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Cotesia invirae, sp. nov., from South Brazil: a new gregarious microgastrine wasp (Hymenoptera: Braconidae) reared from *Opsiphanes invirae* (Nymphalidae) feeding on palms

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ABSTRACT

A new species of microgastrine wasp, *Cotesia invirae* Salgado-Neto & Whitfield, **sp. nov**., is described from southern Brazil in Santa Maria, Rio Grande do Sul. This species is a koinobiont gregarious larval endoparasitoid and spins a common mass of cocoons underneath the host caterpillars of *Opsiphanes invirae* (Huebner, 1818) (Lepidoptera: Nymphalidae), feeding on the palm trees *Syagrus romanzoffianum* (Cham.) Becc.; *Livistona chinensis* (N.J. Jacquim) R. Brown; *Roystonea regia* (HBK) O.F. Cook; *Archontophoenix cunninghamiana* Wendland & Drude (Palmaceae). Morphological, molecular, biological, ecological and geographical data are integrated to describe the new species.

Introduction

Cotesia Cameron, 1891 (Braconidae: Microgastrinae) is the second largest genus of microgastrine wasps in terms of described species, encompassing roughly 300–400 described species (Shaw and Huddleston, 1991; Yu et al., 2016). However, this number will increase in the coming years, since it has been estimated that nearly 1000 species of *Cotesia* exist worldwide (Mason, 1981; Michel-Salzat and Whitfield, 2004). This increase will increase especially in the Neotropical region, where a relatively small number of studies recording species of *Cotesia* and their biology are available (Whitfield, 1997), particularly in South America.

Cotesia is recognizable among the microgastrines by the following features: (1) forewing with second r-m vein absent, so that the small areolet is open distally; (2) propodeum coarsely sculptured, with medial longitudinal carina rather than medial areola; (3) first and second metasomal tergites usually rather quadrate in form and coarsely sculptured; and (4) ovipositor and sheaths short and barely exserted (Whitfield et al., 2009). These wasps have a koinobiont habit (Kankare and Shaw, 2004) and can produce both single adults or gregarious broods of offspring. The new species described here was recorded from caterpillars of Lepidoptera (Nymphalidae) in Brazil, Peru and Venezuela (Yu et al., 2016).

Opsiphanes invirae (Huebner 1818) is widespread in Brazil but is most commonly found between the States of Rio de Janeiro (Southeastern Region) and Rio Grande do Sul (Southern Region) (Silva et al., 1968; Ferreira et al., 1998). This species is considered a pest of the "açaí" palm tree (*Euterpe oleraceae*) in Eastern Brazilian Amazonia (Northern Region), according to Souza and Lemos (2007). In Rio Grande do Sul, this species was recorded by Link and Alvarez Filho (1979) and Link et al. (1980), more recently, Lamas (2004) also reported the occurrence of this species in Paraguay.

Five species of Braconidae have been recorded as endoparasitoids of *Opsiphanes* (larvae stage): *Apanteles biezankoi* Blanchard 1954, *Apanteles opsiphanis* Schrottky 1909, *Cotesia* sp., *Cotesia alius* (Muesebeck, 1958a,b) (Penteado-Dias, 1987; De Santis, 1989; Mason, 1981), *Rhysipolis* sp. (Sauer, 1946; Costa Lima, 1950, 1962; Silva et al., 1968; Briceño-Vergara, 1978; De Santis, 1980; Penteado-Dias, 1987; Mason, 1981; Briceño-Vergara, 1997; Mexzón, 1997; Rodríguez et al., 2006).

As *Cotesia* species have appeared to be highly host specialized (Kankare and Shaw, 2004), with many cryptic species and allopatric distributions (Fiaboe et al., 2017), the use of an integrative taxonomic approach (combining morphological, molecular, biological and geographical data) is critical for recognizing and distinguishing these parasitoid wasps (Smith et al., 2008; Kaiser et al., 2017).

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Using an integrative taxonomic approach, this paper provides a description of a new species of *Cotesia*, whose brood was produced from *Opsiphanes invirae* (Huebner, 1818) (Lepidoptera: Nymphalidae), feeding on palm trees (Palmaceae) in southern Brazil in Santa Maria, Rio Grande do Sul. Additionally, we compared it with other formally described species for the Neotropical region.

Materials and methods

Between March 2006 and March 2007, we collected 35 larvae of *Opsiphanes invirae*. This survey was carried out on native and exotic palms in the Universidade Federal de Santa Maria (UFSM) campus (29°42′19″S, 53°42′57″W – 90 m. elev.). Avenida Roraima (Campus – UFSM), Jardim da Escola Agrícola (Campus – UFSM). The larvae of *O. invirae* were found on *Syagrus romanzoffianum* (Cham.) Becc. (Arecaceae; Gerivá); *Livistona chinensis* (N. J. Jacquim) R. Brown (Chinese Range); *Roystonea regia* (HBK) O. F. Cook (Cuban Real Palm); and *Archontophoenix cunninghamiana* Wendland & Drude (Australian Real Palm).

Upon collection, larvae were kept in an environmental chamber (25 ± 1 °C; 70% UR; photoperiod of 14 h of light) and observed daily until the emergence of the butterflies or parasitoids, which were preserved in 70% ethanol. Voucher specimens were deposited in the collection of the Laboratório de Biologia Evolutiva of the Centro de Educação, Universidade Federal de Santa Maria (UFSM). (Rocco A. Di Mare curator), number GSAL10.01.2006.

Illustrations were made using digital camera photography setups attached to stereoscopic microscopes, both in Brazil and at the University of Illinois. Morphological terms and measurements of structures are mostly those used by Fernandez-Triana et al. (2014).

To check the molecular-specific characterization of the new species, the mitochondrial gene Cytochrome Oxidase I (COI) was analyzed. For the amplification of a fragment of approximately 460 bp of this gene, we used the following primer pair: COI-F (5′-GATTTTTTGGKCAYCCMGAAG-3′) and COI-R (5′-CRAATACRGCTCCTATWGATAAWAC-3′) (Gusmão et al., 2010). DNA extraction of one specimen was performed with the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma–Aldrich®) and followed the manufacturer's protocol. The product was amplified *via* Polymerase Chain Reaction (PCR) according to the following schedule: 94 °C for 2 min, 40 cycles of 94 °C for 30 s, 54 °C for 30 s, 72 °C for 40 s and 72 °C for 4 min. Then the PCR product was purified using polyethylene glycol precipitation (PEG; Schmitz and Riesner, 2006). These samples were sequenced using the Big Dye 3.1 reagent (Life Technologies®) and 3500 × L automatic sequencer (Life Technologies®).

Descriptive taxonomy

Cotesia invirae *Salgado-Neto & Whitfield, sp. nov.*

(Figs. 1 and 2)

Holotype. Female, Brazil: Rio Grande do Sul, Santa Maria, Universidade Federal de Santa Maria (UFSM) (29°42′19″S, 53°42′57″W – 90 m. elev.). Deposited in the Collection of Entomophagous Insects "Oscar Monte" of the Instituto Biológico de Campinas, São Paulo. (IB-CBE; Valmir Antônio Costa curator), number IB-CBE#215-C, GSAL10.01.2006 and also deposited in the Hymenoptera collection of the Museum of Zoology of the University of São Paulo (MZUSP; Carlos Brandão curator), number MZSP62057, September 2018, coll. G. Salgado-Neto, ex larva *Opsiphanes invirae* (Huebner, 1818) (Lepidoptera: Nymphalidae).

Paratypes. 2 males, deposited in MZUSP, numbers MZSP62066 and MZSP62067. Same data as holotype.

Diagnosis. The combination of characters presented below is sufficient to separate *C. invirae* from all other described native species of *Cotesia* for the Neotropical region. In particular, few neotropical *Cotesia* have the anterior metasomal tergites largely orangish rather than black (and even those are mostly undescribed). However, several widespread agricultural species, *C. marginiventris* (Viereck) and *C. flavipes* (Cameron), have current distributions extending into Brazil and are known to be highly variable in tergite colour. *C. marginiventris* differs in having the anterior margin (roughly 1/3 of length) of tergite 3 with rougher sculpture (smooth in *C. invirae*), and *C. flavipes* (along with other members of its species complex) is extremely distinctive in having a relatively depressed mesosoma that is usually significantly narrower in dorsal view than the width of the head (in addition to having the face somewhat bulging below the antennae in lateral view). *C. alius*, previously recorded from the same host, has essentially black tergites, although the laterotergites are bright yellowish as in *C. invirae*. *C. alius* also appears to have a slightly longer first tergite than *C. invirae*, but otherwise the two species are quite similar morphologically.

Description. Female (Fig. 1a). Body colour: head and mesosoma mostly black except pale, almost whitish palpi, most of all legs honey-yellow except darkened junction of trochanter with femur, distal quarter of hind femur, distal 0.3–0.4 of hind tibia, and most of hind tarsus; metasoma mostly light honey-yellow to orange except dark brown first tergite and orange to brown second tergite. Antenna colour: scape, pedicel and flagellum dark. Tegulae colour: dark brown, relatively opaque. Pterostigma colour: dark greyish brown. Fore wings colour: partially pigmented but pigmented veins dark brown (a few veins may be dark but most pale). Antenna length/body length: antenna just slightly shorter than body (head to apex of metasoma). Body in lateral view: not distinctly flattened dorso-ventrally. Body

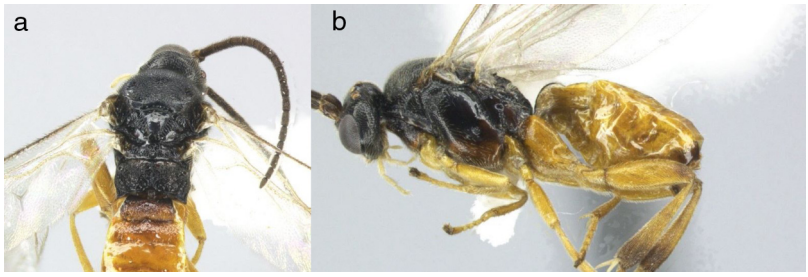

Fig. 1. (a) Lateral view of female *Cotesia invirae*; (b) view of the labrum, mandibles and labial palps; (c) front view of the face; (d) lateral view of the antenna; (e) dorsal view of the mesosoma; (f) dorsal view of abdomen; (g) view of the forewing *in situ*; (h) details of the spurs of the tibia (inner and outer); (i) larvae of *C. invirae*; (j) front view of the pupa of *C. invirae*; (k) dorsal view of pupa of *C. invirae*; (l) lateral view of the pupa of *C. invirae*; (m) front view of head, the larvae of *C. invirae*; (n) view of the cocoons of *C. invirae*, arranged regularly and secured with silk threads to each other in the form of palisades on the host. Some material here previously reported in Salgado-Neto (2013) as from *C. alius*.

Fig. 2. (a) Dorsal habitus of female *Cotesia invirae*, showing sculpturing of mesoscutum, scutellum, propodeum and anterior metasomal tergites; (b) lateral habitus of *C. invirae*; (c) wings of *C. invirae*.

length (head to apex of metasoma): 2.5 – 2.7 mm. Fore wing length: 2.4–2.6 mm. Ocular-ocellar line/posterior ocellus diameter 1.7–1.9. Interocellar distance/posterior ocellus diameter: 1.9–2.1. Antennal flagellomere 2 length-width: 2.9–3.1. Antennal flagellomere 14 length/width 1.4–1.6. Length of flagellomere 2/length of flagellomere 14: 2.2–2.3. Metafemur length/width 3.0–3.2. Metatibia inner spur length/metabasitarsus length: roughly 0.5–0.6. Anteromesoscutum: anteriorly with distinct dense punctures, with only a very narrow smooth band posteriorly anterior to scutoscutellar sulcus. Mesoscutellar disc: sparsely punctured and mostly shiny. Number of pits in scutoscutellar sulcus: 8–10. Propodeum carina: with complete medial longitudinal carina that is nearly obscured by coarse background suggesting medial areola. Propodeum background sculpture: mostly coarsely rugulose.

Mediotergite 1 length/width at widest point: 1.0–1.1. Mediotergite 1 shape: evenly widening from anterior margin to 0.8–0.9 of mediotergite length (widest point), then rounding towards posterior margin. Mediotergite 1 sculpture: mostly rugulose except anteromedially smooth. Mediotergite 2 nearly quadrate with width at posterior margin/length: 2.1–2.3. Mediotergite 2 sculpture: mostly rugose, smoewhat polished laterally. Hypopygium: evenly sclerotized but folded medially, posteriorly forming a strongly obtuse angle in lateral view. Ovipositor thickness: tapering gradually to tip. Ovipositor sheaths: short, approximately 1/3 of hind tibia length but only slightly exposed towards tip. Length of fore wing veins r/2Rs: 1.0–1.1. Pterostigma length/width 2.0–2.2. Point of insertion of vein r in pterostigma: just beyond half way point of pterostigma length. Angle of vein r with fore wing anterior margin: angling 5–10 degrees towards wing tip posteriorly. Shape of junction of veins r and 2RS in forewing: r weakly arched, junction distinctly angled.

Molecular data. COI barcode deposited in GenBank (Accession MK040535). When the Barcode of Life Database (BOLD) was interrogated with this sequence, the closest matches (between 1 and 2% sequence divergence) were two currently undescribed species from the Janzen and Hallwachs ACG project (Janzen et al., 2009). Both of these species were reared from Opsiphanes on palms (albeit different species). They are referred to informally as *Cotesia* Whitfield78 and *Cotesia* Whitfield20 in Smith et al. (2008). As *C. alius* is also from the same basic host group, it appears that there is an international cluster of closely related species, still mostly undescribed, to be sorted out eventually based on a broad study.

Host: *Opsiphanes invirae* (Huebner, 1818) (Lepidoptera: Nymphalidae).

Biology/ecology. *Cotesia invirae* is a gregarious parasitoid wasp that occurs mainly in the wet season (March–May); however, its host, *O. invirae*, occurs throughout the year, mainly in the autumn season (March–April–May). *Cotesia invirae* larvae kill the host larva before the end of the last instar and form their cocoons in regular mass of dirty whitish cocoons, regularly arranged under the host. The larvae of this gregarious species all emerge from the host in a short time through many different holes in the host cuticle and spin a common woolly cocoon mass, within which the individual cocoons can be distinguished. Some previous records attributed to *C. alius* (*e.g.* Salgado-Neto, 2013) actually refer to this new species.

Based on 35 analyzed reared broods, each one referring to a larva of *O. invirae* from nature, were 4581 registered cocoons, averaging 130.88 ± 49.02 per host. Taking into account that the number of cocoons is the number of eggs laid per host-larva, whose average number of eggs laid was 87. Based on seven broods, with a total of 789 cocoons (112.71 ± 31.21 cocoons/posture), it was found that 104 cocoons (13%) from two broods of *C. invirae* were parasitized by Eulophidae. We observed four species of hyperparasitoids (Eulophidae) exploiting the cocoon mass of *C. invirae* collected in the field, two from the Entedoninae (*Horismenus opsiphanis* Schrottky, 1909 and *Horismenus* sp. nov.) and two from Tetrastichinae (*Oomyzus sokolowskii* Kurdjumov, 1912 and *Aprostocetus* sp. nov.). All hyperparasitoids were identified by Dr. Christer Hansson (Lund University, Sweden) and Dr. Valmir Antonio Costa (Instituto Biológico de Campinas, Brazil) (Salgado-Neto & Di Mare, 2010).

Distribution. Known so far from Santa Maria, Rio Grande do Sul, Brazil.

Etymology. The specific epithet *invirae*, from the Latin meaning turn around, (the same as: bickering, return, bend, reverse, spin, put yourself in a new position) is a reference to *Opsiphanes invirae* (Huebner, 1818) (Lepidoptera: Nymphalidae) in tribute to the movement of the host caterpillar to avoid the oviposition of parasitoids.

Discussion

Unlike the recently described *Cotesia* species from Geometridae, *C. itororensis* Sousa-Lopez and Whitfield (Sousa-Lopes et al., 2016; Souza-Lopes et al. 2018), *Cotesia invirae* falls squarely within the usual morphological and definition of Cotesia, rather than somewhat intermediate between the closely related genera *Cotesia* and *Protapanteles*. *Cotesia* are often reared from Nymphalidae, although the only species so far recorded from South America is a *Protapanteles*, *P. eryphanidis* (Whitfield), reared from *Eryphanis greeneyi* (Penz and Devries, 2008) (Nymphalidae) in Ecuador (Greeney et al., 2011). With respect to molecular data, *C. invirae* also fits squarely within *Cotesia* according to BLAST hits, closely related to two ecologically similar species from Central America.

Species of *Cotesia* are important agents of biological control against pest insects, so that the correct identification of them is critical to success in pest management programmes. We reiterate here that the use of an integrative taxonomic approach (combining morphological, molecular, biological and geographical data) is essential for accurate species delineation.

Conflicts of interest

The authors declare no conflicts of interest.

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