Sensation of the tiniest kind: the antennal sensilla of the smallest free-living insect *Scydosella musawasensis* (Coleoptera: Ptiliidae)

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ABSTRACT

Miniaturization is a major evolutionary trend prominent in insects, which has resulted in the existence of insects comparable in size to some unicellular protists. The adaptation of the complex antennal multisensory systems to extreme miniaturization is a fascinating problem, which remains almost unexplored. We studied the antennal sensilla of *Scydosella musawasensis Hall, 1999* (Coleoptera: Ptiliidae), the smallest free-living insect, using scanning electron microscopy. The antenna of *S. musawasensis* bears 131 sensilla; no intraspecific variation in the number or position of the sensilla has been revealed. Nine different morphological types of sensilla are described according to their external morphological features and distribution: four types of sensilla trichodea, one type of sensilla chaetica, two types of sensilla styloconica, and two types of sensilla basiconica. Morphometric analysis of the sensilla of *S. musawasensis*, based on measurements of the lengths and diameters of sensilla and their location and number, showed the absence of significant differences between females and males. Comparative allometric analysis of *S. musawasensis* and larger Coleoptera showed that the number of sensilla and the size of sensilla chaetica decrease with decreasing body size. However, the number of the types of sensilla and the length and diameter of the multiporous sensilla basiconica revealed no correlation with the body size. Comparison of the acquired data with the results of our earlier study of the antennal sensilla of some of the smallest parasitic wasps is used to put forward hypotheses on the common principles of miniaturization of the antennal sensory systems of insects.

INTRODUCTION

Antennae are present in a vast majority of Hexapoda (*Schneider, 1964*). The antennae are complex multimodal organs involved in such processes as the search for a sexual partner, a host, or food, or as intraspecific communication, enemy detection, orientation and navigation during flight, and many others (*Altner, Sass & Altner, 1977*; *Guerenstein et al., 2004*; *Kamikouchi et al., 2009*). The antennal sensory system consists of many components and has an extremely complex organization (*Gao, Yuan & Chess, 2000*; *Xie et al., 2016*). The number of antennal sensilla in insects can reach 100,000, and the number of their types can reach 17 (*Shields & Hildebrand, 2001*; *Di Giulio et al., 2012*). The study of the

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antennal sensilla of insects representative of various lineages and size classes started a long time ago, and the number and quality of such studies is growing with the advent and spread of new opportunities and techniques in microscopy (*Callahan, 1975*; *Hallberg, Hansson & Lofsted, 2003*). However, relatively few studies are devoted to the most speciose order of insects, Coleoptera. Most of those few studies use scanning electron microscopy and describe the external morphology of the antennal sensilla (*Skilbeck & Anderson, 1996*; *Merivee et al., 2002*). The results of such studies are often used for taxonomic purposes as diagnostic characters (*Pérez-González & Zaballos, 2013*). Even fewer studies analyze the internal structure and innervation of the sensilla (*Moeck, 1967*). Several studies deal with the involvement of the antennal sensory system in interspecies communication and behavioral responses in Coleoptera (*Lopes, Marques & Araújo, 2005*; *Ali et al., 2016*).

The evolutionary decrease in the body size, down to extreme miniaturization, is one of the principal directions of insect evolution and has recently been the subject of intense research (*Polilov, 2015a*; *Minelli & Fusco, 2019*). Body size largely determines the morphology, physiology, ecology, and behavior of insects (*Eberhard & Wcislo, 2011*; *Polilov, 2016*). Decrease in body size results in various morphological adaptations of the antennal sensory system. Thus, in Chalcidoidea, a correlation has been found between body size and the surface area of the antenna (*Symonds & Elgar, 2013*). A decrease in the number of antennomeres with a decrease in the body size has been shown in many of the smaller insects (*Polilov, 2015a*), down to only one antennomere in the male *Dicopomorpha echmepterygis* (*Mockford, 1997*) (Hymenoptera: Mymaridae). Allometric analysis has shown a decrease in the number of antennal sensilla and in the length of the mechanoreceptor sensilla with a decrease in the body length in parasitic wasps (*Diakova, Makarova & Polilov, 2018*). Studies on the intraspecific ranges of body sizes analyze the optimization of the antennal sensory system within the limits of intraspecific variation. A study on the olfactory system of the parasitic wasp *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) revealed that the number and length of the olfactory antennal sensilla and the number of glomeruli in the antennal lobes positively correlates with the body size and that the absolute and relative sizes of the glomeruli correlate with the body length (*Van der Woude & Smid, 2016*). It has been shown that in the housefly *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae), in which adult body size depends on the larval population density, the size of the antenna correlates with body size; moreover, although the antennae of larger and smaller flies bear the same types of sensilla, the antennae of larger houseflies bear nearly twice as many sensilla as those of smaller conspecifics (*Smallegange, Kelling & Otter, 2008*). A study on the size characteristics of worker ants of the species *Solenopsis invicta* Buren, 1972 (Hymenoptera: Formicidae) has shown that the length of the antennae relative to body length decreases with increasing body size (*Tschinkel, Mikheyev & Storz, 2003*).

The effects of extreme miniaturization on the antennal sensory system has barely been studied. We have analyzed earlier the antennal sensilla of three species of parasitic wasps of the genus *Megaphragma* Timberlake, 1924 (Hymenoptera: Trichogrammatidae), which have bodies only 0.2 mm long (*Diakova, Makarova & Polilov, 2018*). We found that due to miniaturization there is a significant decrease in the number of antennal sensilla: there are

fewer than 50 sensilla per antenna in the studied species. Number and position of sensilla are the same in individuals of the same species and sex, which indicates a high degree of optimization of the antennal sensory system.

The effects of extreme miniaturization on the antennal sensory system in free-living insects have never been studied to date. The smallest free-living insect is *Scydosella musawasensis* (*Hall, 1999*) (Coleoptera: Ptiliidae), the body length of which is only about 0.3 mm (*Polilov, 2015b*). Only the general morphology of the antennae has been described in this species. The aim of this study is to analyze the arrangement and the structure of the antennal sensilla in *S. musawasensis*.

MATERIALS & METHODS

Materials
We used adult specimens of *Scydosella musawasensis* (*Hall, 1999*) (Coleoptera: Ptiliidae) collected for earlier projects (*Polilov, 2015b*). A total of 15 females and 12 males were fixed in the alcoholic Bouin liquid for studying the general morphology or in glutaraldehyde for studying ultrastructure.

Scanning electron microscopy
The fixed material was gradually dehydrated through a series of ethyl alcohols and then acetone, critical point dried (Hitachi HCP-2), and sputtered with gold (Giko IB-3). The specimens were studied and photographed using Jeol JSM-6380 and FEI Inspect F50. The methods have been described in detail earlier (*Diakova, Makarova & Polilov, 2018*).

Morphometry
All measurements were performed on SEM images, using the measurement tool in the Fiji package of ImageJ. Shapiro–Wilk normality test, descriptive statistics, ANOVA, SMA, and OLS were performed using R software (*R Core Team, 2020*). The *sma* and *slope.test* functions of the *smatr* package in R were used to determine the presence of allometry.

Terminology
The nomenclature of the sensilla used in this study is described in Diakova et al. 2019; it is a system developed by us in accordance with earlier studies of the antennal sensilla of Staphylinoidea (*Skilbeck & Anderson, 1996*; *Oliva, 2012*).

RESULTS

General description of the antenna of *S. musawasensis*
The antenna of *S. musawasensis* consists of ten antennomeres: the scape, the pedicel, and eight flagellomeres, which include the two-segmented club (Fig. 1). No differences were found in the structure of the antennae or shape of the antennomeres between the males and females. The antennae are 74.03 ± 2.93 μm long in the males and 73.02 ± 3.59 μm long in the females (Table 1). The antenna of *S. musawasensis* bears 131 sensilla (Figs. 2 and 3A). No intraspecific variation was found in the number and location of the sensilla.
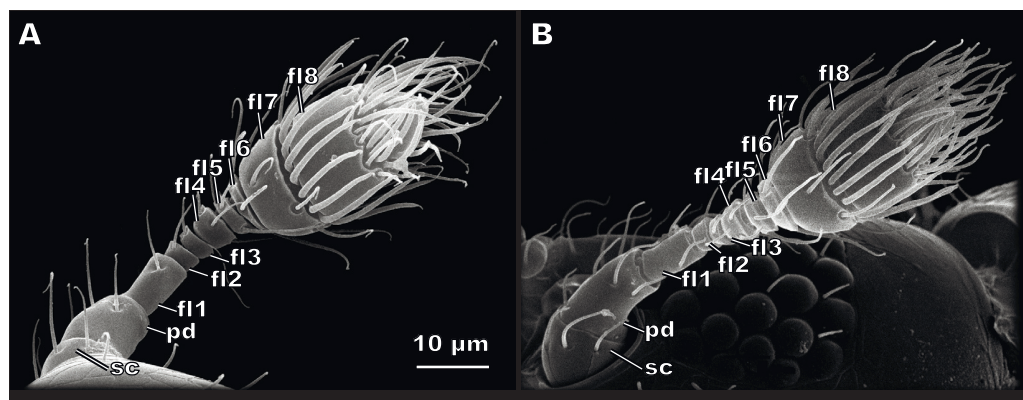
Figure 1 Female (A) and male (B) antennae of *Scydosella musawasensis.* sc, scape; pd, pedicel; fl1, flagellomere 1; fl2, flagellomere 2; fl3, flagellomere 3; fl4, flagellomere 4; fl5, flagellomere 5; fl6, flagellomere6; fl7, flagellomere 7; fl8, flagellomere 8.

Table 1 Length (L) and diameter (D) of antennal segments (mean ± sd) in *Scydosella musawasensis,* in μm.

	sc L	sc D	pd L	pd D	fl L	fl D	club L	club D
♂	9.21 ± 1.44	12.01 ± 0.93	14.32 ± 1.33	9.75 ± 0.67	50.49 ± 3.1	14.2 ± 1.27	27.7 ± 1.44	14.2 ± 1.27
♀	9.73 ± 1.23	12.21 ± 1.02	14.54 ± 1.45	8.75 ± 1.12	48.75 ± 2.81	15.26 ± 1.69	28.36 ± 2.02	15.25 ± 1.69

Notes.
sc, scape; pd, pedicel; fl, flagellum.
For additional details, see Table S1.

The morphological types of the antennal sensilla and their distribution

Nine morphofunctional types of sensilla were identified based on the details of their external morphology, shape, and location. Since one-way ANOVA revealed no significant differences in the size of the sensilla between the males and females (Table S1), the mean values of the lengths and diameters of the sensilla of both sexes is given, ± standard deviation. No differences were revealed between the sexes also in the number of sensilla or in the number of their types.

Sensillum trichodeum type 1 (TS1)

Very small aporous sensilla, each of which is located in a small depression (Fig. 4C). Four such sensilla are situated at the base of the pedicel (Fig. 2). This type of sensilla is the shortest, 0.63 ± 0.11 μm long. The diameter of TS1 is 0.44 ± 0.07 μm (Table 2; Table S1).

Sensillum trichodeum type 2 (TS2)

Elongated aporous sensilla located in small cuticular depressions (Fig. 3C). The most numerous type of sensilla: each antennomere bears from two (flagellomere 3) to 15 (segment 2 of the club) TS2 (Fig. 2). There are a total of 61 TS2 on the antenna. TS2 are 8.38 ± 1.98 μm long and 0.52 ± 0.1 μm in diameter (Table 2; Table S1).
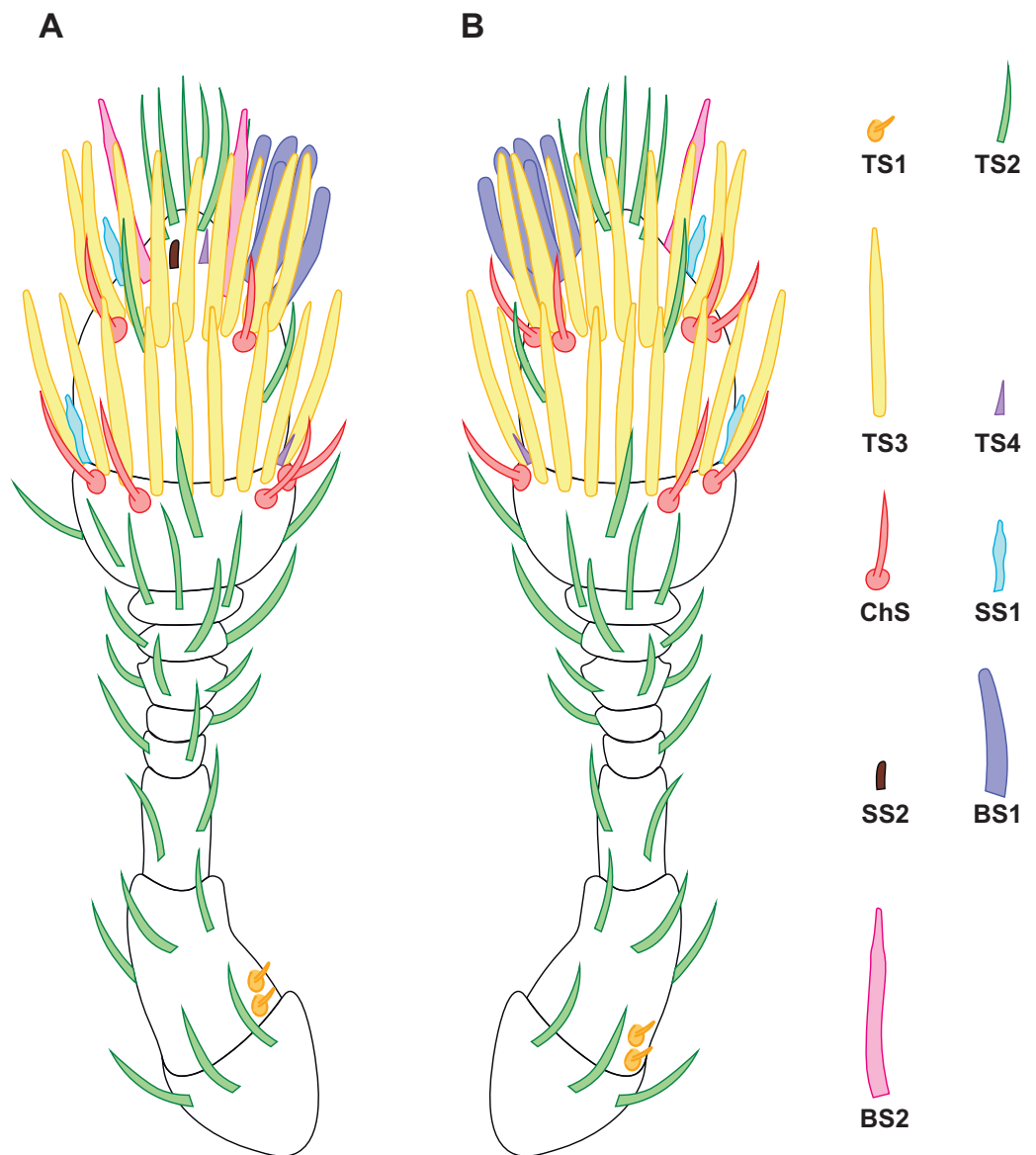
Figure 2 **Distribution of antennal sensilla in *Scydosella musawasensis*.** (A) Ventral view; (B) dorsal view.

Sensillum trichodeum type 3 (TS3)

Large sensilla narrowing apically, with many pores arranged in rows over the entire surface (Fig. 4D). TS3 are located along the distal margin of segments 1 and 2 of the club, 20 and 18, respectively (Fig. 2). TS3 are the largest type of sensilla both in length (13.14 ± 1.66 μm) and in diameter (0.88 ± 0.11 μm) (Table 2; Table S1).
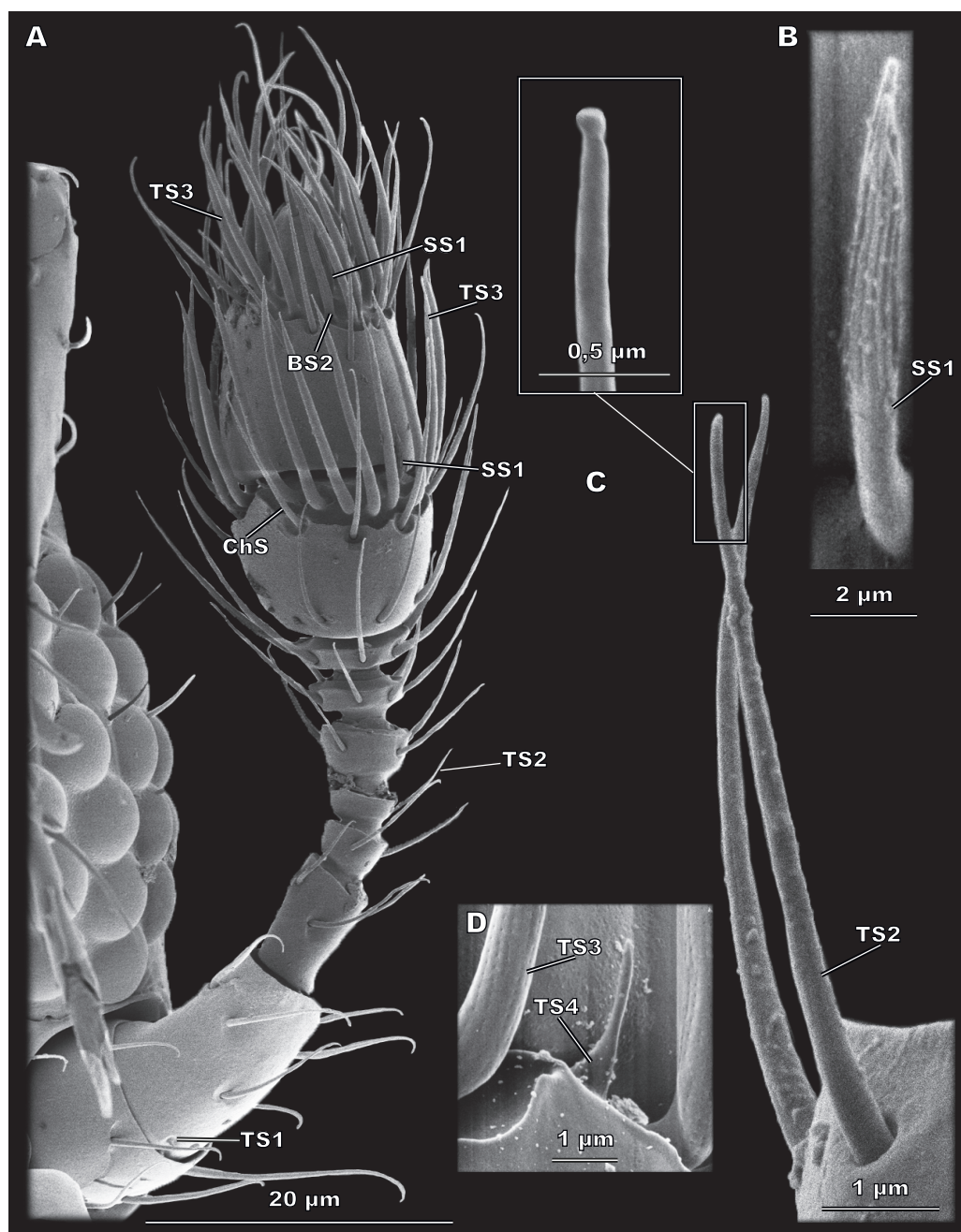
Figure 3 **Morphological types of antennal sensilla in *Scydosella musawasensis* (SEM).** (A) Whole antenna, ventral view, demonstrating TS1, TS2, TS3, ChS, SS1 and BS2; (B) aporous SS1 with longitudinal grooves; (C) TS2 with close-up showing its aporous tip; (D) aporous TS4 surrounded by TS3 with porous walls.

Sensillum trichodeum type 4 (TS4)

Small elongated poreless sensilla located at the distal margin of segments 1 and 2 of the club, one sensillum per segment (Figs. 3D and 2). Their length is 2.47 ± 0.7 µm, and their diameter is 0.44 ± 0.09 µm (Table 2; Table S1).
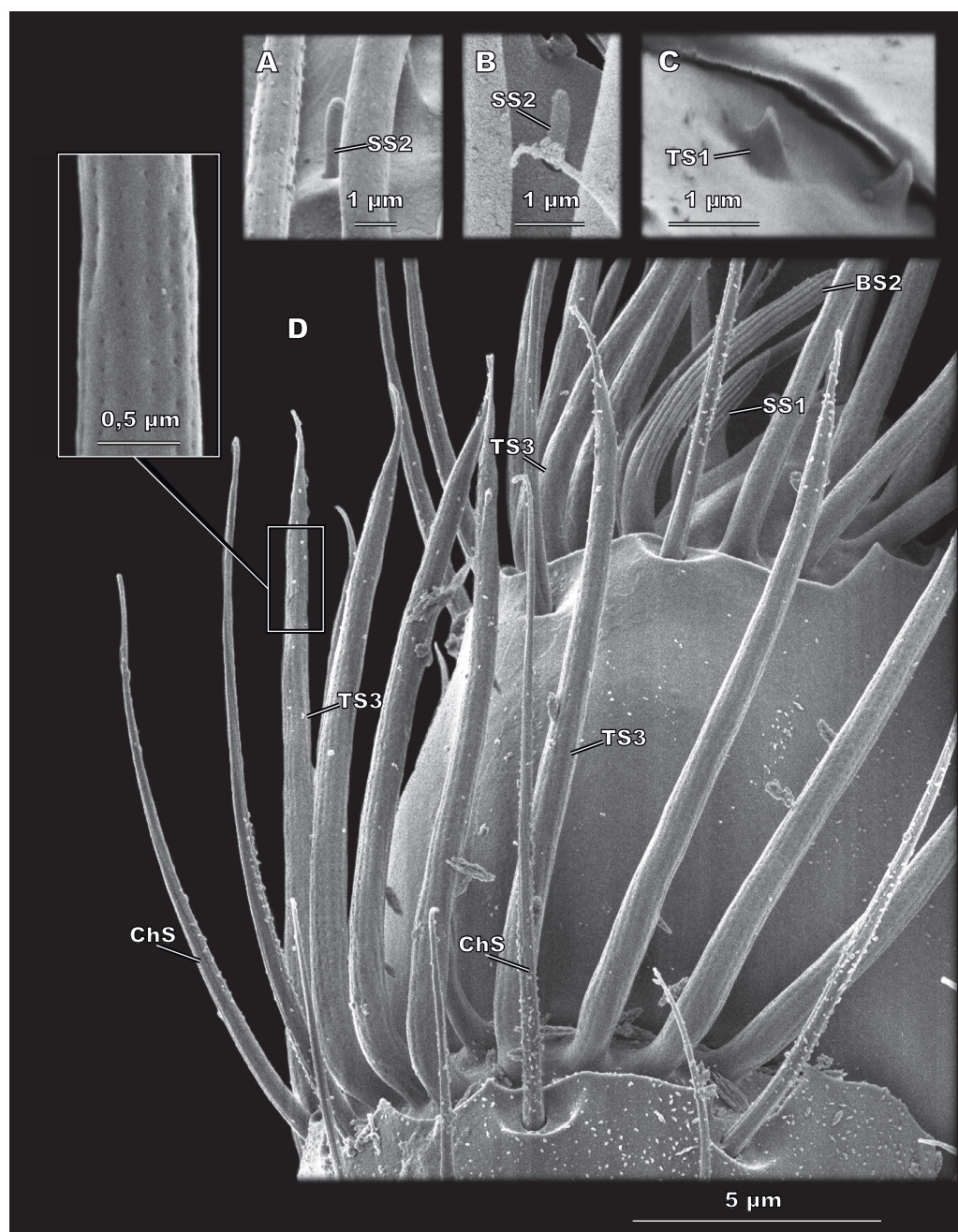
Figure 4 **Club sensilla and proprioceptors of *Scydosella musawasensis* (SEM).** (A) Aporous SS2 surrounded by porous TS3; (B) a small and inconspicuous SS2 is observed on the club; (C) Proprioceptive aporous TS1 situated on pedicel near the margin of scape; (D) detailed ventral view of the club. ChS, SS1, BS2, and TS3 with a close-up showing its porous wall are shown.

Sensillum chaeticum (ChS)

Elongated aporous sensilla with small longitudinal depressions, located in a wide and distinct cuticular socket (Figs. 4D and 5B). Arranged in a circle on segments 1 and 2 of the

Table 2 Number (n) per antenna and length (L) and diameter (D) of antennal sensilla (mean ± sd) in *Scydosella musawasensis*, in μm.

	Sensillum type								
	TS1	TS2	TS3	TS4	ChS	SS1	BS1	BS2	SS2
n	4	61	38	2	14	2	1	7	2
L	0.63 ± 0.11	8.38 ± 1.98	13.14 ± 1.66	2.47 ± 0.7	9.61 ± 1.92	3.87 ± 0.72	7 ± 0.94	8.61 ± 1.1	0.72 ± 0.15
D	0.44 ± 0.07	0.52 ± 0.1	0.88 ± 0.11	0.44 ± 0.09	0.58 ± 0.06	0.6 ± 0.12	0.78 ± 0.08	0.71 ± 0.08	0.33 ± 0.05

Notes.
For more details, see Table S1.

club, eight and six, respectively (Fig. 2). ChS are 9.61 ± 1.92 μm long and 0.58 ± 0.06 μm in diameter (Table 2; Table S1).

Sensillum styloconicum type 1 (SS1)
Small apically narrowing aporous sensilla with many longitudinal depressions in the apical half (Fig. 3B). One SS1 is located laterally on the distal edge of each of segments 1 and 2 of the club (Fig. 2). SS1 are 3.87 ± 0.72 μm long and 0.60 ± 0.12 μm in diameter (Table 2; Table S1).

Sensillum styloconicum type 2 (SS2)
Very small and inconspicuous aporous sensillum with a rounded apex (Figs. 4A and 4B). A single sensillum of this type is situated ventrally on segment 2 of the club (Fig. 2). SS2 have the smallest diameter, 0.33 ± 0.05 μm. Their length is 0.72 ± 0.15 μm (Table 2; Table S1).

Sensillum basiconicum type 1 (BS1)
Curved sensilla with a rounded tip and many pores all over the surface (Fig. 5B). Located in a group of seven sensilla on the medial surface of the segment 2 of the club (Fig. 2). BS1 are 7 ± 0.94 μm long and 0.78 ± 0.08 μm in diameter (Table 2; Table S1).

Sensillum basiconicum type 2 (BS2)
Large aporous sensilla with a narrow elongated apical narrowing and rows of pronounced longitudinal grooves (Figs. 5A and 5B). Two BS2 are located on segment 2 of the club, on the medial and lateral surfaces (Fig. 2). Their length is 8.61 ± 1.1 μm, and their diameter is 0.71 ± 0.08 μm (Table 2; Table S1).

DISCUSSION

The structure of the antenna
The structure of the antenna of *S. musawasensis* is typical of the tribe Nanosellini. Most species of the family Ptiliidae have 11 antennomeres, which is the most common number of antennomeres in Staphylinoidea, but in *S. musawasensis,* and in many other miniature representatives of Nanosellini, the number of antennomeres is reduced to ten (*Sörensson, 1997*; *Hall, 1999*; *Polilov et al., 2019*).
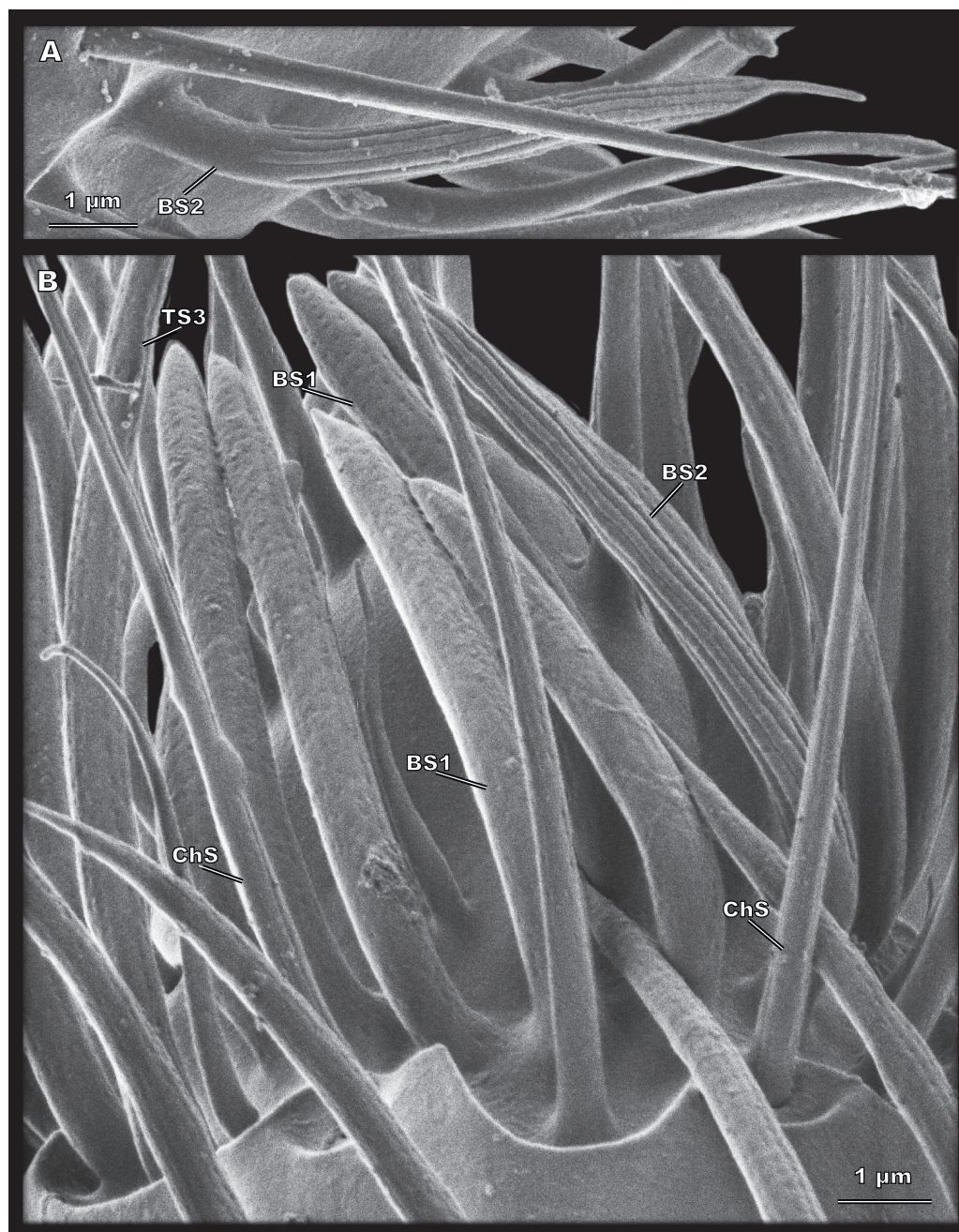
Figure 5 **Sensilla of flagellomere 8 of *Scydosella musawasensis* (SEM).** (A) BS2 with grooves and elongated tip situated on the club; (B) detailed medial view of segment 2 of the club. ChS with cuticular sockets, porous BS1 and TS3 and grooved BS2 are shown.

The morphological types of the antennal sensilla and their supposed functions

The antennae of Coleoptera usually bear many different types of sensilla; the greatest number of types of antennal sensilla among insects was described in a representative of this

order, *Rhynchophorus palmarum* Linnaeus, 1758 (Coleoptera: Curculionidae) (*Di Giulio et al., 2012*). The antennal sensilla of Coleoptera are represented by many morphological types, in some cases unique to the species described (*Newman, McDonald & Triebold, 1993*). In the case of *S. musawasensis*, most of the revealed types of sensilla have been previously described in Coleoptera and have a typical structure and location. The few exceptions are TS4, SS2, and BS2.

Sensillum trichodeum type 1

Sensilla of this type, also known as the "Böhm bristles" (*Böhm, 1911*), are proprioceptors found in many insects, including the previously studied species of the genus *Aleochara* Gravenhorst, 1802 (Coleoptera: Staphylinidae), in which they have been found not only on the scape and pedicel, but also on the flagellum (*Skilbeck & Anderson, 1996*). They are usually located in groups on the articular surfaces between antennomeres. The movement of one of the antennomeres deflects the cuticular hair, signaling the position of the antennomeres relative to each other (*Schmidt & Smith, 1986*).

Sensillum trichodeum type 2

This is the most abundant type of sensilla on the antenna of *S. musawasensis*. A small depression at the base of the sensilla suggests a mechanosensory function (*Zhantiev, 1969*). The ultrastructural organization of such sensilla usually includes the dendrite of the sensory neuron, which reaches the cuticular depression and thus perceives the deviations of the long cuticular hair when it touches external objects (*Consoli, Kitajima & Parra, 1999*). These are common sensilla, found, among other insects, in many coleopterans (*Payne et al., 1973*; *Merivee et al., 2002*; *Di Giulio et al., 2012*).

Sensillum trichodeum type 3

The many regular pores on the cuticular surface of these sensilla indicate their olfactory function (*Ivanov, 2000*). Many branched dendrites of sensory neurons are usually located inside such sensilla. Similar sensilla have been described in the parasitic wasps *Tetrastichus hagenowii* Ratzeburg, 1852 (Hymenoptera: Eulophidae) and *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera: Trichogrammatidae), in which they are also located distally on the antenna (*Barlin, Vinson & Piper, 1981*; *Romani, Isidoro & Bin, 2010*). Sensilla similar in morphology and location have also been found in 16 species of Scolytidae (Coleoptera), in which they were described as sensilla basiconica long type (*Payne et al., 1973*).

Sensillum trichodeum type 4

These small inconspicuous sensilla resemble TS1 in structure, but they have no cuticular depression at the base and are located on the club, rather than on the articular surfaces. These characteristics suggest that these sensilla are not proprioceptors. There are no published data on this type of sensilla, but due to their small size it is possible that they were overlooked in larger insects. Their ultrastructure has to be studied to accurately determine their function.

Sensillum chaeticum

The sensilla chaetica of *S. musawasensis* appear as typical insect mechanoreceptors: they have a pronounced cuticular socket at the base and a long bristly cuticular hair and are located at an obtuse angle to the antennal axis (*Ivanov, 2000*). Such sensilla are known in most insects, including even the extremely simplified miniature male of *D. echmepterygis*, in which the only sensillum on the miniaturized antenna is a sensillum chaeticum (*Mockford, 1997*). Sensilla chaetica have been found in many species of Coleoptera (*Dyer & Seabrook, 1975*; *Harbach & Larsen, 1977*; *Handique et al., 2017*).

Sensillum styloconicum type 1

Such sensilla are found in many very different insects, usually located on the club, if it is present, or distally on the antenna, as in *S. musawasensis* (*Altner & Loftus, 1985*; *Chiappini, Solinas & Solinas, 2001*; *Diakova, Makarova & Polilov, 2018*). Sensilla of this type usually contain processes of several neurons and are thermo/hygroreceptors (*Yokohari, 1999*). Such sensilla have been described in some coleopterans; usually only one such sensillum is present, or very few of them (*Smith, Carolina & Frazier, 1976*).

Sensillum styloconicum type 2

Such sensilla are barely mentioned in the available publications. A similar sensillum has been found in *Oxelytrum erythrurum* Blanchard, 1849 (Coleoptera: Silphidae), in which it was also located on the ventral surface of the club (*Oliva, 2012*). The lack of data on their ultrastructure makes it impossible to propose a possible function for them at the moment.

Sensillum basiconicum type 1

The multiporous sensilla basiconica are a typical example of olfactory receptors found in many insects, including coleopterans (*Borden, 1968*; *Kim & Yamasaki, 1996*). In a study on the honeybee *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae), it was found that they are distance chemoreceptors (*Lacher, 1964*). They contain multiply branching processes of many sensory neurons (*Chiappini, Solinas & Solinas, 2001*).

Sensillum basiconicum type 2

These sensilla are very similar in shape and location to BS1, but the absence of pores on their surface suggests that they are not chemoreceptors. They could be thermo/hygroreceptors, but their exact function cannot be determined without studying their ultrastructure.

The effects of miniaturization on the antennal sensilla of Coleoptera

Analysis of the dependence of the principal morphometric characteristics of coleopteran antennae on body size revealed the consequences of reduction in the latter and especially of extreme miniaturization. For the allometric analyses, in addition to the data obtained on the sensilla of *S. musawasensis*, we used published data from 16 sources on 33 species of beetles of eight families (Table S2); the body sizes of the beetles included in our analyses ranged from 0.3 to 40.0 mm (for references, see Appendix 1). We used the equation $y = ax^b$, where y is the variable in question, a is elevation, x is body length, and b is slope (terminology follows the one described in *Warton et al., 2006*).

The number of sensilla correlates positively with body size (SMA $358.1x^{1.31}$, OLS $473.1x^{1.16}$; the slopes of both regressions are significantly different from 0, $p < 0.01$) (Fig. 6A; Table S3). The model explains a majority of the variability the data ($R^2 = 0.79$ for SMA and OLS). The range is from 131 (*S. musawasensis*) to 57 370 sensilla (*Rhynchophorus palmarum* Linnaeus, 1758, Coleoptera: Curculionidae) per antenna (*Saïd et al., 2003*). Decreasing numbers of antennal sensilla with decreasing body size have been shown earlier in parasitic wasps (*Diakova, Makarova & Polilov, 2018*). Comparisons of larger and smaller individuals of *T. evanescens* (*Van der Woude & Smid, 2016*) and *M. domestica* (*Smallegange, Kelling & Otter, 2008*) also revealed a correlation between the body size and the number of antennal sensilla. The number of sensilla types in the analysis showed no significant correlation with body size (Fig. 6B; Table S3), same as found in parasitic wasps (*Diakova, Makarova & Polilov, 2018*). In a study on the intraspecific variation of *M. domestica,* no variation in the number of the types of sensilla was found either (*Smallegange, Kelling & Otter, 2008*). The base length and diameter of ChS show positive correlations with body size (length: SMA $14.55x^{0.81}$, OLS $27.99x^{0.42}$; diameter: SMA $0.91x^{0.60}$, OLS $1.04x^{0.54}$) (Figs. 6C and 6D; Table S3). The slopes for both length and diameter are significantly smaller than 1, $p < 0.01$, which indicates allometry and an increase in the relative sizes of ChS with decrease in body size. The model explains a majority of the variation in ChS diameter ($R^2 = 0.79$). However, model for ChS length demonstrates low $R^2 = 0.27$, which implies a possibility that the model is imprecise. The ranges are 9.6 to 280.0 μm for length and 0.6 μm to 8.0 μm for diameter. Length and diameter of BS1 showed no significant correlation with body size (Figs. 6C and 6D; Table S3); the slope did not significantly differ from 0, which indicates that the sizes of sensilla do not change with decreasing body size. In the study on the antennal sensilla of parasitic wasps, a weak correlation of the length of ChS with the body length has been revealed, but no correlation has been found between the diameter of ChS and body length or between the size characteristics of BS1 and body length (*Diakova, Makarova & Polilov, 2018*).

Comparison of the effects of extreme miniaturization on the antennal sensilla in the free-living *S. musawasensis* vs. the parasitic wasps of the genus *Megaphragma*

Comparison with the results of our earlier study on the antennal sensilla of three species of miniature parasitic wasps of the genus *Megaphragma* (*Diakova, Makarova & Polilov, 2018*) reveals universal adaptations of the antennal sensory systems to extreme miniaturization common to parasitic and free-living insects. As in *S. musawasensis*, very few antennal sensilla were found in *Megaphragma* wasps. Species of the genus *Megaphragma* have 39–49 antennal sensilla per antenna (depending on the species and sex), while each antenna of larger parasitic wasps can bear up to 9000 sensilla (*Das et al., 2011*). In both beetles and parasitic wasps the number of antennal sensilla decreases with decreasing body size.

No correlation between the body size and the number of the types of sensilla has been revealed in either beetles or parasitic wasps. The diversity of sensory information perceived by the insects depends on the number of the types of antennal sensilla. A small number of types and subtypes of sensilla indicates a low diversity of sensory information, which
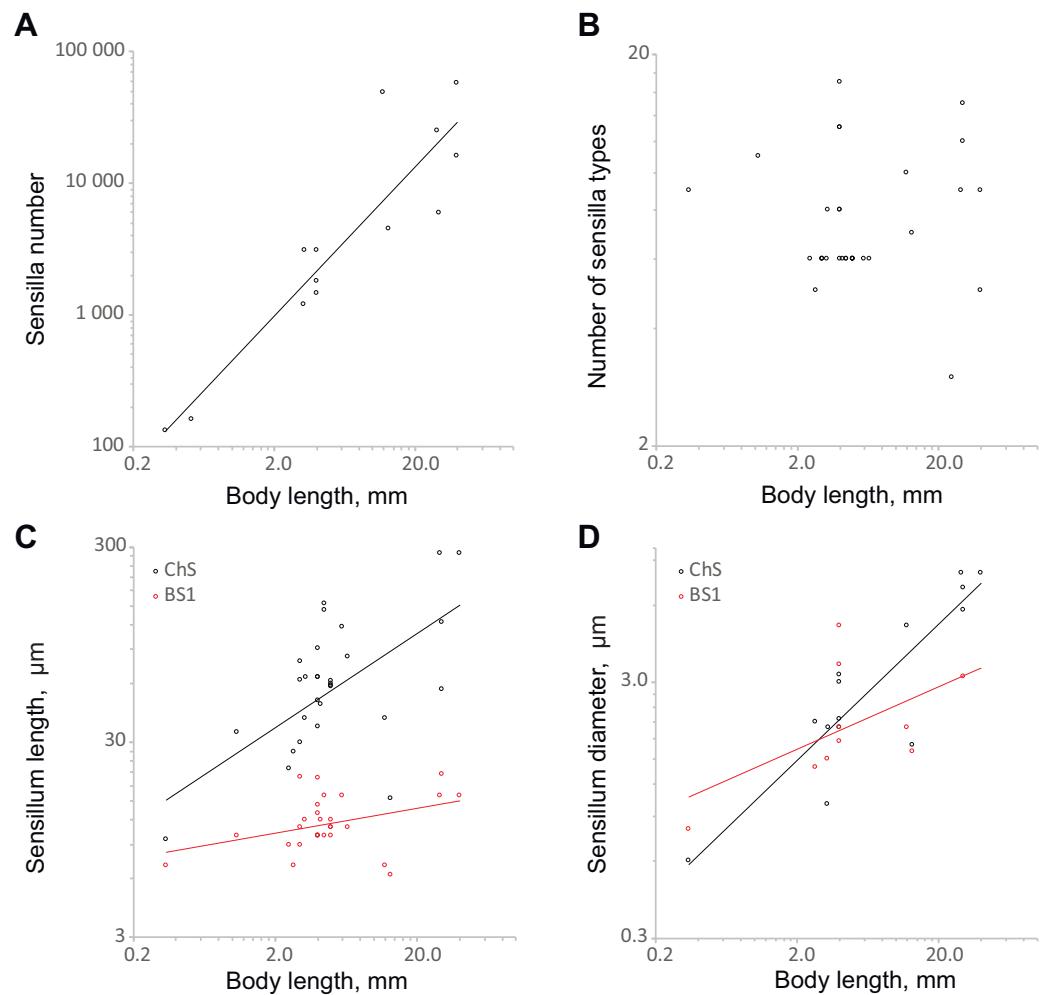
Figure 6 **Changes in antennal sensilla properties in Coleoptera.** (A) Number of antennal sensilla as a function of body length; (B) the number of antennal sensilla types as a function of body length; (C) mechanoreceptive ChS and olphactory BS1 length as a function of body length; (D) ChS and BS1 diameter as a function of body length. For references, see Table S3.

usually includes tactile information and detection of temperature and humidity (*Kapoor, 1985*; *Gaino & Rebora, 1998*; *Derr & Cook, 2005*; *Fialho et al., 2014*). Insects with many types of sensilla, regardless of body size, have the entire possible spectrum of receptors: mechanoreceptors, contact and distant chemoreceptors, thermo/hygroreceptors, and proprioceptors.

The scaling of the dimensional characteristics of the sensilla depends on their type: while for ChS in both beetles and parasitic wasps a correlation of the length and diameter with the body size has been shown, for BS1 no significant correlation has been found. The reason why ChS are scaled but BS1 are not could be the greater complexity of the internal structure of BS1. Neurons containing specific olfactory receptor proteins, their projections, and the glomeruli of the antennal lobe constitute a complex system with an odotopic organization

(*Gao, Yuan & Chess, 2000*), which is difficult to optimize and miniaturize. It is probably the complexity of the internal structure that limits the optimization of the olfactory BS1, as a result of which there is no correlation between their size characteristics and the body size.

CONCLUSIONS

A decrease in body size leads to a significant decrease in the number of individual sensory units (sensilla) in the antennal sensory systems of both parasitic and free-living insects. A similar trend is also found in the miniaturization of the compound eyes of insects, for which the number of ommatidia has been shown to decrease significantly with decreasing body size. At the same time, miniature ommatidia show a cellular organization similar to that of larger insects and a number of structural adaptations associated with miniaturization (*Makarova, Meyer-Rochow & Polilov, 2019*). The scalability of sensilla during miniaturization depends on the type of the sensilla. Mechanosensory sensilla are more scalable, while olfactory sensilla, which have a more complex ultrastructure, do not get smaller with decreasing body size. The number of functional types does not correlate with body size, which indicates the preservation of the complexity and functionality of the antennal sensory system in most of the smallest insects studied. Thus, a miniature antenna is a highly functional multimodal organ with a small number of different receptors, which respond to a wide range of different sensory information.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Anna V. Diakova performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Alexey A. Polilov conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The raw measurements are available in the Supplementary File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.10401#supplemental-information.

REFERENCES

Ali SAI, Diakite MM, Ali S, Wang M-Q. 2016. Effects of the antennal sensilla distribution pattern on the behavioral responses of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Florida Entomologist* **99(1)**:52–59 DOI 10.1653/024.099.0110.

Altner H, Loftus R. 1985. Ultrastructure and function of insect thermo- and hygroreceptors. *Annual Review of Entomology* **30(1)**:273–295 DOI 10.1146/annurev.en.30.010185.001421.

Altner H, Sass H, Altner I. 1977. Relationship between structure and function of antennal chemo-, hygro-, and thermoreceptive sensilla in *Periplaneta americana*. *Cell and Tissue Research* **176(3)**:389–405 DOI 10.1007/BF00221796.

Barlin MR, Vinson SB, Piper GL. 1981. Ultrastructure of the antennal sensilla of the cockroach-egg parasitoid, *Tetrastichus hagenowii* (Hymenoptera : Eulophidae). *Journal of Morphology* **108**:97–108.

Böhm L. 1911. Die antennalen Sinnesorgane der Lepidopteren. *Arbeiten Aus Dem Zoologischen Instituten Der Universität Wien Und Der Zoologischen Station in Triest* **2(22)**:219–246.

Borden JH. 1968. Antennal morphology of *Ips confusus* (Coleoptera: Scolytidae). *Annals of the Entomological Society of America* **61(1)**:10–13 DOI 10.1093/aesa/61.1.10.

Callahan PS. 1975. Insect antennae with special reference to the mechanism of scent detection and the evolution of the sensilla. *International Journal of Insect Morphology and Embryology* **4(5)**:381–430 DOI 10.1016/0020-7322(75)90038-0.

Chiappini E, Solinas C, Solinas M. 2001. Antennal sensilla of *Anagrus atomus* (L.) (Hymenoptera: Mymaridae) female and their possible behavioural significance. *Entomologica* **35**:51–76.

Consoli FL, Kitajima EW, Parra JR. 1999. Sensilla on the antenna and ovipositor of the parasitic wasps *Trichogramma galloi* Zucchi and *T. pretiosum* Riley (Hym. Trichogrammatidae). *Microscopy Research and Technique* **324**:313–324.

Das P, Chen L, Sharma KR, Fadamiro HY. 2011. Abundance of antennal chemosensilla in two parasitoid wasps with different degree of host specificity may explain sexual and species differences in their response to host-related volatiles. *Microscopy Research and Technique* **74(10)**:900–909 DOI 10.1002/jemt.20974.

Derr DP, Cook JL. 2005. Morphology of the antenna of Caenocholax fenyesi pierce (Strepsiptera: Myrmecolacidae) based on scanning electron microscopy. *Proceedings of the Entomological Society of Washington* **107(4)**:762–769.

Diakova AV, Makarova AA, Polilov AA. 2018. Between extreme simplification and ideal optimization: antennal sensilla morphology of miniaturized *Megaphragma* wasps (Hymenoptera: Trichogrammatidae). *PeerJ* **6**:e6005 DOI 10.7717/peerj.6005.

Di Giulio A, Maurizi E, Rossi Stacconi MV, Romani R. 2012. Functional structure of antennal sensilla in the myrmecophilous beetle *Paussus favieri* (Coleoptera, Carabidae, Paussini). *Micron* **43(6)**:705–719 DOI 10.1016/j.micron.2011.10.013.

Dyer LJ, Seabrook WD. 1975. Sensilla on the antennal flagellum of the sawyer beetles *Monochamus notatus* (Drury) and *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae). *Journal of Morphology* **146(4)**:513–531 DOI 10.1002/jmor.1051460407.

Eberhard WG, Wcislo WT. 2011. Grade changes in brain-body allometry: morphological and behavioural correlates of brain size in miniature spiders, insects and other invertebrates. *Advances in Insect Physiology* **40**:155–214 DOI 10.1016/B978-0-12-387668-3.00004-0.

Fialho MDCQ, Guss-Matiello CP, Zanuncio JC, Campos LAO, Serrão JE. 2014. A comparative study of the antennal sensilla in corbiculate bees. *Journal of Apicultural Research* **53(3)**:392–403 DOI 10.3896/IBRA.1.53.3.07.

Gaino E, Rebora M. 1998. Ultrastructure of the antennal sensilla of the mayfly Baetis rhodani (Pictet) (Ephemeroptera: Baetidae). *International Journal of Insect Morphology and Embryology* **27(2)**:143–149 DOI 10.1016/S0020-7322(97)00001-9.

Gao Q, Yuan B, Chess A. 2000. Convergent projections of Drosophila olfactory neurons to specific glomeruli in the antennal lobe. *Nature Neuroscience* **3(8)**:780–785 DOI 10.1038/77680.

Guerenstein PG, Yepez E, Van Haren J, Williams DG, Hildebrand JG. 2004. Floral CO_2 emission may indicate food abundance to nectar-feeding moths. *Naturwissenschaften* **91(7)**:329–333 DOI 10.1007/s00114-004-0532-x.

Hall E. 1999. Generic revision of the tribe nanosellini (Coleoptera: Ptiliidae: Ptiliinae). *Transactions of the American Entomological Society* **125(1)**:39–126.

Hallberg E, Hansson BS, Lofsted C. 2003. Sensilla and proprioceptors. In: Kristensen NP, ed. *Lepidoptera, moths and butterflies 2: morphology, physiology, and development.* Berlin: Walter de Gruyter, 265–278 DOI 10.1515/9783110893724.

Handique G, Bhattacharyya B, Baruah AALH, Boruah R. 2017. Antenna morphology and sensilla microstructure of the male and female scarab beetle, Lepidiota mansueta Burmeister (Coleoptera: Scarabaeidae). *Invertebrate Reproduction and Development* **61(3)**:200–205 DOI 10.1080/07924259.2017.1287784.

Harbach RE, Larsen JR. 1977. Fine structure of antennal sensilla of the adult mealworm beetle, Tenebrio molitor L. (Coleoptera: Tenebrionidae). *International Journal of Insect Morphology and Embryology* **6(1)**:41–60 DOI 10.1016/0020-7322(77)90029-0.

Ivanov VP. 2000. In: Orlova GM, ed. *Organy chuvstv nasekomykh i drugikh chlenistonogikh (The sense organs of insects and other arthropods).* Moscow: Nauka.

Kamikouchi A, Inagaki HK, Effertz T, Hendrich O, Fiala A, Göpfert MC, Ito K. 2009. The neural basis of Drosophila gravity-sensing and hearing. *Nature* **458**:165–171 DOI 10.1038/nature07810.

Kapoor NN. 1985. External morphology and distribution of the antennal sensilla of the stonefly, Paragnetina media (Walker) (Plecoptera: Perlidae). *International Journal of Insect Morphology and Embryology* **14(5)**:273–280.

Kim JL, Yamasaki T. 1996. Sensilla of *Carabus* (*Isiocarabus*) *fiduciarius saishutoicus* Csiki (Coleoptera: Carabidae. *International Journal of Insect Morphology and Embryology* **25(1–2)**:153–172 DOI 10.1016/0020-7322(95)00015-1.

Lacher V. 1964. Elektrophysiologische Untersuchungen an einzelnen Rezeptoren fur Geruch, Kohlendioxyd, Luftfeuchtigkeit und Tempratur auf den Antennen der Arbeitsbiene und der Drohne (*Apis mellifica* L.). *Zeitschrift Fur Vergleichende Physiologie* **48(6)**:587–623 DOI 10.1007/BF00333743.

Lopes O, Marques PC, Araújo J. 2005. The role of antennae in mate recognition in *Phoracantha semipunctata* (Coleoptera: Cerambycidae). *Journal of Insect Behavior* **18(2)**:243–257 DOI 10.1007/s10905-005-0478-7.

Makarova AA, Meyer-Rochow VB, Polilov AA. 2019. Morphology and scaling of compound eyes in the smallest beetles (Coleoptera: Ptiliidae). *Arthropod Structure and Development* **48**:83–97 DOI 10.1016/j.asd.2019.01.001.

Merivee E, Ploomi A, Rahi M, Bresciani J, Ravn HP, Luik A, Sammelselg V. 2002. Antennal sensilla of the ground beetle *Bembidion properans* Steph. (Coleoptera, Carabidae). *Micron* **33(5)**:429–440 DOI 10.1016/S0968-4328(02)00003-3.

Minelli A, Fusco G. 2019. No limits: breaking constraints in insect miniaturization. *Arthropod Structure and Development* **48**:4–11 DOI 10.1016/j.asd.2018.11.009.

Mockford EL. 1997. A new species of *Dicopomorpha* (Hymenoptera: Mymaridae) with diminutive, apterous males. *Annals of the Entomological Society of America* **90(2)**:115–120 DOI 10.1093/aesa/90.2.115.

Moeck A. 1967. Electron microscopic studies of antennal sensilla in the ambrosia beetle *Trypodendron lineatum* (Olivier) (Scolytidae). *Canadian Journal of Zoology* **46**:521–556.

Newman S, McDonald I, Triebold B. 1993. Antennal sexual dimorphism in *Diabrotica virgifera virgifera* (Le Conte) (Coleoptera : Chrysomelidae): male specific structures, ultrastructure of a unique sensillum, and sites of esterase activity. *International Journal of Insect Morphology and Embryology* **22(5)**:535–547 DOI 10.1016/0020-7322(93)90039-4.

Oliva A. 2012. The antennal sensilla of *Oxelytrum erythrurum* (Blanchard) and *Oxelytrum apicale* (Brullé) (Coleoptera: Silphidae). *Neotropical Entomology* **41(5)**:395–403 DOI 10.1007/s13744-012-0060-x.

Payne TL, Moeck HA, Willson CD, Coulson RN, Humphreys WJ. 1973. Bark beetle olfaction-II. Antennal morphology of sixteen species of Scolytidae (Coleoptera). *International Journal of Insect Morphology and Embryology* **2(3)**:177–192 DOI 10.1016/0020-7322(73)90027-5.

Pérez-González S, Zaballos J. 2013. Antennal morphology of the endogean carabid genus *Typhlocharis* (Coleoptera: Carabidae: Anillini): Description of sensilla and taxonomic implications. *Journal of Morphology* **274(7)**:809–823 DOI 10.1002/jmor.20140.

Polilov AA. 2015a. Small is beautiful: features of the smallest insects and limits to miniaturization. *Annual Review of Entomology* **60(1)**:103–121 DOI 10.1146/annurev-ento-010814-020924.

Polilov AA. 2015b. How small is the smallest? New record and remeasuring of Scydosella musawasensis (*Hall, 1999*) (Coleoptera, Ptiliidae), the smallest known free-living insect. *ZooKeys* **2015(526)**:61–64 DOI 10.3897/zookeys.526.6531.

Polilov AA. 2016. *At the size limit—effects of miniaturization in insects.* Berlin: Springer DOI 10.1007/978-3-319-39499-2.

Polilov AA, Ribera I, Yavorskaya MI, Cardoso A, Grebennikov VV, Beutel RG. 2019. The phylogeny of Ptiliidae (Coleoptera: Staphylinoidea)—the smallest beetles and their evolutionary transformations. *Arthropod Systematics and Phylogeny* **77(3)**:433–455 DOI 10.26049/ASP77-3-2019-4.

R Core Team. 2020. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. *Available at http:// www.R-project.org/*.

Romani R, Isidoro N, Bin F. 2010. Antennal structures used in communication by egg parasitoids. In: *Egg parasitoids in agroecosystems with emphasis on trichogramma.* Berlin: Springer, 57–96 DOI 10.1007/978-1-4020-9110-0.

Saïd I, Tauban D, Renou M, Mori K, Rochat D. 2003. Structure and function of the antennal sensilla of the palm weevil *Rhynchophorus palmarum* (Coleoptera, Curculionidae). *Journal of Insect Physiology* **49(9)**:857–872 DOI 10.1016/S0022-1910(03)00137-9.

Schmidt JM, Smith JJB. 1986. Correlations between body angles and substrate curvature in the parasitoid wasp *Trichogramma minutum*: a possible mechanism of host radius measurement. *Journal of Experimental Biology* **125**:271–285.

Schneider D. 1964. Insect antennae. *Annual Review of Entomology* **9(1)**:103–122 DOI 10.1146/annurev.en.09.010164.000535.

Shields VDC, Hildebrand JG. 2001. Recent advances in insect olfaction, specifically regarding the morphology and sensory physiology of antennal sensilla of the female sphinx moth *Manduca sexta. Microscopy Research and Technique* **55(5)**:307–329 DOI 10.1002/jemt.1180.

Skilbeck CA, Anderson M. 1996. The ultrastructure, morphology and distribution of sensilla on the antennae of the adult parasitoids *Aleochara bilineata* Gyll. and *Aleochara bipustulata* L. (Coleoptera: Staphylinidae). *International Journal of Insect Morphology and Embryology* **25(3)**:261–280 DOI 10.1016/0020-7322(96)00005-0.

Smallegange RC, Kelling FJ, Den Otter CJ. 2008. Types and numbers of sensilla on antennae and maxillary palps of small and large houseflies, Musca domestica (Diptera, Muscidae). *Microscopy Research and Technique* **71(12)**:880–886 DOI 10.1002/jemt.20636.

Smith CM, Carolina N, Frazier JL. 1976. Antennal sensilla of the clover head weevil *Hypera meles* (F.) (Coleoptera: Curculionidae). *International Journal of Insect Morphology and Embryology* **5**:349–355 DOI 10.1016/0020-7322(76)90010-6.

Sörensson M. 1997. Morphological and taxonomical novelties in the world's smallest beetles, and the first Old World record of Nanosellini (Coleoptera: Ptiliidae). *Systematic Entomology* **22(3)**:257–283 DOI 10.1046/j.1365-3113.1997.d01-40.x.

Symonds MRE, Elgar MA. 2013. The evolution of body size, antennal size and host use in parasitoid wasps (Hymenoptera: Chalcidoidea): a phylogenetic comparative analysis. *PLOS ONE* **8(10)**:e78297 DOI 10.1371/journal.pone.0078297.

Tschinkel WR, Mikheyev AS, Storz SR. 2003. Allometry of workers of the fire ant, solenopsis invicta. *Journal of Insect Science* **3(2)**:1–11 DOI 10.1673/031.003.0201.

Warton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. *Biological Reviews of the Cambridge Philosophical Society* **81**:259–291 DOI 10.1017/S1464793106007007.

Van der Woude E, Smid HM. 2016. How to escape from Haller's rule: olfactory system complexity in small and large *Trichogramma evanescens* parasitic wasps. *Journal of Comparative Neurology* **524(9)**:1876–1891 DOI 10.1002/cne.23927.

Xie G-Y, Zhao X-C, Ma B-W, Guo P, Li G-P, Feng H-Q, Wu G-L. 2016. Central projection of antennal sensory neurons in the central nervous system of the mirid bug *Apolygus lucorum* (Meyer-Dür). *PLOS ONE* **11(8)**:e0160161 DOI 10.1371/journal.pone.0160161.

Yokohari F. 1999. In: Eguchi E, Tominaga Y, eds. *Atlas of Arthropod sensory receptors: dynamic morphology in relation to function.* Berlin: Springer, 191–210.

Zhantiev RD. 1969. Ultrastructural organization of the mechanoreceptor sensilla of insects. *Zhurnal Obshchei Biologii* **30**:224–231 (In Russian).