

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

The abundance of sensilla of various types appears to be related to several ecological factors including the dietary range (i.e., monophagous, oligophagous, polyphagous:

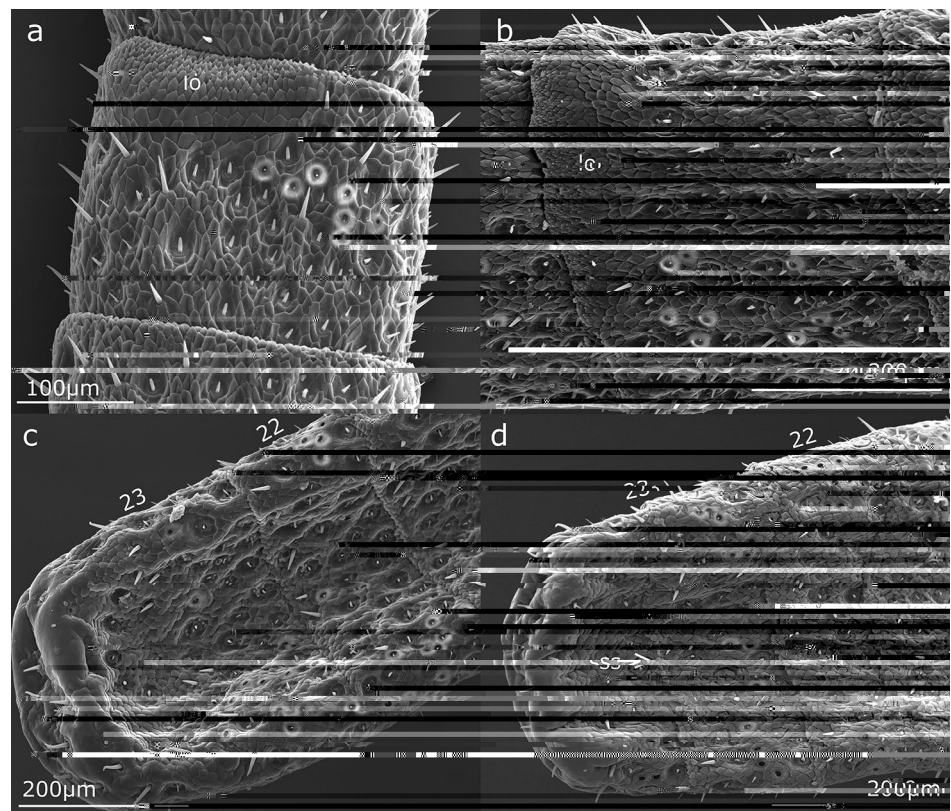
Scanning electron microscopy (SEM)

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



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

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

$p=0.90$ 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

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 chaetica 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).

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

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.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00435-022-00579-z>.

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