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The 'hairwheels' in *Strumigenys* ants are not glandular

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ABSTRACT. Ants of the myrmicine genus *Strumigenys* are characterized by the occurrence of a number of exocrine glands that are exclusive to this genus. One of these structures is the presumed 'mesopleural gland', although its real existence has never been documented. Examination of this conspicuous mesopleural 'hairwheel' organ in 19 *Strumigenys* species with further scanning microscopy and histology revealed that this structure does not contain any glandular tissue. The peculiar bristle-lined hairwheels of *Strumigenys* are not covering lateral cavities but are part of the typical transversal ventral groove lying behind the forecoxal insertions, present across most ants. A survey across non-*Strumigenys* ants using scanning microscopy showed a great variation in the shape of the cuticle and the pilosity surrounding the equivalent mesopleural area, from bare lateral openings to rudimentary hairwheel-like structures in some myrmicine genera. The precise function of the hairwheels remains unknown.

Keywords	Mesopleural excavation, thorax, ants, exocrine glands, histology, SEM
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INTRODUCTION

The more than 800 described species of *Strumigenys* ants are small predators that feed mainly on collembola and other small arthropods (Masuko 1984; Dejean 1987). The genus is a highly diversified clade within which "trap-jaw" mandibles - specialized mousetrap-style mandibles for fast prey-catching - evolved many times independently (Larabee & Suarez 2014; Booher et al. 2021). Most of them form small colonies in leaf litter or decaying wood in warm temperate regions. *Strumigenys* for long has been the largest genus in the myrmicine tribe Dacetini (Bolton 1999; Baroni Urbani & de Andrade 2007), a tribe recently synonymized within the enlarged tribe Attini based on molecular evidence (Ward et al. 2015). Bolton (1999) stated that *Strumigenys* ants are characterized by a basic set of five exocrine glands that are exclusive to this genus, and made this claim based solely on scanning microscopy observations. Among these presumed exocrine structures, the apicofemoral and apicotibial glands in the legs were soon histologically confirmed (Billen et al. 2000; Billen 2009). Evidence for the existence of the ventral scape gland (Wang et al. 2021a) and basimandibular gland (Wang et al. 2021b) was added recently. All four confirmed exocrine structures correspond with a thickened glandular differentiation of the tegumental epidermis (class-1 according to the universally used gland classification by Noirot & Quennedey 1974). The cuticle overlaying the glandular epithelium externally appears under a scanning microscope as a smooth patch that led Bolton (1999) to the conclusion about its glandular nature. Only at sufficiently high magnification of the surface of this smooth patch, hundreds of minute pores of around 100 nm can be seen, that represent the cuticular openings through which the glandular secretions are released to the exterior (Billen et al. 2000; Wang et al. 2021a,b).

The fifth of Bolton's presumed glands is the 'mesopleural gland' that has a very different and conspicuous external appearance, and was described as "s*hape and size variable but usually a roughly circular excavation in the anterior margin of the mesopleuron at its junction with the posterolateral pronotal margin, posterior to the insertion of the first coxa. The excavation is frequently lined with small setae that are directed towards the central point of the cavity*" (p. 1665 in Bolton 1999). An even more noticeable mesopleural excavation was already found by Brown in the very rare and aberrant myrmicine *Pilotrochus besmerus* Brown, 1978 from Madagascar, who referred to it as a 'hairwheel' and also interpreted it as a possible gland organ (Brown 1978). We here report on our examination of these mysterious hairwheels, and clarify the apparent confusion that was created by observations with scanning microscopy versus histological sections.

Table 1. Survey of ant material with collection locality and number of workers (W), queens (Q) and males (M) examined using scanning microscopy (SEM) and histological sections.

	species	**Collection locality**	**SEM**			**histology**		
			W	**Q**	**M**	**W**	**Q**	**M**
short-mandibulate *Strumigenys*	*S. benten* (Terayama, Lin & Wu, 1996)	Yuchi Township, Nantou County, Taiwan	4		2	5		
	S. elegantula (Terayama & Kubota, 1989)	Yuchi Township, Nantou County, Taiwan	3			2	1	
	S. emmae (Emery, 1890)	Baihe District, Tainan City, Taiwan	1			2		
	S. leptothrix Wheeler, 1929	Ren'ai Township, Nantou County, Taiwan	1	1		3	1	
	S. membranifera Emery, 1869	Ren'ai Township, Nantou County, Taiwan	2	1				
	S. mutica (Brown, 1949)	Yuchi Township, Nantou County, Taiwan	5	3	2	2		
	S. sauteri (Forel, 1912)	Lugu Township, Nantou County, Taiwan	1					
	S. sydorata Bolton, 2000	Bogor, Indonesia					1	

long-mandibulate *Strumigenys*	*S. chuchihensis* Lin & Wu, 2001	Jianshi Township, Xinchu County, Taiwan	1				
	S. formosensis Forel, 1912	Ren’ai Township, Nantou County, Taiwan	1			3	
	S. hispida Lin & Wu, 1996	Yuchi Township, Nantou County, Taiwan	1			2	1
	S. lewisi Cameron, 1886	Matsuyama-shi, Japan	1	1			
	S. liukueiensis Terayama & Kubota, 1989	Jiji Township, Nantou County, Taiwan	1	1			
	S. minutula Terayama & Kubota, 1989	Hengchun Township, Pingtung County, Taiwan	4			1	
	S. nanzanensis Lin & Wu, 1996	Lanyu Township, Taitung County, Taiwan	3			1	
	S. orchidensis Lin & Wu, 2001	Lanyu Township, Taitung County, Taiwan	3	1		1	
	S. perplexa (F. Smith, 1876)	Clyde Mountain, NSW, Australia				2	
	S. rogeri Emery, 1890	Jiji Township, Nantou County, Taiwan	1	1			
	S. solifontis Brown, 1949	Lugu Township, Nantou County, Taiwan	4	2		2	2
non-*Strumigenys*	*Dolichoderus laminatus* (Mayr, 1870)	Yutajé, Amazonas, Venezuela	1				
	Eciton hamatum (Fabricius, 1782)	Yutajé, Amazonas, Venezuela	1				
	Ectatomma tuberculatum (Olivier, 1792)	Corcovado National Park, Puntarenas, Costa Rica	1				
	Eurhopalothrix procera (Emery, 1897)	Hengchun Township, Pingtung County, Taiwan	1				
	Formica fusca Linnaeus, 1758	Macomb Township, New York, USA	1				
	Heteroponera brouni (Forel, 1892)	Cascade Kauri, Waitakere, New Zealand	1				
	Manica rubida (Latreille, 1802)	Albula Pass, Switzerland	1				
	Myrmica americana Weber, 1939	Macomb Township, New York, USA	1				
	Pogonomyrmex barbatus (F. Smith, 1858)	Antigua, Veracruz, Mexico	1				
	Ponera pennsylvanica Buckley, 1866	Ithaca, New York, USA	1				
	Prionopelta antillana Forel, 1909	San Ramón de Pangoa, Junin, Peru	1				
	Proceratium croceum (Roger, 1860)	Tillman, South Carolina, USA	1				

MATERIAL AND METHODS

We examined 19 mainly Taiwanese *Strumigenys* species with scanning microscopy and histology, and include SEM-observations on 12 species of other genera for comparison. A survey list of the ants examined in this study is given in Table 1. For histological examination, the anterior part of the thorax was fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Postfixation was carried out in 2% osmium tetroxide in the same buffer. After dehydration in a graded acetone series, tissues were embedded in Araldite and sectioned with a Leica EM UC6 ultramicrotome. Serial semithin 1 µm sections were stained with methylene blue and thionin and viewed in an Olympus BX-51 microscope. Entire ants for scanning microscopy were attached to aluminium stubs with double-adhesive tape, gold-sputtered and examined in a JEOL JSM-6360 scanning microscope or a Hitachi S4700 field emission scanning microscope.

RESULTS

Profile views under the scanning microscope of all examined *Strumigenys* species (except *Strumigenys mutica* (Brown, 1949), see further) show a pronounced round to oval excavation with a diameter of 25-40 µm depending on the overall size of the ant (Fig. 1A-F). The anterior side of the structure is formed by the ventroposterior margin of the pronotum, the upper and posterior side is formed by the mesopleura. The lower part is often formed by a cone-like protuberance that projects anterad, and that in many species almost touches a similar but smaller protuberance that projects posterad from the ventrolateral corner of the pronotum, thus making the entire structure appear like a hole. Long and slender bristle hairs originate from the mesopleural part. These hairs point anteriorly and are directed towards the central anterior part of the excavation (Fig. 1A-F). The structure exists in workers, queens and males. Only in *S. mutica*, this region in all castes appears as a narrow vertical slit of 5-10 µm without any hair (Fig. 1G-I).

As there is no external indication such as pores that may be linked to the presence of gland cells in any of the investigated species, we extended our search with a histological examination of thorax sections. This confirmed that no glandular tissue occurs in this mesopleural region (Fig. 2). The epithelium underneath the cuticle of the excavation region is hardly visible with a thickness of only 1-2 µm, and there is no trace of any ducts that could connect to class-3 secretory cells. The only tissue that can be observed in this region is the mesothoracic ganglion, the ventral nerve cord (Fig. 2B,C) and flight muscles (in queens, Fig. 2D,E). The serial sections in addition reveal that there is no confined cavity at all behind the externally visible hairs, as could possibly be expected from SEM-observation. Instead, the excavations at both sides are transversely connected by a wide and hairy ventral groove (Fig. 2C,E). The presence of the groove and the hairs distributed in it are also clearly visible on longitudinal sections (Fig. 2F,G). Only in *S. mutica*, the groove is more narrow and devoid of hairs in both workers and queens (Fig. 2H).

The absence of any glandular tissue and the apparent existence of a transverse ventral groove rather than two lateral cavities, as revealed by histological observations, prompted us to re-analyse the mesopleural region with scanning microscopy. In order to have a proper ventral view, we completely removed the forelegs, as these in their natural position obstruct such view. These new SEM-observations clearly confirmed the existence of a transverse groove lined with anteriorly directed hairs (Fig. 3A-I), except in *S. mutica*, where a groove without hairs is found (Fig. 3J-L). It is noteworthy that dirt particles can sometimes be seen near the hairs on sections (Fig. 2E) as well as on scanning micrographs (Fig. 1F,3E-H,I).

Examination of the mesopleural region with scanning microscopy in workers of non-*Strumigenys* ants shows that many ants show some sort of mesothoracic protuberance (Fig. 4). Especially in some myrmicine genera as *Manica* and *Myrmica*, lateral openings can be observed that resemble rudimentary hairwheels (Fig. 4K,L).

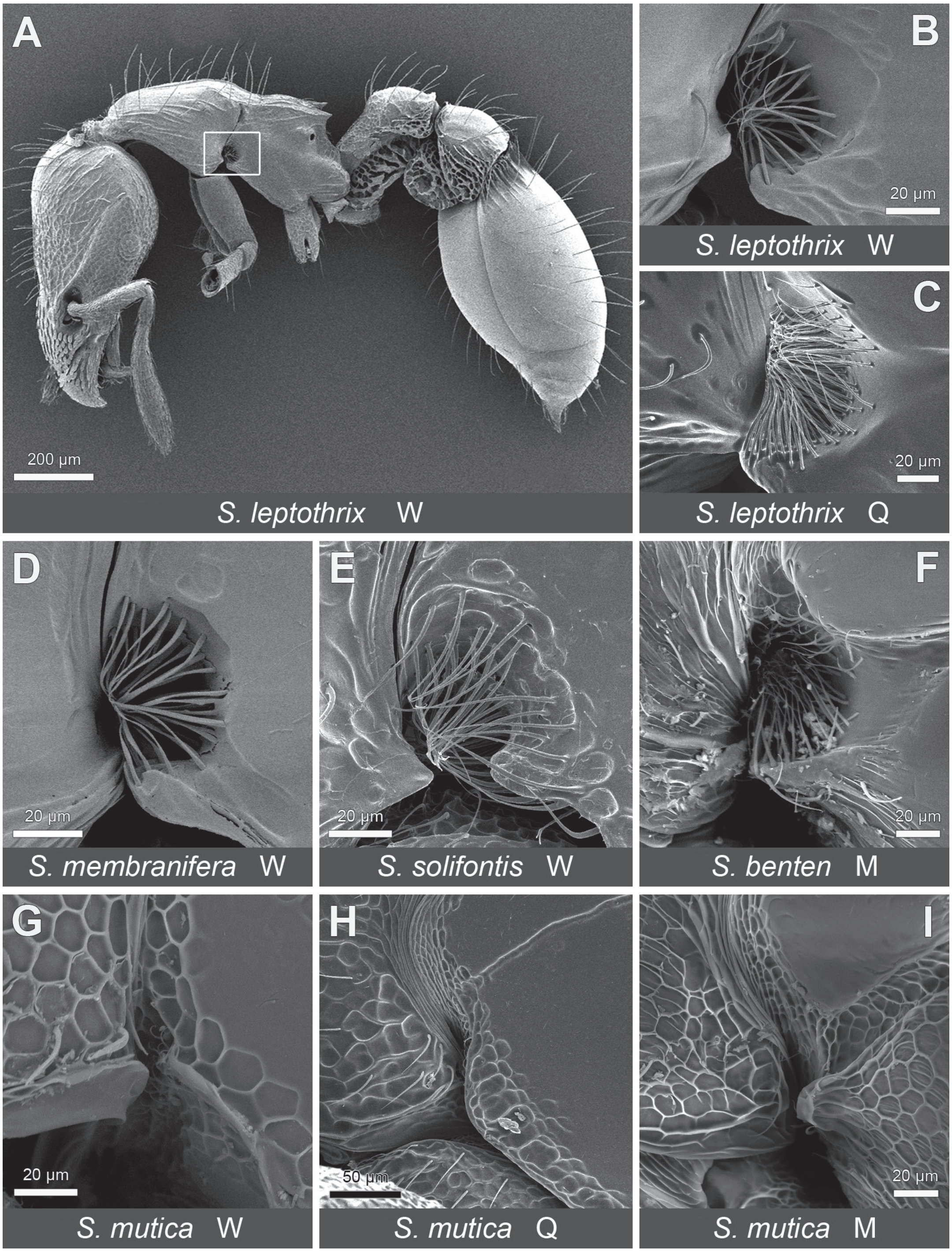

Fig. 1. Scanning micrographs of the left side profile view of a *S. leptothrix* worker (**A**, framed part is enlarged in **B**) and close-up views of hairwheels in other species and castes (**B**-**I**). M: male, Q: queen: W: worker.

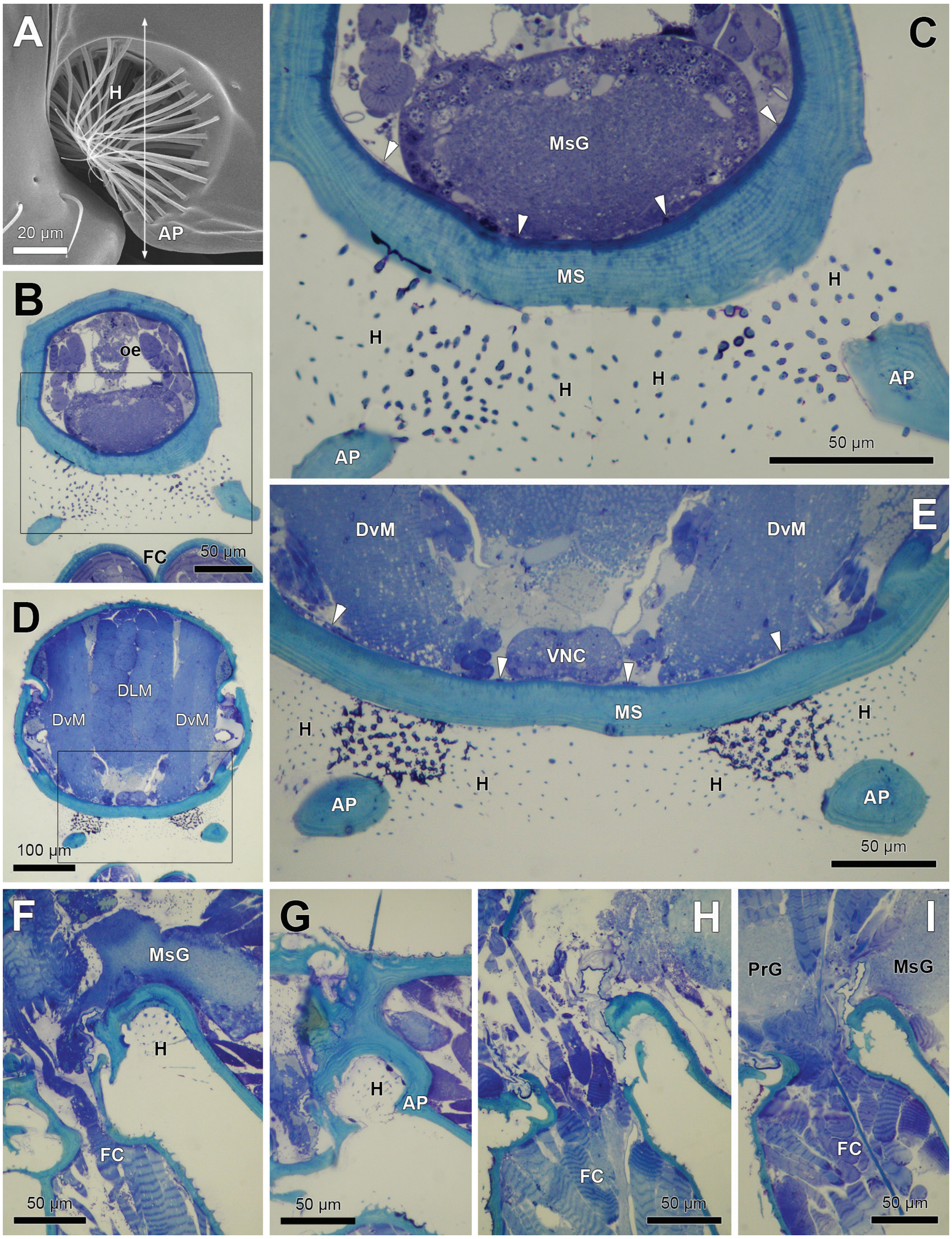

Fig. 2. A. Scanning micrograph showing mesopleural excavation of *S. benten* worker with anterior protuberance (AP) and hairs (H), double arrow indicates sectioning plane of transverse sections in B-E. **B**. Transverse section of *S. benten* worker (frame enlarged in C). **C**. Detail of ventral anteromesothoracic region showing flat tegumental epidermis (arrowheads), mesothoracic ganglion (MsG) and hairs (H) underneath anterior mesosternum (MS). **D**. Transverse section of *S. elegantula* queen (DLM: dorsal longitudinal flight muscles, DvM: dorsoventral flight muscles; frame enlarged in E). **E**. Detail of anteromesothoracic region showing flat tegumental epidermis (arrowheads). VNC: ventral nerve cord; note dirt accumulation on hairs. F-G. Longitudinal sections of *S. solifontis* worker in central region of hairy groove (**F**) and at level of anterior protuberance (AP) in **G**. H-I. Longitudinal sections of *S. mutica* queen (**H**) and worker (**I**) in central region of groove, note absence of hairs. FC: foreleg coxa, oe: oesophagus, PrG: prothoracic ganglion.

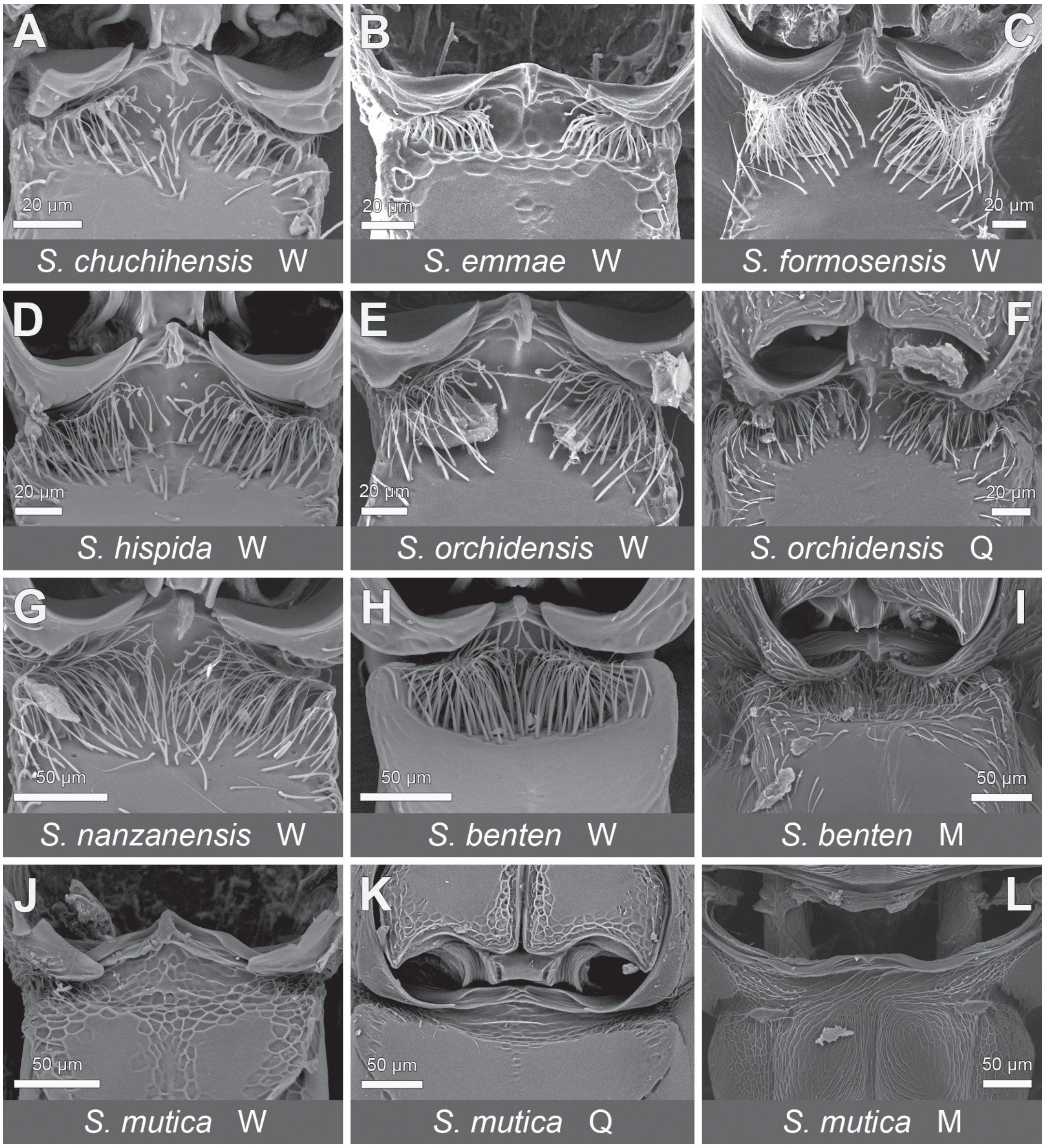

Fig. 3. Ventral view scanning micrographs of the transverse groove at the anteroventral region of the mesothorax, behind the procoxal insertions (forelegs have been removed to expose the groove). Notice the anteriorly directing bristle hairs in all species and castes examined (**A**-**I**), except in *S. mutica* (**J**-**L**).

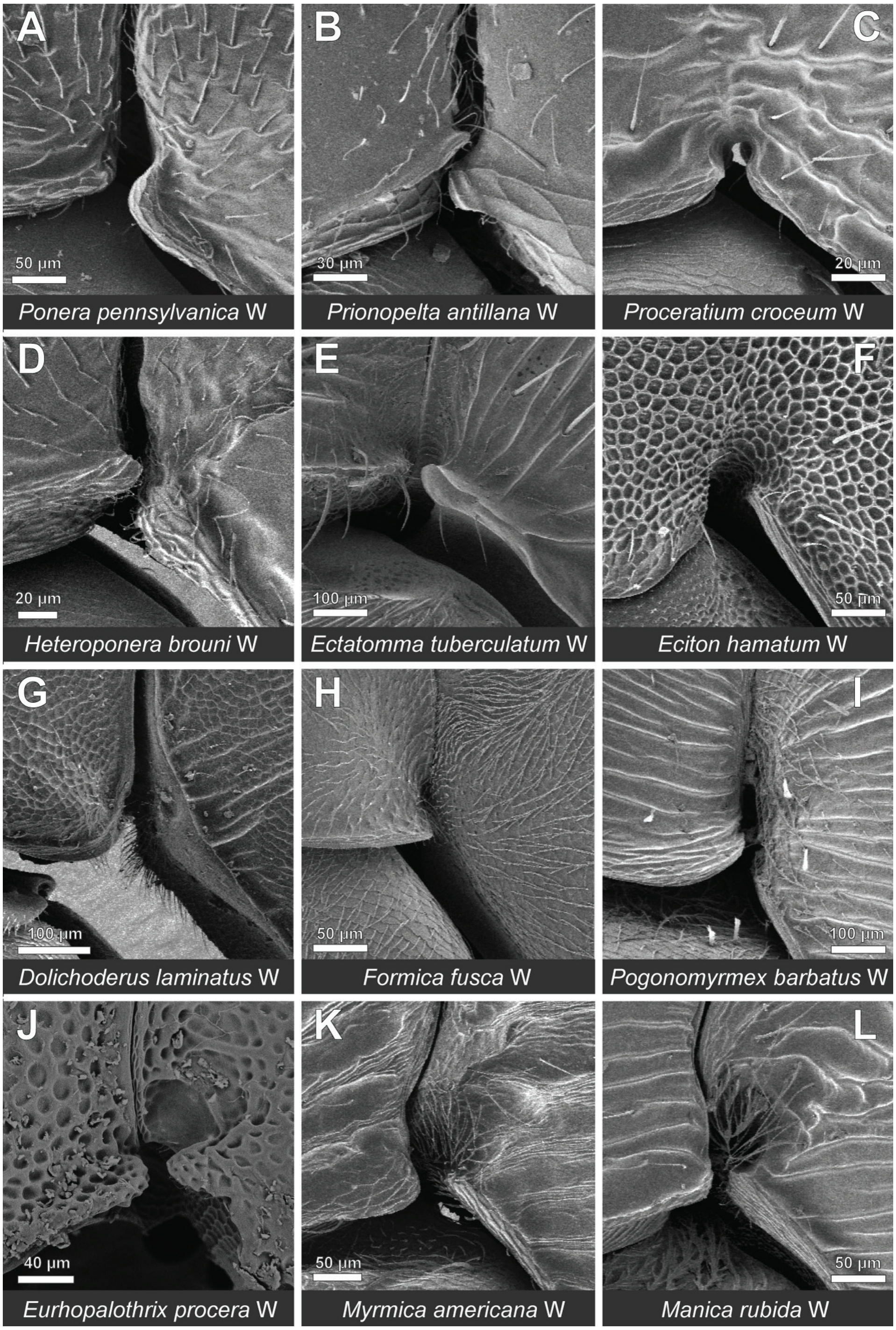

Fig. 4. **A**-**L**. Scanning micrographs of the left side profile view of various ant workers in different species. Notice that some myrmicine genera (**K**,**L**) have lateral opening resembling rudimentary hairwheels.

DISCUSSION

The appearance of the hairwheel-like mesopleural excavation in all examined *Strumigenys* ants (except *S. mutica*, see further) as a conspicuous tuft of hairs that cover an excavation under the scanning microscope may well give the impression of this being some form of gland opening. In particular, the opening of the metapleural gland of some ant species (e.g. *Technomyrmex* (Pech & Billen 2017) and *Aneuretus* (Billen 2017)) or the acidopore of formicine ants (see Keller 2011) has a fairly similar appearance, and may have led Bolton (1999) to his erroneous conclusion that the mesopleural excavation of *Strumigenys* represented the opening of a presumed mesopleural gland.

Histological sections, however, revealed that the region of the mesopleural excavation is not associated with any glandular tissue, neither of class-1 nor of class-3 (which are the two categories of exocrine glands following the standard classification by Noirot & Quennedey 1974). In case of a class-1 gland, the tegumental epidermis would appear considerably thickened (as e.g. for the mandibular pit gland (Billen & Al-Khalifa 2016) and the basitarsal sulcus gland (Billen et al. 2021) underneath pit-like and groove-like excavations, respectively). The mesopleural epidermis in *Strumigenys*, however, is extremely thin and therefore non-glandular. In case of a class-3 gland, duct cells and pores with a diameter around 0.5-1 µm that connect to secretory cells should be found (as e.g. for the metapleural gland (Hölldobler & Engel-Siegel 1984; Pech & Billen 2017) and the propleural pit gland and posterolateral pronotal gland in *Myopias hollandi* (Forel, 1901) (Billen & Ito 2018)); however, such duct cells or pores do not occur at all in the mesopleural excavation region of *Strumigenys*.

While the absence of glandular tissue rules out the erroneous interpretation of a ‘mesopleural gland’ (Bolton 1999), the question about the real nature of this peculiar hairwheel organ remains. Mesopleural excavations represent a normal part of the ant thorax. The anterior margin of the mesothorax, which articulates inside the back of the prothorax in a ‘telescopic’ fashion, is delimited behind by a transverse girding ridge or groove, which essentially is an inflection of the cuticle to provide strength. The groove structure can also be recognized on 3D surface renders generated from microCT scans such as these available online of *Strumigenys avatar* and *S. gunter* in Sarnat et al. (2019). As these surface renders are derived from relatively low-resolution scans, the hairs are not visible, which has the interesting effect that when looking under the right angle in profile view, one can look through the ventral groove. The dorsal and lateral portions of this groove are usually concealed underneath the pronotum, and in workers with promesonotal fusion (e.g., Myrmicinae, Proceratiinae, Ectatomminae), the groove is usually absent. However, the ventral portion of this groove is always present to some degree and is laterally visible as the mesopleural excavation, and can appear as a prominent hairwheel. This configuration is especially conspicuous in *Strumigenys*, although it also occurs in other myrmicine ants such as *Pilotrochus* (Brown 1978), *Myrmica*, and *Manica*. Especially in the wingless workers, the thorax has become a rigid box containing strong neck and leg muscles that allow them to carry heavy loads (Keller et al. 2014; Peeters et al. 2020). In spite of the rigidity of this thoracic box, efficient articulation with the coxae forms an essential condition for proper leg movements. The ventral groove lying just behind the procoxal articulations forms a tight space that presumably needs to be kept clean against dirt and debris, as it otherwise compromises the movement of the forecoxae. The accumulation of dirt that can sometimes be seen on sections and scanning micrographs can be an indication of such filtering function. In this context, the hairs may also help to protect the ants against parasitism by small mites, that can sometimes be found living in *Strumigenys* nests (C.-C. Lin, pers. obs.). The configuration of the circular hairwheel becomes particularly obvious by the presence of an anterad projecting cone-like protuberance as in most *Strumigenys* species. Although many ants show some sort of mesothoracic protuberance to block the sides of the confined space behind the forecoxae, this is more common in subterranean or ground-nesting genera as *Eciton*, *Ectatomma* or *Ponera*, than in arboreal ants as *Dolichoderus*.

It remains unclear as yet why *Strumigenys* ants have such obvious mesopleural excavations. Keeping the area clean through grooming perhaps is difficult as their long, specialized mandibles may get in the way of the maxillolabial complex that is used for grooming. This, however, does not apply to the short-mandibulate species although these too have the peculiar hairwheel structures. Another purely hypothetical function could be that the hairwheel structure acts as an auditory organ, although this can also be ruled out as this would require the presence of specialized sensilla and/or neural connections; however, no such tissue exists. It is equally unclear why workers as well as queens of *S. mutica* are aberrant in not having the hairy mesopleural groove that is so characteristic in all other investigated *Strumigenys* species. It is interesting that *S. mutica* is a temporary social parasite of other *Strumigenys* species (Wilson & Brown 1956; Wang et al. 2021b), even though we do not see any apparent link between this social parasitism and the absence of the hairwheel. Although our study does clarify the erroneous interpretation of the hairwheels as glandular organs, divining the real function of these structures remains an intriguing challenge.

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