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SHORT COMMUNICATION

# *Sphaerularia bombi* (Nematoda: Sphaerulariidae) parasitizing *Bombus atratus* (Hymenoptera: Apidae) in southern South America

**Santiago Plischuk · Carlos E. Lange**

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**Abstract** Bumble bees are some of the most important insect pollinators. However, knowledge on parasites associated to bumble bees in South America is very limited. This study reports the first isolation of a sphaerularid nematode parasitizing queens of the native bumble bee *Bombus atratus* in Argentina. Measurements and morphological characters of eggs, juveniles, and adults strongly suggest that the species is *Sphaerularia bombi*, a parasite that affects the reproduction and foraging behavior of the host. The nematode was detected in bumble bees of San Carlos de Bariloche, northwestern Patagonia region, and the surroundings of La Plata, northeastern Pampas region. Prevalence varied between 8% and 20%.

## Introduction

Pollination by bumble bees is essential in both natural environments and agro-ecosystems. Several studies have shown links between bumble bee population declines and losses in quality–quantity of agricultural production (Potts et al. 2010). About 240 species of *Bombus* are distributed in mostly temperate areas of North America and Eurasia (Williams 1998), while 42 species have been reported in a variety of habitats in the Neotropical region (Abrahamovich et al. 2007). In Argentina, native *Bombus atratus* shows the widest geographic distribution of all ten known species of the genus. This species is found in a diverse range of habitats North of parallel 41°10′S, reaching altitudes of more than 2,000 m. in the Andes. Families of plants primarily visited by this species are Asteraceae and Fabaceae (Abrahamovich et al. 2007). Knowledge on parasitism of bumble bees in South America is very limited. Only recently the presence of *Nosema ceranae* (Microsporidia) in Argentina has been reported in three native *Bombus* species (Plischuk et al. 2009), as well as *Crithidia bombi* (Euglenozoa: Kinetoplastea) and *Apicystis bombi* (Apicomplexa: Neogregarinorida) in invasive *B. terrestris* (Plischuk and Lange 2009; Plischuk et al. 2011). The aim of this contribution is to report the presence of a parasite not previously known to occur in bumble bees of South America.

## Materials and methods

A total of 214 queens of native *Bombus* species (*B. atratus*, *N*=167; *B. morio*, *N*=26; *B. bellicosus*, *N*=16; *B. tucumanus*, *N*=2; *B. opifex*, *N*=2; *B. dahlbomii*, *N*=1) were collected in different regions of the country between March 2005 and January 2010 (Plischuk and Lange 2009; Plischuk et al. 2009, 2011). The insects were captured with entomological nets while foraging. Immediately after collection, bumble bees were frozen (−32°C) until their identification (Abrahamovich et al. 2007) and processing. Dissections and initial observations were performed under a stereoscopic microscope (×10–×40).

S. Plischuk · C. E. Lange
Centro de Estudios Parasitológicos y de Vectores – CEPAVE (CONICET CCT La Plata – UNLP) La Plata, Buenos Aires, Argentina

C. E. Lange
Comisión de Investigaciones Científicas (CIC) de la Provincia de Buenos Aires, Buenos Aires, Argentina

S. Plischuk (✉)
CEPAVE,
Calle 2 # 584 (B1902CHX),
La Plata, Argentina
e-mail: santiago@cepave.edu.ar

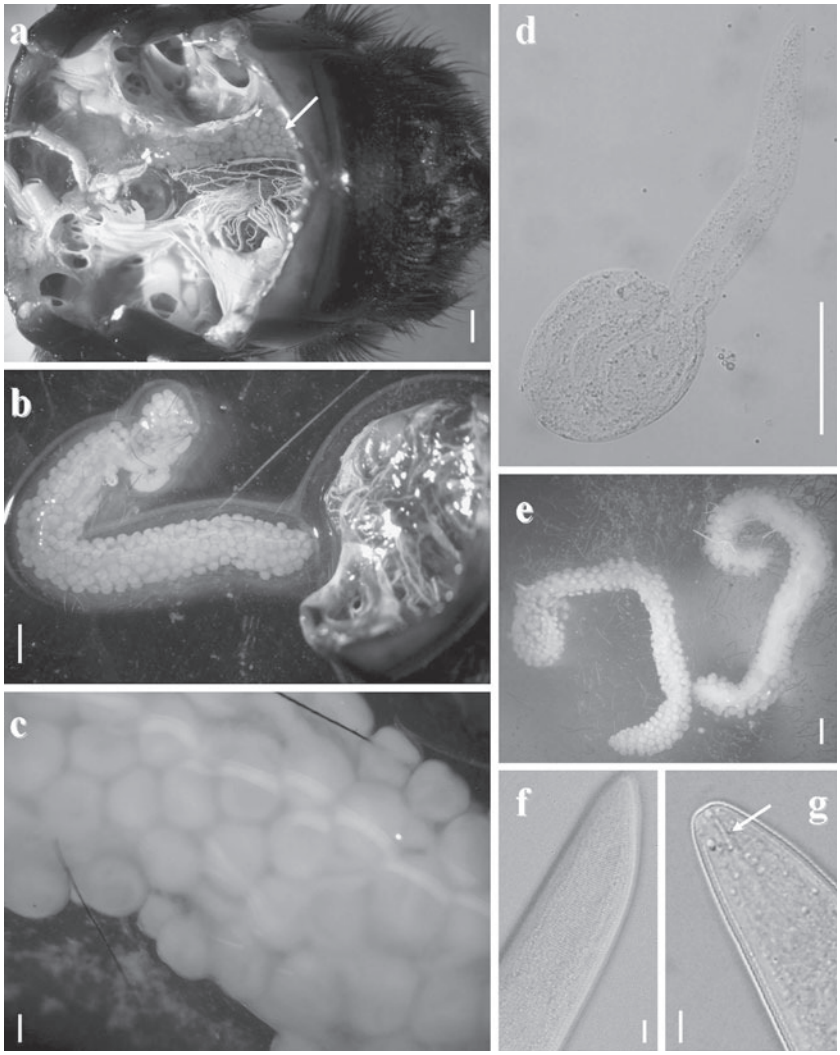


**Fig. 1** *Sphaerularia bombi* in *Bombus atratus* of Argentina. **a** Dissected bumble bee showing sac in the hemocoel (*arrow*) (bar: 1 mm). **b** Ovarian sac (bar: 1 mm). **c** Closer view of part of an ovarian sac (bar: 100 μm). **d** Juvenile emerging from an egg (bar: 100 μm). **e** Two ovarian sacs, numerous juveniles and eggs (bar: 1 mm). **f** Anterior end of a juvenile showing annulated cuticle (bar: 10 μm). **g** Anterior end of a juvenile showing stylet (*arrow*) (bar: 10 μm)

Further observations and identification of nematodes were conducted as fresh preparations under a compound microscope (×400, ×1,000). Measurements were taken with an ocular micrometer and are expressed as mean±SE and range.

**Table 1** Comparison of measurements of *Sphaerularia bombi* in *Bombus atratus* of Argentina and data from other reports

| Host | Egg | | Juvenile | | | | Adult | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | Length | Width | Length | Width | Tail | Stylet | Length | Width | |
| *B. atratus* | 120–196 | 76–152 | 752 –1080 | 26–46 | 34–50 | 6–13.5 | 13–20 | 0.8–1.4 | Present |
| *B. terrestris*<br>*B. lucorum* | | | | | | | | | Pouvreau (1962)[a] |
| *B. lapidarius* | | | 770–932 | 24–27 | 27–32 | 12–14 | | | |
| *B. pratorum* | – | – | | | | | – | – | |
| *B. hypnorum* | | | 840–1138 | 21–28 | 26–34 | 15–17 | | | |
| *B. agrorum* | | | | | | | | | |
| *Psithyrus* spp | | | | | | | | | |
| Not specified | 160 | 110 | 900–1500 | 28–34 | 32–59 | 7–11 | 2–20 | 1.2–1.5 | Siddiqi (2000) |

Egg and juvenile: measurements in μm; adult: measurements in mm

[a] Male measures in upper line, female measures in lower line

## Results and discussion

Nematodes were observed in queens of *B. atratus* only. They were isolated from bumble bees collected in the surroundings of La Plata (34°54′41″S; 57°55′37″W), northeastern Buenos Aires province during October 2008 (two positives) and 2009 (three positives), and November 2009 (two positives). Only one positive sample was detected in San Carlos de Bariloche, western Rio Negro province (41°07′33″S; 71°23′55″W) in January 2010. In all cases, the parasites were present as adult females (one to three per bumble bee) and as numerous juveniles and eggs (hundreds of each per bumble bee) in the metasomal hemocoel (Fig. 1).

Characteristics and measurements of eggs are as follows: length, 153±9 (120–196) μm; width, 109±9 (76–152) μm ($N$=10; Fig. 1d, e); for juveniles, length, 873±24 (752–1,080) μm. Width at mid-body was 35±1 (26–46) μm; tail 43±1 (34–50) μm in length. Stylet not knobbed 10±0.5 (6–13.5) μm in length. Cuticle with fine annulations, excretory pore in advanced forms ($N$=30; Fig. 1e, f, g), and everted ovarian sacs of adult female (length 16.7±0.8 [13–20] mm; width 1.2±0.1 [0.8–1.4] mm) ($N$=10; Fig. 1a, b, c, e) clearly place this nematode in the Family Sphaerulariidae as *Sphaerularia bombi*, a known nematode species occasionally detected in bumble bees in other regions of the world (Poinar and van der Laan 1972; Lundberg and Svensson 1975; Siddiqi 2000).

*S. bombi* is one of the first discovered entomoparasitic nematodes. In previous reports, it was associated with more than 25 species of genus *Bombus* (including the socially parasitic subgenus *Psithyrus*) (Pouvreau 1962; Poinar and van der Laan 1972; Lundberg and Svensson 1975; Macfarlane 1975; MacFarlane and Griffin 1990; McCorquodale et al. 1998; Rutrecht and Brown 2008). A major diagnostic character of the species is the small female with an everted and hypertrophied genital tract, sausage-shaped, showing a surface with rows of rounded elevations into the host hemocoel (Poinar and van der Laan 1972; Siddiqi 2000), that have been observed in this study (Fig. 1). The life cycle is relatively well known, but knowledge about effects on the host is limited. It is believed that *S. bombi* affects the reproductive behavior of queens, disrupting the *corpora allata*, and stunting the oviducts and the uterus. Other effects appear to be difficulties in construction of the brood nests, in flight ability, and finally death (Pouvreau 1962; Poinar and van der Laan 1972; Lundberg and Svensson 1975). MacFarlane and Griffin (1990) have estimated mortality levels (3–10 %) in *B. terrestris* and *B. hortorum* in New Zealand.

In the cases reported here, the number of nematode uteri found per host (1 to 3) appears to agree with those obtained by Lundberg and Svensson (1975) in Sweden and by Macfarlane and Griffin (1990) in New Zealand, 1 to 5 in *B. alpinus*, *B. jonellus*, *B. pascuorum*, *B. pratorum*, *B. hortorum*, and *B. hypnorum*. On the other hand, studies elsewhere have shown levels of parasitism between 7% and 93% in *B. hortorum*, *B. pratorum*, *B. lapidarius*, *B. terrestris*, and *B. agrorum* (Pouvreau 1962; Macfarlane and Griffin 1990; Rutrecht and Brown 2008). Prevalence in *B. atratus* from the surroundings of La Plata city was 8% in 2009 ($N$=63), and 20% in 2008 ($N$=10).

The size variations registered in the juvenile stages (e.g., tail, body width) between our observations and those by Pouvreau (1962) and Siddiqi (2000) (Table 1) could be attributed to the possible existence of intraspecific variation associated to soil characteristics and/or hosts involved, as has been suggested by Poinar and van der Laan (1972). In this sense, methods based on comparative DNA (e.g., DNA micro- and macro-arrays, DNA barcoding; Janzen et al. 2005; Hajibabaei et al. 2007) could be useful to contrast South American populations of sphaerularids with those from other regions of the world, as has been demonstrated in taxonomic studies with other nematode species (Powers 2004).

Although there are reports of this species in North America (Goldblatt and Fell 1984; McCorquodale et al. 1998), to our knowledge this is the first record of a sphaerularid nematode parasitizing bumble bees in South America, and we include *B. atratus* as a new host of *S. bombi*. Since the high impact of *S. bombi* on their hosts has been reported (Pouvreau 1962; Rutrecht and Brown 2008), we believe that recording its presence in Argentina is relevant. We envisage further studies of this parasite–host association by increasing the number of samples as well as expanding the survey area and prospecting potential new hosts.

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