle structures were measured

Family	Species	Body size (mm)	Comb diameter (nm)	Side length of primary granules (nm)	Secondary granule distance (nm)	Secondary granule height (nm)
Entomobryidae	*E. corticalis* (Nicolet, 1842)	1.4	1,200	210		
Entomobryidae	*E. muscorum* (Nicolet, 1842)	2.8	670	180		
Entomobryidae	*L. paradoxus* (Uzel, 1890)	2.0	1,200	200		
Entomobryidae	*O. flavescens* (Bourlet, 1839)	2.5	790	190		
Entomobryidae	*S. domestica* (Nicolet, 1842)	2.5	1,600	330		
Entomobryidae	*S. tenebricosa* (Folsom, 1902)	1.1	720	190		
Tomoceridae	*P. flavescens* (Tullberg, 1871)	4.6	1,000	220		
Tomoceridae	*T. minor* (Lubbock, 1862)	1.2	870	180		
Oncopoduridae	*O. crassicornis* (Shoebotham, 1911)	0.3	620	160		
Isotomidae	*H. schaefferi* (Krausbauer, 1898)	1.5	620	170		
Isotomidae	*D. violacea* (Tullberg, 1876)	1.3	570	210		
Isotomidae	*F. candida* (Willem, 1902)	1.1	440	190		
Isotomidae	*F. quadrioculata* (Tullberg, 1871)	0.9	570	180		
Isotomidae	*A. coiffaiti* (Cassagnau & Delamare, 1955)	0.7	700	320		
Isotomidae	*I. viridis* (Bourlet, 1839)	2.7	870	330		
Onychiuridae	*S. furcifera* (Börner, 1901)	0.9	680	200	2,900	890
Onychiuridae	*O. stachianus* (Bagnall, 1939)	1.0	670	190	4,700	1,800
Onychiuridae	*K. heterodoxa* (Gisin, 1964)	1.5	580	270	7,500	5,000
Onychiuridae	*K. paradoxa* (Schäffer, 1900)	1.2	720	270	6,800	4,800
Onychiuridae	*T. bielanensis* (Waga 1842)	4.0	610	300	5,100	3,800
Tullbergiidae	*S. quadrispina* (Börner, 1901)	0.7	590	200	2,800	990
Tullbergiidae	*M. macrochaeta* (Rusek, 1976)	0.3	600	180	1,400	570
Tullbergiidae	*T. callipygos* (Börner, 1902)	0.7	830	240	3,100	710
Neanuridae	*N. muscorum* (Templeton, 1836)	1.1	780	270	6,400	4,500
Neanuridae	*D. conjuncta* (Stach, 1926)	1.5	840	300	6,300	5,800
Neanuridae	*F. mirabilis* (Tullberg, 1871)	0.3	650	230	2,200	1,500
Hypogastruridae	*C. denticulata* (Bagnall, 1941)	0.9	810	260	4,300	1,200
Hypogasturidae	*C. scotica* (Carpenter & Evans 1899)	0.8	760	280	3,800	3,000
Brachystomellidae	*B. parvula* (Schäffer, 1896)	0.4	620	200	2,200	1,800
Poduridae	*P. aquatica* (Linneus, 1758)	0.7	700	220	2,200	1,400
Bourletiellidae	*B. hortensis* (Fritch, 1863)	0.7	1,200	350		
Bourletiellidae	*H. insigris* (Reuter, 1876)	0.4	990	220		
Sminthuridae	*A. fusca* (Linnaeus, 1758)	2.1				
Sminthuridae	*L. lubbocki* (Tullberg, 1872)	0.6	730	290	2,700	1,500
Dicyrtomidae	*D. ornata* (Nicolet, 1842)	1.4	1,000	260	1,500	1,200
Dicyrtomidae	*D. fusca* (Lubbock, 1873)	1.6	970	400	1,800	890
Arrhopalitidae	*A. pygmaeus* (Wantzel, 1860)	0.5	620	190	1,500	540
Arrhopalitidae	*A. caecus* (Tullberg, 1871)	0.4	470	160	1,300	430
Katiannidae	*S. aureus* (Lubbock, 1962)	0.3	450	240	1,600	550
Neelidae	*M. minimus* (Willem, 1900)	0.2	380	160	950	300

This table presents an overview about body size, comb diameter, side length of primary granules, distance and height of secondary granules to each species

which are important for the cutaneous respiration. In contrast, species living on the surface (epedaphic) do not show secondary granules. They are characterized by the basic hexagonal pattern only.

The hemiedaphic species, which live in the coarse structure of the soil, are a transitional group covering the largest number of species. Some of the hemiedaphic Collembola show secondary granules, mainly species of the orders Poduromorpha and Symphypleona. As far as secondary granules can be seen as an adaptation to living in soil, these species likely migrate into the soil compared to hemiedaphic species without secondary granules. The family Isotomidae possesses rhombic patterns taking an exceptional position in the order Entomobryomorpha. Their cuticle patterns without secondary granules imply that they are unlikely migrating deeply into the soil and for longer time periods as they may not be adapted to this particular habitat.

Secondary granules might support the forming of plastrons. As observed by Colmer and Pedersen (2008) on submerged plant leaves, plastrons improve the exchange of O_2 and CO_2 and enable a continued gas exchange, which is important for the underwater respiration. In this respect, plastrons of submerged plants are analogous to plastrons surrounding Collembola in the humid soil. Investigations on plastron forming by water insects were done by Thorpe and Crisp (1947) showing that a high density of hairs supports the formation of a plastron. Concerning the low density of hairy structures of euedaphic Collembola (Eisenbeis and Wichard 1985), it is reasonable to assume that the papillose secondary granules in combination with the patterns of primary granules are important for the forming of plastrons in the soil. Regarding Collembola, water-repellency is important for species living in temporarily rain-flooded environments.

In summation, we can assume that the different types of cuticle structures correlate with their life form. Species living euedaphically possess secondary granules most probably as an adaptation to the life in soil.

Based on the current data on life forms available for individual species and the cuticle patterns of the different Collembola orders, which we identified, it is possible to draw conclusion on correlations between cuticle structure and habitat.

Our results prove that different types of cuticle patterns identified within Collembola occur in certain habitats.

It will be the task of future studies to answer the question whether a certain structure evolved in a group of springtails allowed for capturing a new habitat or whether the habitat conditions were the driving force for adaptation in cuticle structure.

The interest and focus of this study was dedicated to the structural variations of collembolan skin and their functions so far providing first indications for answering this question.

Conclusion

Collembola show a wide diversity of cuticle patterns, which are different in size and shape.

Specific cuticle structures can be used to support current hypotheses of taxonomical issues. The orders Poduromorpha and Entomobryomorpha are distinguishable by their cuticle patterns as well as the family Isotomidae whose species show a unique structure.

Furthermore, Collembola are potentially adapted to their habitat due to specific cuticle structures.

The adaptation is necessary to ensure the cutaneous respiration upon flooding. Euedaphic Collembola may have evolved secondary granules to provide a protection against abrasion in soil. In addition, they possibly support the plastron forming when becoming submerged. Species living epedaphically on the soil surface or in plant litter only show comb-like patterns without secondary granules. A large group of species lives hemiedaphically, which is an intermediate life form displaying cuticle structures only partly containing secondary granules.

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