Hirsutella sp. (Hypocreales: Ophiocordycipitaceae) Affecting the Invasive Social Wasp Vespula vulgaris (Hymenoptera: Vespidae) in Southern Chile

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Hirsutella sp. (Hypocreales: Ophiocordycipitaceae) affecting the invasive social wasp *Vespula vulgaris* (Hymenoptera: Vespidae) in southern Chile

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The genus *Vespula* (Hymenoptera: Vespidae) includes wasp species that have painful stings that cause irritating nuisances with impacts on human health and on a range of outdoor activities. These wasps are economically significant pests of such primary industries as beekeeping, forestry, and horticulture (Dymock et al. 1994; Beggs 2000, 2001; Gardner-Gee & Beggs 2012). The stings of the common wasp, *Vespula vulgaris* (L.) (Hymenoptera: Vespidae), are well documented to induce allergic reactions and, occasionally, fatal anaphylaxis (King et al. 1996; King & Spangfort 2000; Fitch et al. 2001; Tankersley & Ledford 2015). This wasp, which is native to Eurasia, has become a notorious pest in Argentina and New Zealand, where it can attain high population densities and cause major ecological consequences such as increased predation pressure on native insect communities (Yamane et al. 1980; Beggs 2001; Baz et al. 2010; Lester et al. 2014).

Vespula vulgaris was first detected in Chile during the summer of 2011, in the mountains of the Araucanía region (Barrera-Medina & Vidal 2013). It is now distributed from the Araucanía region through the southernmost Magallanes region. As a result of a continuous monitoring of Servicio Agrícola y Ganádero (SAG) in Los Lagos region (41.4717°S, 72.9367°W) for early detection of quarantine bark beetles in Chile, a single cadaver of a naturally fungal infected worker adult of *V. vulgaris* was collected during May 2015 (autumn in Chile). This insect was recovered in a funnel trap placed in the southern Chilean city of Puerto Montt (41.453914°S, 72.867700°W).

In the past, several natural enemies that could be useful control agents of this invasive *Vespula* species have been reported (Rose et al. 1999; Singh et al. 2010; Evison et al. 2012). Nonetheless, little is known of the biodiversity of entomopathogenic fungi on wasps in Chile apart from this report of a naturally occurring entomopathogenic fungus affecting *V. vulgaris* in southern Chile.

The identification of the fungus was done using a light microscope (Nikon® Eclipse E600; Nikon Corporation, Tokyo, Japan) and slide mounts in lactophenol-cotton blue were prepared as suggested by Humber (2012). Fifty measurements for each taxonomically significant fungal structure were made using images taken with a digital camera (Nikon® DS-Fi1; Nikon, Tokyo, Japan) and measured with Motic® Images Plus 2.0 software (Motic China Group Co., Ltd, Shenzhen, China). Attempts to cultivate the fungus involved transferring conidia to potato-dextrose-agar medium (PDA; Difco®, Becton, Dickinson and Company, Sparks, Maryland) in 60 × 15 mm Petri dishes. Dishes were sealed with parafilm and incubated at 24 ± 1 °C and natural photophase. The development of fungi and of any contaminants was monitored daily for 15 d. However, none of the 15 attempts to cultivate this fungus on PDA were successful. This result suggested that the specimen might have suffered from some environmentally unfavorable conditions before its collection.

The dead, mycosed *V. vulgaris* adult presented a small number of relatively short, thick white synnemata whose overall appearance was exclusively typical for infections by *Hirsutella* (Hypocreales: Ophiocordycipitaceae) species (Fig. 1A, B) (Hodge 1998; Humber 2012). On the affected wasp, the fungus produced white mycelium and unbranched synnemata, 1,520 ± 340 µm long (overall range: 336–4,460 µm), emerging from the cuticle of the infected host (Fig. 1A, B). The synnemata were seen to be simple (although occasionally sparsely branched), dark colored, slender, leathery to brittle in texture, and bearing a discontinuous layer of conidiogenous cells with elongated and narrowed necks projecting from the synnemal surface. The conidiogenous cells were monophialidic, septate, scattered to moderately crowded, arose laterally from the synnemata, with cylindrical to ellipsoid bases, 25.2 ± 1.6 µm length × 1.5 ± 0.1 µm width (overall range: 16.4–44.1 µm length × 1.2–2.0 µm width) (Fig. 1C, D). The conidia were hyaline, smooth, one-celled and lemon-shaped, 8.5 ± 0.3 µm length × 3.7 ± 0.1 µm width (overall range: 7.1–10.6 µm length × 2.1–5.2 µm width), produced singly, only rarely seen in groups, and occasionally coated by an obvious mucoid sheath (Fig. 1E).

The trap containing the infected wasp was collected by Verónica Cruces (SAG-Puerto Montt), and the infected wasp was deposited in Jun 2016 in the Mycological Collection of the National Museum of Natural History of Chile, Parque Quinta Normal s/n, Santiago, Chile, as accession SGO 166649. The conidiogenous cells of this and other *Hirsutella* species are best described as phialidic, and form one to a few conidia tending strongly to be asymmetrical and often resembling the individual sections of an orange (Minter et al. 1983; Hodge 1998). The great majority of *Hirsutella* species are pathogens of insects; a few are pathogens of mites or spiders, and still fewer are pathogens of nematodes (Shimazu & Glockling 1997; Hodge KA, Plant Pathology and Plant-Microbe Biology Section, Cornell University, personal communication). The slime-embedded spores of many *Hirsutella* species are thought to be adapted for dispersal by contact with a passing invertebrate or in water drops (Evans 1989).

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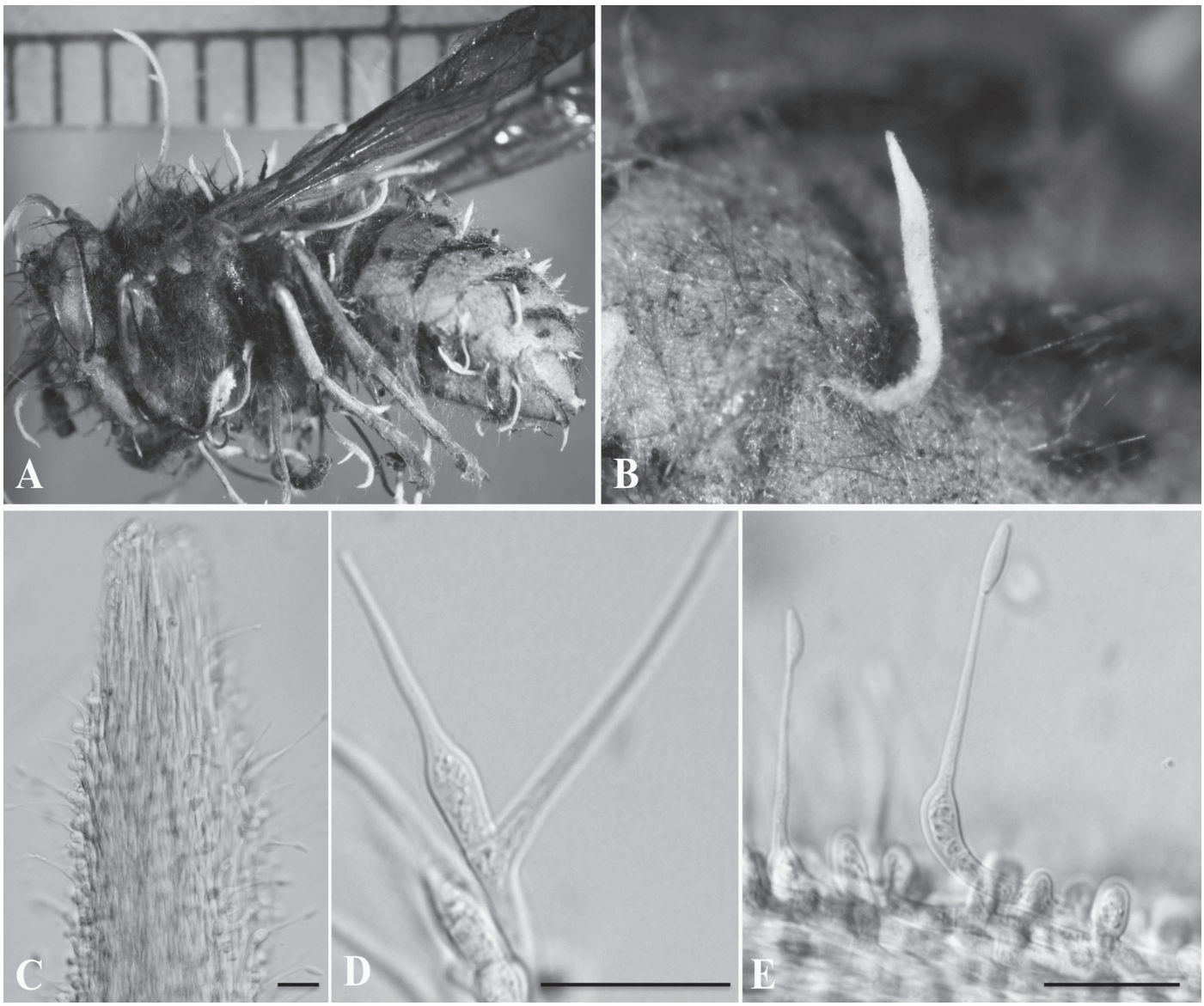

Fig. 1. *Vespula vulgaris* adult infected with *Hirsutella* sp. found in southern Chile. A. Mycotized wasp cadaver; B–C. synnema arising from the abdomen of the wasp; D. conidiogenous cells with cylindrical bases and an elongated, narrowed neck; E. conidiogenous cells and conidia. Horizontal scale bars represent 20 µm in C, D, and E.

The only *Hirsutella* species ever reported from the superfamily Vespoidea is *H. saussurei* (Cooke) Speare (Hypocreales: Ophiocordycipitaceae). This species has been reported from the USA, Honduras, Panama, England, Papua New Guinea, Borneo, and Taiwan (Hodge 1998; Rose et al. 1999). *Hirsutella saussurei* was originally described as *Isaria saussurei* by Cooke (1892) from a *Polistes* sp. (Hymenoptera: Eumenidae) and transferred to *Hirsutella* by Speare (1920). Speare described the color of *H. saussurei* synnemata as brownish. That the synnemata of the Chilean *Hirsutella* species are whitish and smaller in size than noted by Speare (1920) suggests a reasonable possibility that these synnemata were only partially developed when the insect was collected but might have been both larger and darker if the specimen had remained in the field for a longer time.

The synnemata of the Chilean fungus differ from those of typical collections of *H. saussurei* (e.g., Speare 1920; Samson et al. 1988) by their comparatively smaller number, shorter length, comparatively greater thickness, and absence of lateral branchings of the synnemata. Speare (1920) described the conidiogenous cells of *H. saussurei* as simple, sessile, with an inflated short basal portion tapering abruptly to a very long neck (35–70 µm), but the conidiogenous cells of the Chilean *Hirsutella* had a total length of 16.4 to 44.1 µm including the neck. As noted previously, all of these differences between the Chilean collection and other reported examples of *H. saussurei* may indicate that the fungus reported here may be a relatively immature collection of this species. Based on these results, we report the first incidence of an entomopathogenic fungus, *Hirsutella* sp. in this case, as a potential natural enemy of *V. vulgaris* in Chile. The *Hirsutella* found in the study might differ from *H. saussurei*, but more infected wasps from Chile need to be examined to determine whether the variant morphology of the fungus characterized here does, indeed, represent a developmentally early state of sporulation by *H. saussurei*.

The biodiversity of South American entomopathogenic fungi has still received surprisingly little attention. Aruta et al. (1974) and Aruta & Carrillo (1989) summarized the biodiversity of entomopathogenic fungi in Chile, and Sosa-Gomez et al. (2010) provided an extensive summary of these from Argentina and Brazil. Globally, the fungal ento-

mopathogens of *Vespula* species include *Aspergillus flavus* Link (Eurotiales: Trichocomaceae), *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), and *Beauveria brongniartii* (Sacc.) Petch (Cordycipitaceae) as well as a single *Hirsutella* species recorded to attack *Vespula germanica* (F.) (Hymenoptera: Vespidae). Only *B. bassiana* has been reported to infect *V. vulgaris* (Glare et al. 1993, 1996). This finding represents the first report of any wasp-pathogenic *Hirsutella* species from Chile.

We found only a single wasp infected with this *Hirsutella* sp., and we were unable to culture it. Future studies involving more infected individuals will be needed to obtain essential genomic sequence data to confirm our morphologically based identification. However, it must also be noted that no concerted genomic survey of a wide range of species of *Hirsutella* has yet been undertaken, so genome-based identifications for most of these species remain unattainable at this time. Further, the new rules of nomenclature that took effect in 2012 have led to the synonymization of most *Hirsutella* species to species in the very species-rich genus *Ophiocordyceps* (Hypocreales: Ophiocordycipitaceae). In this new classification, *H. saussurei* is now treated as a synonym for its known sexual (teleomorphic) state, *Ophiocordyceps humbertii* (C. P. Robin) G. H. Sung, J.-M. , Hywel-Jones & Spatafora (Hypocreales: Ophiocordycipitaceae) (Sung et al. 2007).

The findings reported here contribute to our knowledge of the natural fungal enemies of *V. vulgaris* in Chile. Our aim is to use this fungus as a biological control agent; therefore, future collections and successful in vitro isolations of this species will be required. Further studies are needed to clarify the identification this fungus, to isolate conidia, and to produce infectious formulations before this pathogen could be applied in the field to limit the populations of this serious invasive pest.

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Summary

In Chile, the invasive and noxious pest *Vespula vulgaris* (L.) (Hymenoptera: Vespidae) was first reported in 2011 in the Araucanía region and is currently distributed between Araucanía and Magallanes regions. In Mar 2015 (autumn), during an ongoing monitoring of funnel traps by the Servicio Agrícola y Ganadero, a fungus-infected individual was collected. The fungus was identified morphologically as a species of *Hirsutella* (Hypocreales: Ophiocordycipitaceae). This is the first report of any *Hirsutella* species on *V. vulgaris* in Chile. No in vitro cultures were successfully established from the infected insect.

Key Words: invasive species; natural enemy; entomopathogenic fungus

Sumario

En Chile la plaga invasora y nociva *Vespula vulgaris* (L.) (Hymenoptera: Vespidae) se reportó por primera vez el año 2011 en la Región de la Araucanía y actualmente se distribuye entre las regiones de la Araucanía y Magallanes. En marzo del 2015 (otoño) durante un monitoreo que estaba en curso con trampas de embudo por el Servicio Agrícola y Ganadero, un individuo micotizado fue colectado. El hongo se identificó morfologicamente como una especie de *Hirsutella* (Hypocreales: Ophiocordycipitaceae). Este es el primer reporte de cualquier *Hirsutella* en *V. vulgaris* en Chile. No se consiguió realizar cultivos *in vitro* desde el insecto infectado.

Palabras Clave: especie invasora; enemigo natural; hongo entomopatógeno

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