



Abundance and distribution of antennal sensilla on males and females of three sympatric species of alpine grasshopper (Orthoptera: Acrididae: Catantopinae) in Aotearoa New Zealand

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Abstract

Brachaspis nivalis, *Sigauss australis* and *Paprides nitidus* are grasshopper species endemic to Aotearoa, New Zealand where they are sympatric in several regions of South Island. On mountains of Kā Tiritiri o te Moana (Southern Alps), *B. nivalis* is more abundant on scree/rock habitat, whereas *S. australis* and *P. nitidus* are prevalent in alpine tussock and herbfields. It is expected, therefore, that these species have different sensory needs that are likely to be apparent in the type, abundance, and distribution of chemo-sensilla on their antennae. It is also likely that natural selection has resulted in sexual differences in sensilla. To test these hypotheses, abundance and distribution of the chemo-sensilla on the dorsal and ventral surfaces of their antennae were characterized in adult males and females of the three species. Five types of chemo-sensilla were identified on the distal portion of their antenna: chaetica, basiconica, trichoidea, coeloconica, and cavity. All species had significantly more chemo-sensilla on the ventral than the dorsal surface of antennae and a similar distribution pattern of chemo-sensilla. Despite having relatively short antenna, *B. nivalis* had the largest number of olfactory sensilla, but the fewest chaetica of the three species studied. A plausible explanation is that *B. nivalis* is abundant on less vegetated habitats compared to the other species, and therefore may rely more on olfaction (distance) than gustatory (contact) reception for finding food. No significant differences were observed between the sexes of *B. nivalis* and *P. nitidus*, however, *S. australis* males had significantly more basiconica sensilla than females.

Keywords Acrididae · Antenna · Sensilla · Sexual dimorphism · Sympatry

Introduction

A sensillum is a sensory organ protruding through the impervious exoskeleton of an insect, allowing detection of chemicals, temperature, and movement (e.g., olfactory, gustatory, mechanical, hygro-receptive and thermo-receptive sensilla). In short-horned grasshoppers (Orthoptera, Acrididae), chemical sensitive sensilla are abundant on structures including antennae (Altner et al. 1981; Bland 1989; Chapman 1989; Chen et al. 2003; Greenwood and Chapman 1984; Li et al. 2007; Ochieng et al. 1998; Roh et al. 2020), mouthparts (Blaney and Chapman 1969; Chapman 1989; Jin et al. 2006), legs (Mücke 1991; Yu et al. 2011)

and wings (Zhou et al. 2008). The function of each sensilla can be inferred from its shape, size, presence and absence of pores and socket type (Bland 1989; Chapman 1989; Chen et al. 2003; Garza et al. 2021; Li et al. 2007; Nowińska and Brożek 2017). For example, sensilla without pores (aporous) and a flexible socket are considered to be mechanoreceptors, whereas sensilla with pore(s) and an inflexible socket are considered to be chemical receptors (Garza et al. 2021; Li et al. 2007; Nowińska and Brożek 2017; Roh et al. 2020). Chemo-sensitive sensilla can have a single hole (uniporous) at the tip of the projection (apical pore) or have many pores (multi-porous or wall-pored), and these sensilla are responsible for gustation (contact chemoreception) and olfaction (distance chemoreception) respectively. The number and proportions of different types of sensilla are likely to be species-specific and comparison of sensilla density and morphology among species can reveal important ecological differences (Nakano et al. 2022).

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The abundance of sensilla of various types appears to be related to several ecological factors including the dietary range (i.e., monophagous, oligophagous, polyphagous: Bland 1989; Chen et al. 2003; Zaim et al. 2013), distribution and abundance of resources (i.e., mates and food: Greenwood and Chapman 1984; Ochieng et al. 1998) and sexual communication (i.e., signalers and receivers: Bland 1989; Chen et al. 2003; Li et al. 2007, 2021a; Malo et al. 2004; Roh et al. 2016). The inference that the sensitivity of an insect to its external environment depends on the abundance of sensilla (Bland 1989; Chapman 1989) is supported by observations using electro-physiological techniques, such as electroantennography (EAG) and single sensillum recordings (SSRs) (Ochieng and Hansson 1999; Chen and Kang 2000; Malo et al. 2004; Li et al. 2021a). For example, the different phases of locusts show characteristic abundance of sensilla on their antenna. Solitary locusts (at low density) possess more olfactory sensilla (Ochieng et al. 1998) with higher electrophysiological responses to some pheromone components compared to their high density gregarious phase (Ochieng and Hansson 1999). This is possibly because the solitary locusts require higher olfactory sensitivity to locate conspecifics under low population density compared to the gregarious phase (Hassanali et al. 2005). Sexual role is also linked to sensilla abundance and distribution, where receivers (typically males) have higher abundance of sensilla with higher olfactory sensitivity than signalers (typically females), as observed in a range of insects including grasshoppers (Chen and Kang 2000), beetles (Li et al. 2021a,b) and moths (Malo et al. 2004). A greater abundance of chemo-receptive sensilla is therefore predicted for those species that live in habitats with sparsely distributed resources and in the sex that is responsible for receiving chemical signals during mating (typically males).

The approximately 12,250 species of grasshoppers (Orthoptera; Caelifera) interact with diverse plant communities around the globe (Husemann et al. 2022; Ibanez et al. 2013; Joern 1979; Welte et al. 2019). However, most current knowledge of the chemical exchanges that underpin these plant–insect interactions is derived from the study of a small number of economically important pest species (locusts) (Nakano et al. 2022). In addition to locust species, representatives of a number of Gomphocerinae, Oedipodinae and Melanoplinae, and a few species from Acridinae (Bland 1982, 1989; Chen et al. 2003; Li et al. 2007) have been examined for sensilla but no representatives of the Euryphyminae, Eyprepocnemidinae, Ommatolampidinae, Spathosterninae, Coptacrinae, or southern Catantopinae.

The alpine environment of Aotearoa/New Zealand has a rich, endemic ecological community including flightless, acridid grasshoppers (Bigelow 1967; White 1975). These species of southern Catantopinae are the products of an endemic radiation associated primarily with Kā Tiritiri o

te Moana, the Southern Alps (Koot et al. 2020). At most locations, several species co-occur on the same plant communities with overlap in their food plants (Watson 1970). Three widespread sympatric species, *Brachaspis nivalis* (Hutton, 1898), *Sigauss australis* (Hutton, 1897) and *Paprides nitidus* (Hutton, 1898), have been shown to have different micro-habitat preferences within scree-shrub-herbfield mosaics (Bigelow 1967; Koot 2018; Watson 1970). Habitat partitioning suggests that these grasshopper species have different sensory requirements relating to the type and distance of cues from potential food plants. Similarly, communication between individual grasshoppers exerts specific demands on sensory ability. The coloring and appearance of these grasshoppers suggests selection on camouflage from predators rather than sexual signals (Fig. 1), and they have reduced wings (tegmina) unsuitable for sound production. Together these limitations in auditory and visual signaling imply that chemical cues may be important for selection of mates as well as food, but direct evidence is lacking.

To explore the chemosensory capabilities of endemic, flightless grasshoppers, we use a comparative approach, hypothesizing that sensilla abundance and distribution among these three species will reflect the putative ecological differences of co-occurring taxa. We focused on antennal sensilla, as the antenna is the major location for chemical receptive sensilla (Bland 1989; Chen et al. 2003). We predicted more sensilla on the antennae of *B. nivalis* that is predominantly in rocky areas of sparse vegetation, compared to *S. australis* and *P. nitidus*. We also expected that sexual dimorphism in antennal chemosensory structures would be apparent with males (potential signal-receivers) having higher densities of sensilla than females (Bland 1989; Chen et al. 2003; Li et al. 2007). We quantified the abundance and distribution of chemo-sensilla in male and female *B. nivalis*, *S. australis* and *P. nitidus*.

Materials and methods

Insects

Adult grasshoppers of *B. nivalis*, *S. australis* and *P. nitidus* (Fig. 1) were collected during the active summer season on the southeast flank of Hamilton Peak in the Craigieburn Range (43°07'3"0.7"S 171°41'1"0.5"E) with approval from the Broken River ski area operators and New Zealand Department of Conservation (authorization number: 97397-FLO). Insect specimens were frozen then preserved in 99% ethanol. Storage in high concentration ethanol preserved DNA and effectively dehydrates tissues for microscopy.



Fig. 1 Three sympatric New Zealand alpine grasshoppers are cryptically colored in their typical habitat. *Brachaspis nivalis* adult male (a), *Sigaus australis* adult female (b), *Paprides nitidus* adult male (c),

B. nivalis adult female (d), *S. australis* adult female (e), *P. nitidus* adult male (f)

Scanning electron microscopy (SEM)

Antennae were examined under a scanning electron microscope (SEM) after being excised from preserved specimens and fixed in fresh 99% ethanol for one to three days to ensure dehydration, and then air-dried for two days. Fixed antennae were mounted on aluminum stubs, and gold-coated for 200 s with a Baltec SCD 050 sputter coater before examination with an FEI Quanta 200 SEM operated in the range of 15–20 kV.

Antenna size and sensilla

Antennal morphology was examined under a Leica stereo microscope (SM225, Olympus, Japan) equipped with a digital camera (SC180, Olympus, Japan) and antennal lengths were measured using imaging software (NIS-Elements 5.01, Nikon Instruments Inc., USA), at The New Zealand Institute for Plant & Food Research Limited, Palmerston North, with

permission from Dr Kambiz Esfandi. The area of each antennal segment was measured using the Measure function on ImageJ/Fiji with SEM images.

Dorsal and ventral surfaces of either a left or right antenna of each adult grasshoppers were examined for 10 or 11 males and 10 or 11 females of each species. The surface of each antenna was identified by its position in relation to the antennal groove on the frons (Fig. 2), with the presence of a lenticular organ on the ventral surface of segment 14 and the dorsal surface of segment 20 providing confirmation (Fig. 3a, b; Chen et al. 2003; Bland 1989). These grasshoppers have 23 segments on their antenna, but some individuals have subsections within particular segments (Fig. 3c, d), but we ensured consistent segment numbering by measuring the area of each segment (Table S1). The thirteen distal segments (segments 11 to 23; counting from scape, 23rd being the most distal) are those on which chemo-sensitive sensilla have been reported as abundant in other grasshopper species, whereas the proximal segments have sensilla usually linked to proprioception (Bland 1982, 1989; Chen et al. 2003; Jin

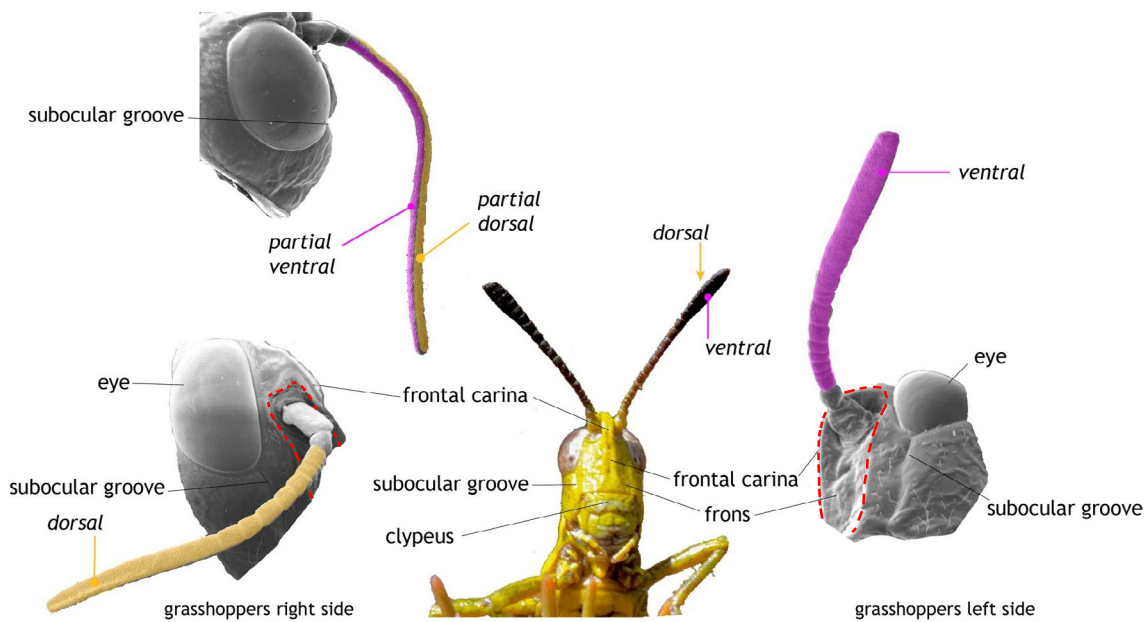
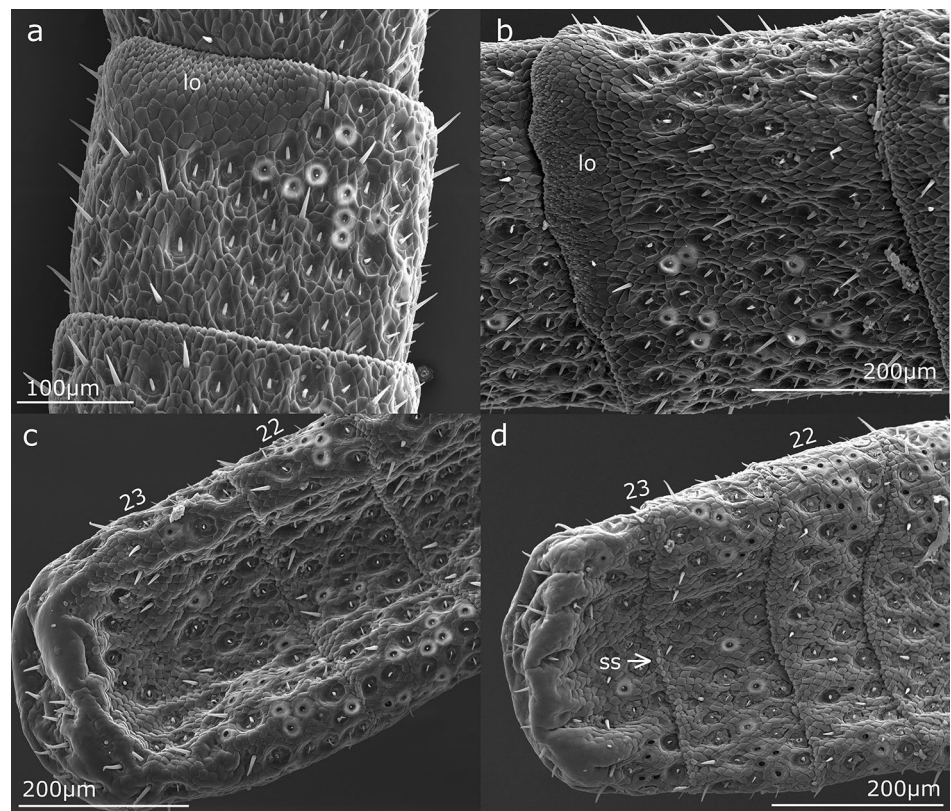


Fig. 2 Identification of ventral (purple) and dorsal (yellow) surfaces of antenna in New Zealand alpine grasshoppers. The surfaces of the antennae were determined by orientation relative to the groove (indicated by red dotted line) between frontal carina and subocular groove

Fig. 3 Antennal morphology of New Zealand alpine grasshoppers (Acrididae; Catantopinae). The lenticular organ (Bland 1989) on the dorsal surface of the 20th segment (a) and the ventral surface of the 14th segment (b). An example of antennae tip (segment 23) without subsection (c) and with subsection (d) in *Brachaspis nivalis*. *lo* lenticular organ, *ss* segment subsection. Numbers indicate segment numberings from attachment to head (most proximal segment)



et al. 2005; Ochieng et al. 1998). Preliminary observations showed a similar pattern of sensilla distribution in *B. nivalis*, *S. australis* and *P. nitidus*, so all sensilla on these thirteen distal segments were recorded.

Sensilla were classified according to the nomenclature used for the locusts *Schistocerca gregaria* and *Locusta migratoria* since these are the most extensively studied taxa (Nakano et al. 2022). The number and size of sensilla was

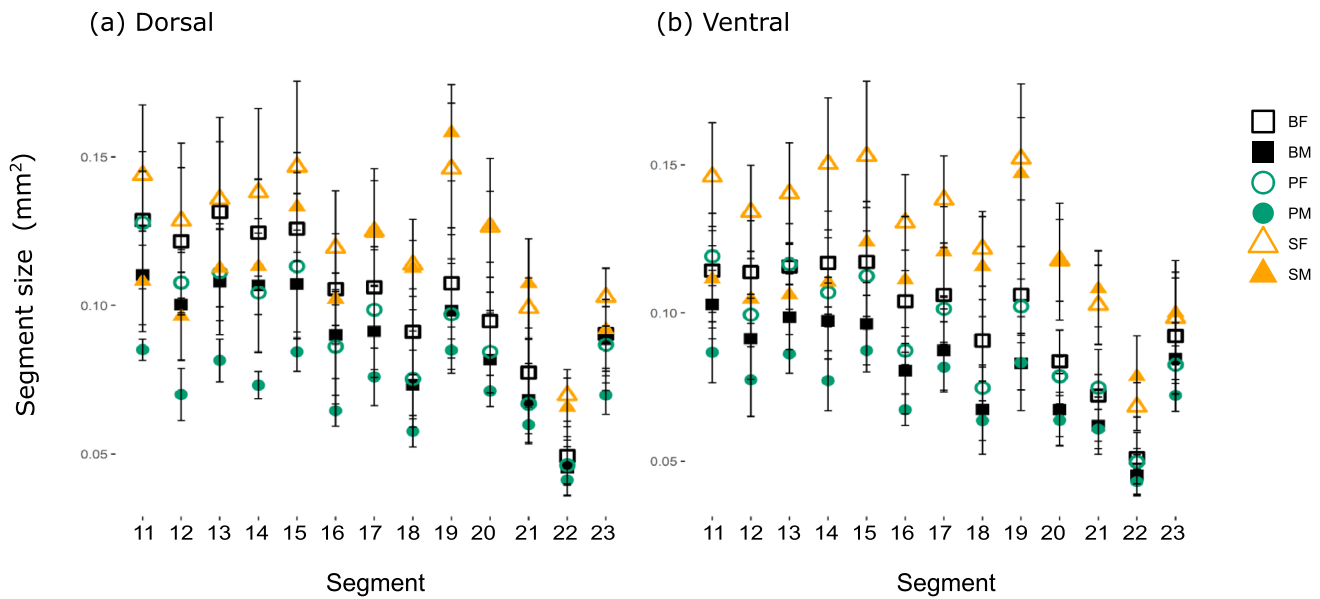


Fig. 4 Variation in antenna size distinguishes three New Zealand alpine grasshopper species. The surface area of each antennal segment (mm^2) for distal half of antennae (segments 11 to 23) on the dorsal (a) and ventral (b) surfaces. Vertical bars indicate stand-

ard deviation. *BF* *Brachaspis nivalis* female, *BM* *B. nivalis* male, *PF* *Paprides nitidus* female, *PM* *P. nitidus* male, *SF* *Sigaus australis* female, *SM* *S. australis* male

counted and measured using the add-in Cell Counter and the Measure functions in Image/Fiji respectively.

Statistical analysis

All statistical analyses were performed in the R statistics environment (R Core Team 2022) using the software platform R Studio 4.0.3 (Boston, MA, USA) and graphics are generated using R Studio 4.0.3 and Inkscape 1.2. Statistical normality was tested by the Kolmogorov–Smirnov test before further analysis. Using a student T-test, the body length (mm), antenna length (mm) and segment area (mm^2) between species of the same sex, and total number and each type of sensilla recorded on the dorsal and ventral surfaces were compared. Differences in total number and number of each type of sensilla on segments 11 to 23 of the dorsal and ventral surfaces were analyzed among species and sexes of the grasshoppers with a linear model using the `lm()` function. This was followed by post hoc Tukey honest significant differences for multiple pair-wise comparisons using the `emmeans` package.

Results

Antennal structure (shape, length, area, and segmentation)

In all three species, an irregular arrangement of sharply pointed cuticular plates known as the lenticular organ

(Fig. 3a, b) was observed on the dorsal surface of the 20th antennal segment and the ventral surface of the 14th segment. The length of antennae ranged between 4.3 and 9.3 mm, with *S. australis* having the longest antennae (male 6.64 ± 0.65 mm, female 7.80 ± 0.96 mm), and similar lengths observed in *P. nitidus* (male 5.54 ± 0.33 , female 7.32 ± 0.63) and *B. nivalis* (male 5.51 ± 0.71 mm, female 6.83 ± 0.96 mm). The surface area of each of the thirteen distal segments (11–23) differed among the three species (Fig. 4). 2D images can potentially underestimate segment area as antennae are not completely flat, in particular, the dorsal surface of *B. nivalis* antennae were often concave (Fig. 3c, d).

No significant difference was observed in the total antennal length between females of *P. nitidus* and *S. australis* ($p=0.22$), but antennae of female *S. australis* were significantly longer than antennae of *B. nivalis* females ($p=0.03$) and the antenna of *S. australis* males were significantly longer than antennae of both *P. nitidus* and *B. nivalis* males ($p<0.01$). Most of the segments were significantly larger in male and female *S. australis* than other species (Fig. 4, Table S1). This is broadly in proportion with their body size as *S. australis* specimens were significantly larger in terms of body length (male 21.04 ± 7.20 mm, female 31.25 ± 2.88 mm) than *P. nitidus* (male 19.08 ± 1.67 mm, female 27.66 ± 2.03 mm) or *B. nivalis* (male 17.68 ± 4.52 mm, female 24.47 ± 2.16 mm). No significant difference in antenna length was observed between *P. nitidus* and *B. nivalis* ($p=0.20$ in females,

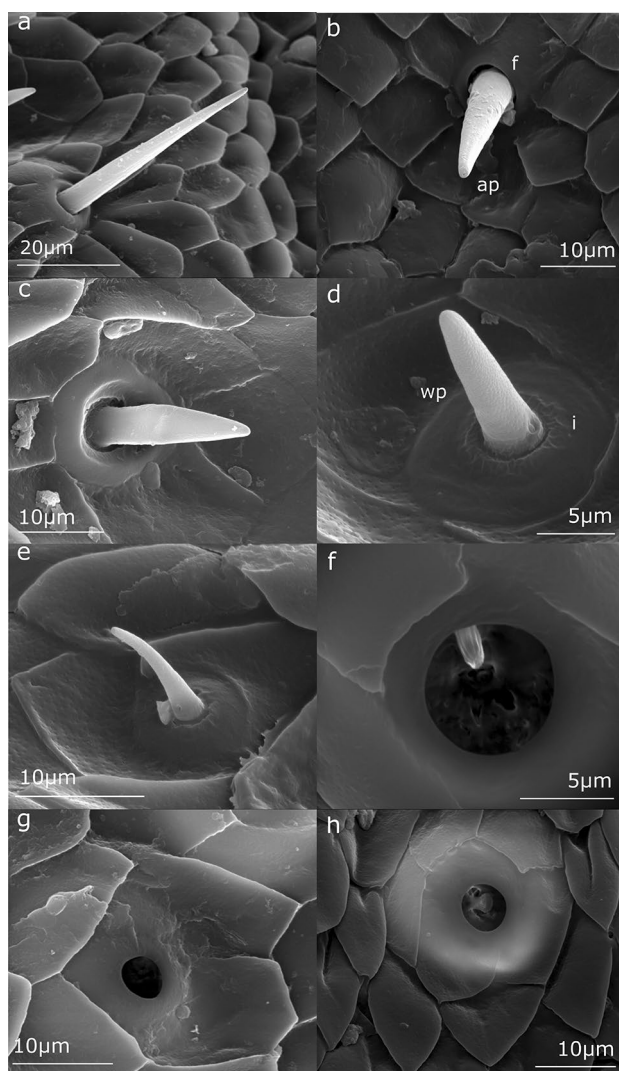


Fig. 5 Types of antennal sensilla found on the antenna of New Zealand alpine grasshoppers (Acrididae; Catantopinae). Chaeticum (a, b), basiconicum (c, d), trichoideum (e), coeloconicum (f), cavity sensillum (g) and cavity sensillum with internal tissue visible (h). *f* flexible socket, *i* inflexible socket, *ap* apical pore, *wp* wall pore

$p = 0.90$ in males) but some segments were significantly larger in both male and female *B. nivalis* compared to *P. nitidus* (Fig. 4, Table S1). In all three species, females had longer antennae (with larger segments) than conspecific males (Fig. 4, Table S1) which is in keeping with their larger body size (Meza Joya et al. 2022).

Sensillatypes, abundance, and distribution

Using sensilla morphology (shape, size, presence/absence of pores, socket types), we recorded five classes of sensilla on the distal antennal segments. These were sensilla chaetica, basiconica, trichoidea, coeloconica and cavity (Fig. 5, Table 1). Within each class of sensilla, size variation was observed (Table 1) and some shape variation was detected in basiconica (Fig. 5c, d), but no species-specific sensilla or shapes were identified. Internal tissue was apparent in images of some cavity sensilla (Fig. 5h) but these were not differentiated from typical cavity sensilla (Fig. 5g).

Males and females of all three species had significantly more chemo-sensilla on the ventral surface of their antennae than on the dorsal surface (Fig. 6a). Three types of olfactory sensilla (basiconica, coeloconica and cavity) were significantly more abundant on ventral surfaces in all species (Fig. 6c, e, f), but significantly more gustatory sensilla (chaetica) were found on the dorsal surfaces of male and female *B. nivalis* antennae (Fig. 6b). No class of sensilla was restricted to a single antennal surface, sex, or species.

The distribution of the five sensilla types along the antennae was consistent among males and females of *B. nivalis*, *S. australis* and *P. nitidus* (Fig. 7). Gustatory sensilla (chaetica) were most abundant at the distal end of each antenna (segment 23) (Fig. 7b, h) in all species. For example, the last antennal segment of *S. australis* had 27–37 chaetica compared to 10–20 on segments 11 to 22 and a similar pattern was seen in *B. nivalis* and *P. nitidus*. Olfactory sensilla consisting of basiconica, coeloconica and cavity were most abundant on the middle antennae segments (especially

Table 1 Types of sensilla, probable function and morphological traits observed on the antennae of three species of New Zealand grasshopper

Sensillum name	Function	Length (µm)	Basal diameter (µm)	Socket type	Pores	Shape
Chaetica (ch)	Gustation & Mechano-reception	15–30	4–6	Flexible	Apical pore	Long peg-like, ribbed wall (Fig. 5a, b)
Basiconica (ba)	Olfaction	10–18	3.5–5.5	Inflexible	Wall-pored	Short and stout peg-like (Fig. 5c, d)
Trichoidea (tr)	Olfaction	11–15	<3	Inflexible	Wall-pored	Thin hair-like (Fig. 5e)
Coeloconica (co)	Olfaction & Thermo-reception	2.5–3.5	3–10 (pit diameter)	Inflexible	Wall-pored	Peg contained within a pit (Fig. 5f)
Cavity (ca)	Olfaction	N/A	3–10 (pit diameter)	N/A	N/A	Pit with (Fig. 5h) or without (Fig. 5g) visible tissue

Identification of chaetica, basiconica, trichoidea and coeloconica is based on locusts (Altner et al. 1981; Ochieng et al. 1998) and cavity sensilla are based on Chinese grasshoppers (Li et al. 2007)

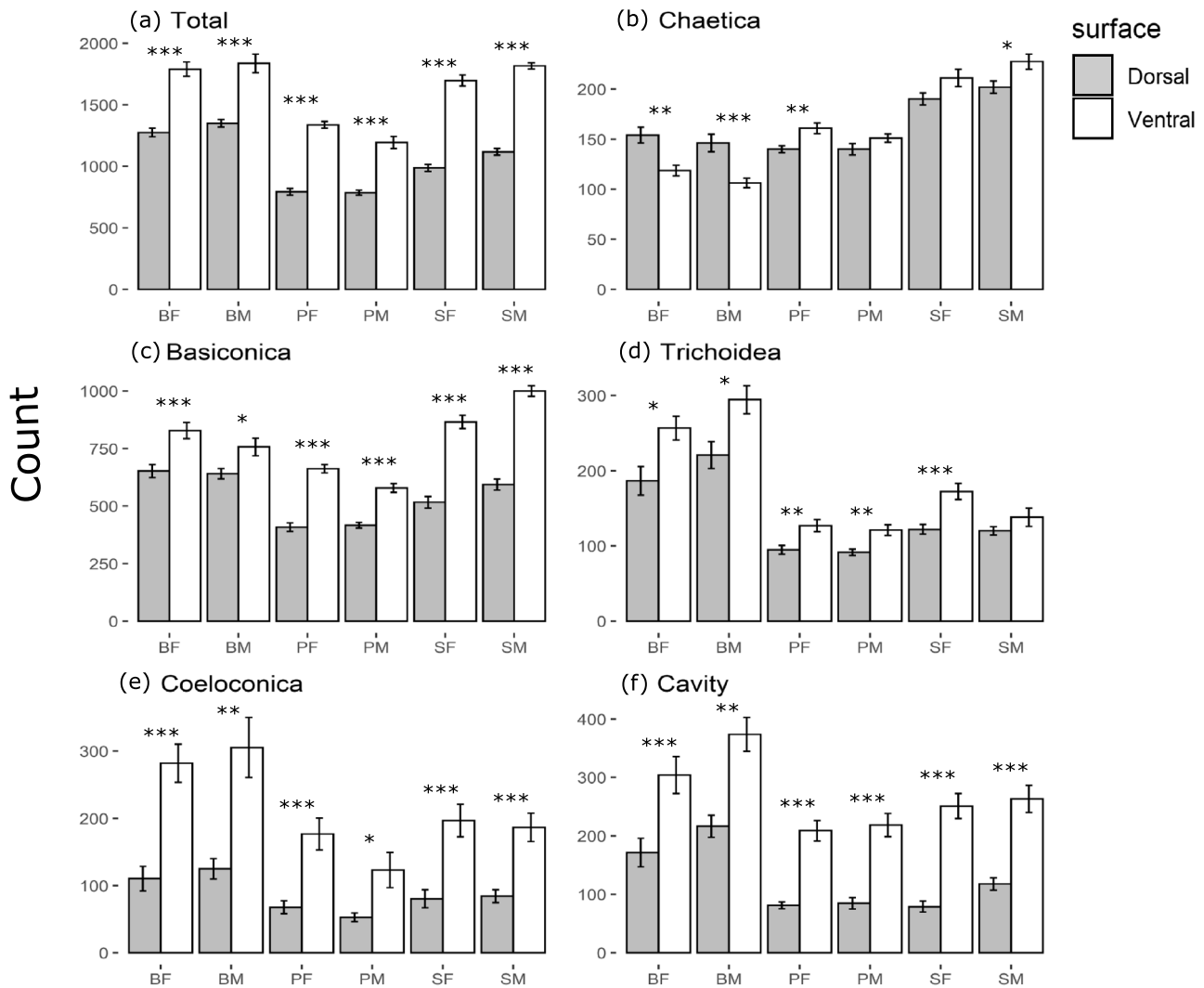


Fig. 6 Mean number of sensilla on dorsal vs. ventral surfaces of distal half of the antennae of three New Zealand alpine grasshopper species (BF *Brachaspis nivalis* female, BM *B. nivalis* male, PF *Paprides nitidus* female, PM *P. nitidus* male, SF *Sigauss australis* female, SM

S. australis male). Vertical bars indicate standard error. *indicates significant difference of sensilla number between dorsal and ventral surface within a group. Significant level: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

on 15 to 20; Fig. 7c, e, f, i, k, l), whereas trichoidea were most abundant on segments 19 or 21 on the dorsal surface (Fig. 7d) and segment 15 on the ventral surface (Fig. 7j).

Comparison of sensilla abundance between species and sexes

The total abundance of sensilla and the proportion of each class on the 13 distal segments of the grasshopper antenna differed between species. *Brachaspis nivalis* had the most chemo-sensilla on their antennae, followed by *S. australis* and *P. nitidus* (Fig. 8a, Table S2). Both male and female *B. nivalis* had significantly more trichoidea than *S. australis* and *P. nitidus* ($p < 0.001$) (Fig. 8d) and *B. nivalis* males

had significantly more coeloconica ($p < 0.02$) and cavity sensilla ($p < 0.001$) than the other species (Fig. 8e, f). *Brachaspis nivalis* and *S. australis* had significantly more basiconica than *P. nitidus* ($p < 0.001$) (Fig. 8c), and *S. australis* (both males and females) had significantly more chaetia than *B. nivalis* or *P. nitidus* ($p < 0.001$) (Fig. 8b).

Female grasshoppers had longer antennae than conspecific males, but no significant differences were observed in the total number of chemo-sensilla between the sexes (Fig. 8a) except for *S. australis* females having fewer basiconica than conspecific males ($p = 0.0138$) (Fig. 8c). Although not statistically significant, the *B. nivalis* males examined possessed more olfactory sensilla than their conspecific females (about 15% more trichoidea, 10% more

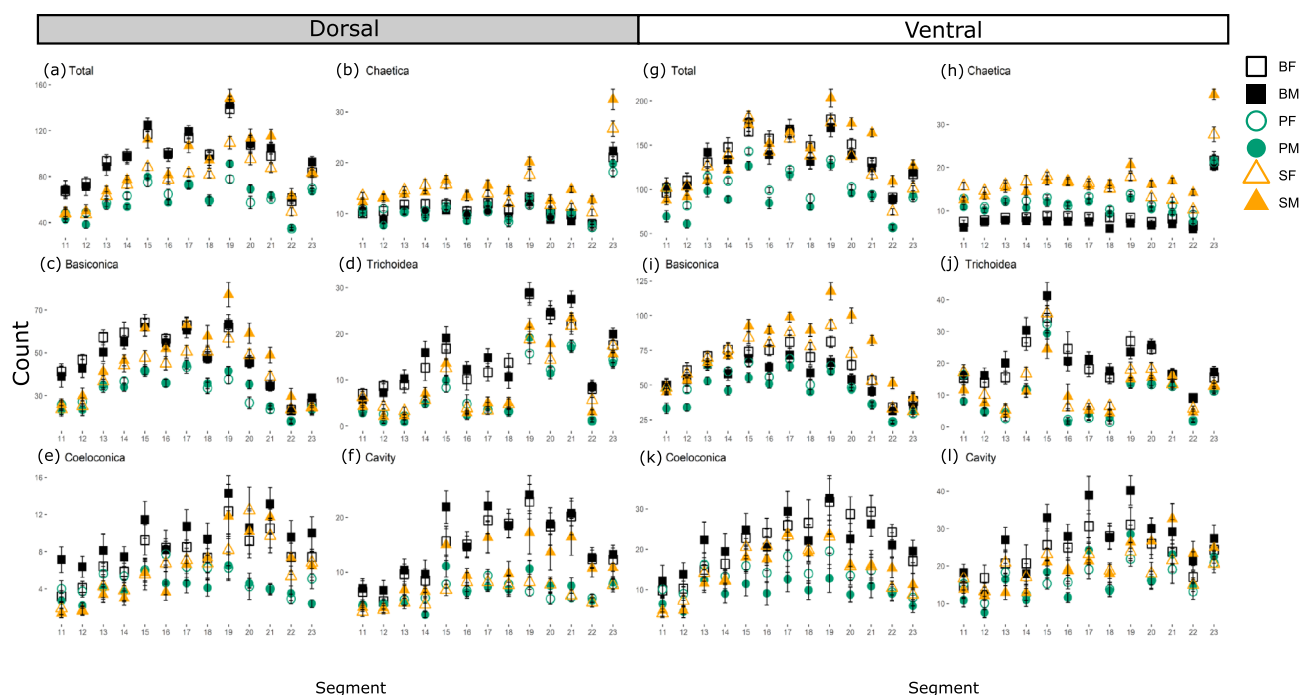


Fig. 7 The distribution of five types of sensilla on the distal half of the antennae of three New Zealand alpine grasshoppers. Mean number of sensilla on the dorsal (a–f) and ventral (g–l) surfaces is shown for each of 13 segments (segment 23 is antennal tip). Vertical bars

indicate standard error. *BF* *Brachaspis nivalis* female, *BM* *B. nivalis* male, *PF* *Paprides nitidus* female, *PM* *P. nitidus* male, *SF* *Sigaia australis* female, *SM* *S. australis* male

coeloconica and 20% more cavity sensilla) while *P. nitidus* females had more olfactory sensilla (about 7% more basiconica and 30% more coeloconica) than their conspecific males (Fig. 8c–f, Table S2).

Discussion

Studies of grasshopper antennal sensilla have focused on particular subfamilies including Gomphocerinae, Oedipodinae and Melanoplinae, and Acridinae. Our comparative study focused on three sympatric and closely related species of Catantopinae. Five classes of chemo-sensilla (chaetica, basiconica, trichoidea, coeloconica and cavity) were identified on the antennae of adult males and females of flightless, alpine, grasshopper species endemic to Aotearoa/New Zealand. The distribution and abundance of sensilla were similar in all three species with sensilla significantly more abundant on the ventral surface of their antennae and chaetica more abundant at their apex. We found that *B. nivalis* had significantly more chemo-sensilla than either *S. australis* or *P. nitidus*. No significant differences in numbers of sensilla were observed between sexes of *B. nivalis* or *P. nitidus*, however, male *S. australis* had more basiconic sensilla than conspecific females.

Sensilla types, abundance, and distribution

Chemo-sensilla are diverse in shape with varying numbers of sensory neurons (Altner et al. 1981; Baker et al. 2008; Jin et al. 2006; Ochieng et al. 1998; Romani and Stacconi 2009; Yang et al. 2012; Zhou et al. 2009) and exhibit sensitivity to different chemical compounds (Altner et al. 1981; Cui et al. 2011; Ochieng and Hansson 1999). Four of the five types of chemo-sensilla present in the grasshopper species examined here (chaetica, basiconica, trichoidea, coeloconica and cavity), have been described and studied in *Schistocerca gregaria* (Cyrtacanthacridinae) and *Locusta migratoria* (Oedipodinae) locusts (Altner et al. 1981; Cui et al. 2011; Jin et al. 2006; Ochieng et al. 1998; Yang et al. 2012; Zhou et al. 2009). In contrast, cavity sensilla were not observed in locusts (Ochieng et al. 1998), but reported from grasshopper species of other subfamilies; *Acrida cinerea* (Acridinae), *Chrysacris changbaishanensis*, *Chrysacris jiamusi*, *Chrysacris heilongjiangensis*, *Chrysacris liaoningensis*, *Mongolotettix angustiseptus*, *Euthystria lueifemora* and *Chrysochraon dispar* (Gomphocerinae) (Li et al. 2007). We identified the rosette of cuticular plates (Bland 1982) or lenticular organ (Bland 1989; Chen et al. 2003), which has previously been recorded on the distal end of antennae in other Acridid species, but its function is unknown.

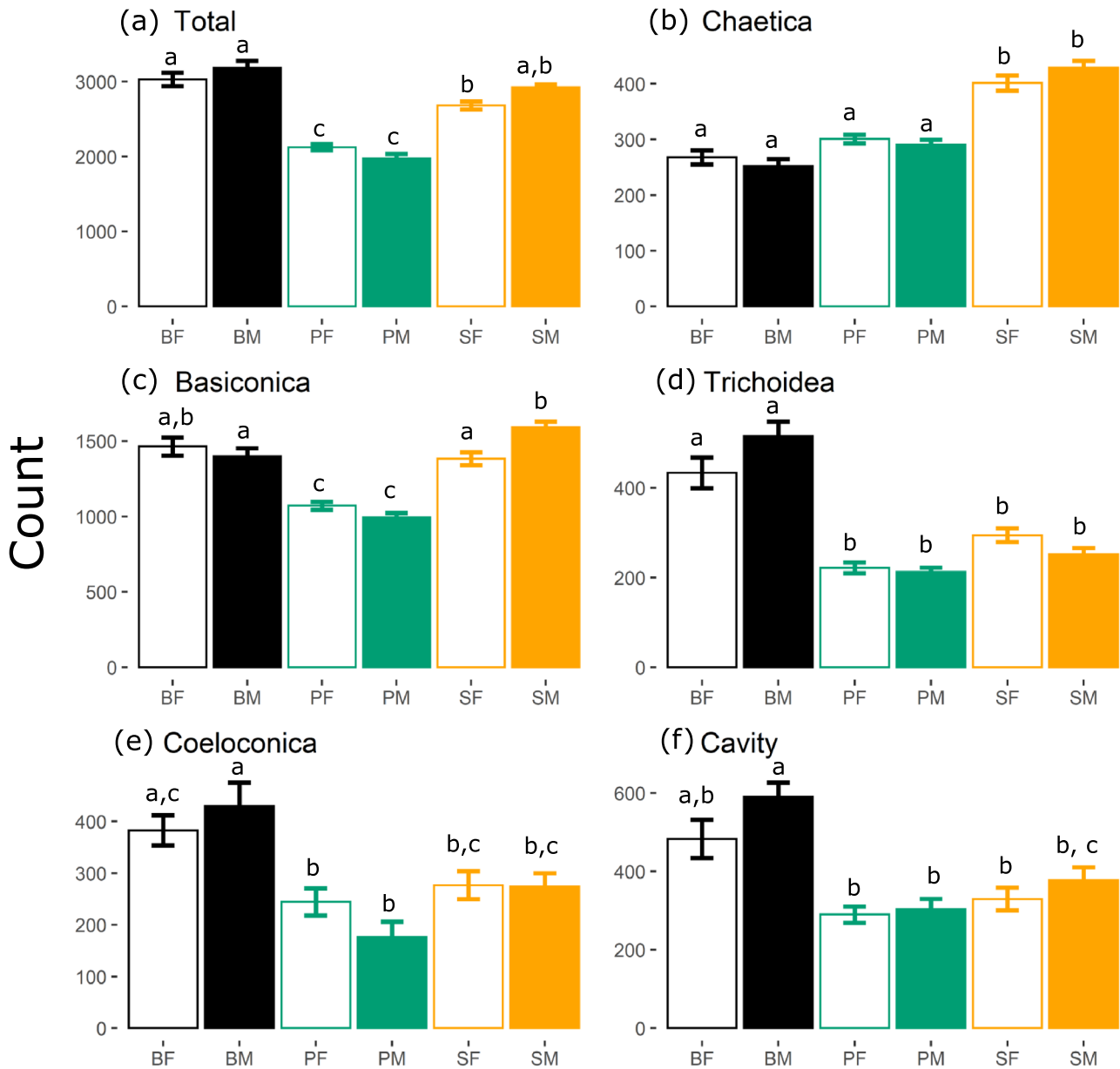


Fig. 8 Variation in abundance of sensilla on the antennae of three New Zealand alpine grasshopper species. Mean number of each sensilla type on the dorsal surface of the distal half of antennae are compared. Vertical bars indicate standard error. Different letters indicate significant differences between males and females within a species

revealed from a linear model followed by a pair-wise post hoc Tukey honest significant test (Table S2). *BF* *Brachaspis nivalis* female, *BM* *B. nivalis* male, *PF* *Paprides nitidus* female, *PM* *P. nitidus* male, *SF* *Sigaus australis* female, *SM* *S. australis* male

Sensilla chaetica have contact-chemical and mechano-receptive functions (with their flexible attachments), whereas basiconica and trichoidea are olfactory (Bland 1989; Chen et al. 2003; Cui et al. 2011; Jin et al. 2006; Li et al. 2007; Ochieng et al. 1998). In short-horned grasshoppers, there are two known types of sensilla coeloconica: one with a blunt tipped peg and an apical pore sensitive to temperature and humidity; and the other with a sharp-tipped

peg and wall pores sensitive to temperature and olfaction (Altner et al. 1981). Sensilla coeloconica in the New Zealand alpine grasshoppers have a sharp-tipped peg and are therefore likely to be thermo- and olfactory-receptors. Although electrophysiological examination of cavity sensilla has never been made, they are considered to be olfactory since they are distributed in a similar pattern to other olfactory sensilla (Li et al. 2007). Some of the cavity sensilla examined

contained visible internal tissue (Fig. 5h) but these were assumed to be typical olfactory sensilla and were not differentiated (Fig. 5g). This unusual form has not been reported before from grasshoppers, but their detection might simply result from cuticle orientation and high resolution imaging.

We detected size and shape variation within types of sensilla as observed in locusts, where they are interpreted as capable of detecting different chemical stimuli and housing different types of chemosensory neurons and proteins (e.g., chaetica: Zhou et al. 2009; trichoidea: Cui et al. 2011, You et al. 2016), and this may be the case for the alpine grasshoppers studied here.

Few studies have compared the ventral and dorsal surfaces of grasshopper antennae (Bland 1982) or those of other insects (Liu et al. 2021; Romani and Stacconi 2009; Yuvaraj et al. 2018). Comparisons can reveal complex specialization, for example, female mugwort grasshoppers *Hypochlora alba* (Melanoplinae) have 25% more coeloconica on their ventral surface compared to their dorsal surface, but males of the species have 10% more on the dorsal than the ventral surface (Bland 1982). With the exception of chaetica on *B. nivalis* antennae, chemo-sensilla were significantly more abundant on the ventral surface compared to the dorsal surface of all three New Zealand alpine grasshopper species. In live grasshoppers, the ventral surface of the erect antennae face forward, detecting stimuli in front of them (as shown in Fig. 1).

Patterns of sensilla distribution along the antennae of the three New Zealand alpine grasshoppers were broadly similar to observations of other grasshopper species. For example, high abundance in olfactory sensilla (basiconica, trichoidea, coeloconica) at the middle to distal portion has been observed in other species (Bland 1989; Chapman 1989; Li et al. 2007; Ochieng et al. 1998). At the most distal end of the antenna, sensilla chaetica are most abundant, and therefore it is likely that this segment is predominantly involved in gustation (contact chemoreception). Watson (1970) observed New Zealand alpine grasshoppers touching plants with their antennae, suggesting that touch (either mechanical or contact chemoreception) is used for food selection.

Comparison of sensilla abundance between species and sexes

The abundance of sensilla is often linked to species-specific characteristics in the distribution of food and the roles of the two sexes (Bland 1989; Chen et al. 2003; Malo et al. 2004; Li et al. 2007, 2021a; b). Notable in this study was how few species-specific or sex-specific differences we detected. We saw few differences when sensilla of *S. australis* and *P. nitidus* were compared, however, we found that *B. nivalis* have distinct sensilla abundance when compared to *S. australis* or *P. nitidus*. *Brachaspis nivalis* have large distal segments

although their antennae are the same length as *P. nitidus*. Enlarged segments at the distal end of antenna facilitate more olfactory sensilla, where *B. nivalis* have significantly more trichoid sensilla than either *S. australis* or *P. nitidus*, and male *B. nivalis* have significantly more coeloconica and cavity sensilla than other species. The number of chaetica (gustatory sensilla) in *B. nivalis* is significantly lower than seen on *S. australis* antennae. These sensilla differences suggest that *B. nivalis* may rely more on olfaction (i.e., distance cues) than gustation (i.e., contact cues) compared to *S. australis*. This is consistent with their association with rocky/scree habitat where food plants are sparser than the habitats of *S. australis* and *P. nitidus* (Bigelow 1967; Koot 2018; Watson 1970). On scree slopes, *B. nivalis* may be more reliant on long-range signals than short-range signals to find food sources. Both *S. australis* and *P. nitidus* are commonly found in mixed shrub, herb and scree habitats than scree-only habitats (Koot 2018; Watson 1970), but *S. australis* (both males and females) have significantly more chemosensilla on their antennae than *P. nitidus*. *Paprides nitidus* antennae are also shorter and have significantly smaller segments than those of *S. australis*.

In many grasshopper species, males have more sensilla on their antennae than females (80% of 75 species examined by Bland 1989, Chen et al. 2003 and Li et al. 2007). These sexual differences are attributed to natural selection on males to have high sensitivity to pheromones released by females (Chen et al. 2003; Malo et al. 2004; Wee et al. 2016; Li et al. 2021b). As the New Zealand alpine grasshoppers tend to be visually cryptic (to avoid visual predators) and do not generate acoustic signals with wings when searching for mates (Watson 1970; personal observation), we expect chemical communication to be important in all three species. In the present study, however, the number of sensilla displayed by males and females differed very little. We did find that male *S. australis* had significantly more basiconica than females. Basiconica, also called short basiconica (Bland 1989; Chen et al. 2003) or basiconic sensilla I–V (Li et al. 2007) have been reported as more abundant in males of other species belonging to Melanoplinae, Cyrtacanthacridinae, Oedipodinae, Gomphocerinae, northern Catantopinae, Pamphaginae and Acridinae, in 36/55 species examined by Bland (1989), all 12 species by Chen et al. (2003), and all eight species by Li et al. (2007). Sex-biased abundance of sensilla type may be due to sex-specific requirements to detect particular stimuli, such as sex pheromones and oviposition-site selection (Rai et al. 1997; Chen et al. 2003; Malo et al. 2004; Wee et al. 2016; Roh et al. 2020; Li et al. 2021b).

No significant difference was observed between male and female *P. nitidus* although females usually had more basiconica and coeloconica than males. An equal number of sensilla with similar olfactory sensitivity between sexes observed in *A. barbensis* is thought to reflect their reliance

on visual and auditory cues when finding mates (Chen and Kang 2000). However, solitary *S. gregaria* males showed higher electrophysiological responses to potential sex pheromones than solitary females (Ochieng and Hansson 1999) despite the equal abundance of sensilla in males and females (Ochieng et al. 1998). Detailed investigations using neurological and electro-physiological studies are required to further characterize sexual differences in the olfactory sensitivity and functional diversity of sensilla. All three grasshopper species studied here have relatively large eyes, and it is possible that despite their disruptive and camouflage color patterning they signal visually to one another. This study serves as a base for further behavioral and electrophysiological (electroantennography or single sensillum recordings) analysis to elucidate the chemical ecology of endemic New Zealand grasshoppers and contribute to understanding of their evolution and diversity.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Inkscape 1.0, R Studio 4.0.3.

Declarations

Conflict of interest The author declare that they have no conflict of interest.

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