Systematics, Morphology and Biogeography

First record of Cotesia scotti (Valerio and Whitfield, 2009) (Hymenoptera: Braconidae: Microgastrinae) comb. nov. parasitising Spodoptera cosmioides (Walk, 1858) and Spodoptera eridania (Stoll, 1782) (Lepidoptera: Noctuidae) in Brazil

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ABSTRACT

This is the first report of Cotesia scotti (Valerio and Whitfield) comb. nov. in Brazil, attacking larvae of the black armyworm, Spodoptera cosmioides, and the southern armyworm, S. eridania. The moth larvae were found respectively, infesting a protected cropping of organic tomato in Hidrolândia, Goiás, Brazil, and a transgenic soybean crop in Sáo José dos Pinhais, Paraná, Brazil. Biological, molecular and morphological characters were used to confirm the identity of the specimens. Parasitoid identification presented a challenge since the species has most diagnostic characters of the genus Cotesia Cameron, but few in the poorly defined genus Parapanteles Ashmead. Based on morphological and molecular evidence, we transfer Parapanteles scotti to the genus Cotesia. The new combination is discussed by comparison with morphologically similar species and available molecular data.

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Introduction

The genus Spodoptera Guenée (Lepidoptera, Noctuidae, Amphipyrae) comprises 31 valid species (Pogue, 2002, 2011), commonly known as ‘armyworms’. It has a cosmopolitan distribution with higher diversity found in the tropics and subtropics (Pogue, 2002). Most of the New World species with known hosts are polyphagous on dicots, with the exception of Spodoptera frugiperda, commonly known as the fall armyworm, which also feeds on monocots (Kergoat et al., 2012). Roughly half of the known species are important or even key pests in several cultivated plants. This status can, in great extent, be attributed to their high biotic potential and polyphagy, frequently leading to population outbreaks. The importance of some species as pests is aggravated by natural tolerance or resistance to some Cry and Vip3A proteins of Bacillus thuringiensis (Bt) Berliner (Eubacteriales: Bacillaceae) expressed in transgenic plants (Luttrell et al., 1999; Bernardi et al., 2014; Horikoshi et al., 2016).

The black army worm, Spodoptera cosmioides (Walk, 1858), is a polyphagous species, native of South America, feeding on a great variety of cultivated and native plants. In Brazil, it is a pest on several crops, especially pineapple (seedlings), cotton (bolls), rice, and soybean (leaves and pods). Specht and Roque-Specht (2016) list 126 species in 40 different plant families as hosts for S. cosmioides, being Solanaceae (with 15 spp.) and Fabaceae (14 spp.), the families with most species recorded. Previous records of S. cosmioides feeding on tomato crops are given by Biezanko et al. (1974), Pastrana (2004) and Silva et al. (1968). The southern armyworm, Spodoptera eridania (Stoll, 1782), occurs throughout the Americas, and is recorded from as many as 202 host plant species, in 58 families (Montezano
et al., 2014), being Asteraceae and Fabaceae the most frequently recorded hosts (with 20 and 19 species respectively). It is reported as pest for many crops, being cotton and soybean the most economically important in Brazil (Parra et al., 1977; Quintella et al., 2007; Santos et al., 2005).

A large number of dipteran and hymenopteran parasitoids are known to attack species of the genus Spodoptera, in all immature stages of development. Molina-Ochoa et al. (2003) listed ca. 150 species parasitizing the fall armyworm, of which 86 are described from South America. Compared to S. frugiperda, literature on parasitoids of S. cosmioides and S. eridania is scarcer, especially for the former. Most of the parasitoid wasps (Hymenoptera) reported for these two species are either highly polyphagous (e.g. Yu et al., 2016; Zaché et al., 2012) and/or associated with S. frugiperda as well (see Molina-Ochoa et al., 2003). For instance, several studies report the egg parasitoid Telenomus remus Nixon, 1937 (Platygastridae) as a potential biological control agent for these three Spodoptera species (Bortolotto et al., 2014; Goulart et al., 2011; Pomari et al., 2013). Larval parasitoids for S. cosmioides and S. eridania are mostly restricted to a few Ichneumonoidea and one Eulophidae (Hymenoptera) species, all of them also associated with S. frugiperda (Capinera, 2018; Tingle et al., 1978; Yu et al., 2016 and references within). For Ichneumonoidea this includes the Braconidae: Meteorus autographae Muesebeck, 1923, Meteorus laphygmae Viereck, 1913 (Euphorinae), Chelonus texanus Cresson, 1872 (Cheloniinae), Cotesia marginiventris (Cresson, 1865) (Microgastrinae), Aleiodes laphygmae (Viereck, 1912) (Rogadinae) for S. eridania, and Aleiodes vaughani (Muesebeck, 1960) (Rogadinae) for S. cosmioides and S. eridania; and the Ichneumonidae: Campopleis flavicincta (Ashmead, 1890) (Campopleginae) (but see Camargo et al., 2015 for a discussion on identity of C. flavicincta and Campoleis sonorensis (Cameron 1886), Ophion Fabricius, 1798 spp. (Ophioninae), and Euphasoma dentator (Fabricius, 1804) (Cremastinae), for S. eridania.

Microgastrinae (Hymenoptera, Braconidae) is the second largest subfamily of braconids, and the single most important group of parasitoids of Lepidoptera, with more than 100 species being used in biological control programs worldwide (Whitfield, 1997). Despite their ecological and economic importance, the majority of the species is still unknown, and the limits of many of its genera are blurred as they are defined by few diagnostic characters (Whitfield et al., 2018a). The genus Cotesia Cameron, 1891 is one of the largest in Microgastrinae, with about 300 species described (Yu et al., 2016). Currently their diversity is concentrated in the Holarctic region, where species are some of the most common parasitoids of macrolepidopteran larvae (Whitfield et al., 2018b). Their importance as biological control agents is well illustrated by the biological control program with Cotesia flavipes Cameron, 1891; imported from the Oriental region for the control of the sugarcane borer, Diaatraea saccharalis (Fabricius, 1794) (Lepidoptera, Crambidae), this is considered the most successful example of biological control in Brazil (Parra, 2014). Other examples in the New World include the control of the cabbage moth, Pieris rapae (L., 1758) (Lepidoptera, Pieridae), by Cotesia glomerata (L., 1758) and Cotesia rubecula (Marshall, 1885), and the control of the gypsy moth, Lymantria dispar (L., 1758) (Lepidoptera, Erebidae) by Cotesia melanoscela (Ratzelburg, 1844) (Vail et al., 2001). All seven species of Spodoptera recorded as hosts for Cotesia in the New World are parasitized by C. marginiventris, including S. eridania and S. frugiperda. There is one additional record of C. congregata on S. frugiperda (Yu et al., 2016), and another two exotic species in the same host (Molina-Ochoa et al., 2003). Here, we present a taxonomic contribution and new host records for a potentially important biological control agent of the black armyworm, S. cosmioides, and the southern armyworm, S. eridania.

Material and methods

Sampling

A large population of S. cosmioides was observed in an organic protected cropping of tomato – Lycopersicon esculentum Mill (Solanaceae) – at Fazenda Orgânica Nossa Senhora Aparecida, Hidrolândia, Goiás, Brazil (16°57’58”S; 49°11’13”W), in the year 2017. From August of that year, many caterpillars produced cocoon masses typical of microgastrine parasitoids (Hymenoptera, Braconidae). Sampling of caterpillars was performed weekly, until the end of the occurrence of the target insect. The samples were taken at random, walking inside the greenhouse where the caterpillars were hand-collected and taken to the Entomology Laboratory of the Plant Protection Department of the School of Agronomy at the Federal University of Goiás. Larvae were kept in individual 9 x 12 cm plastic containers and fed with tomato leaves until emergence of adult moth or parasitoid. Along with the collection of caterpillars we also collected cocoon masses of the parasitoid which were taken to the laboratory until adult wasps emerged.

A survey of caterpillars and their larval parasitoids was conducted in soybeans, Glycine max (L.) Merrill (Fabaceae) in the municipality of São José dos Pinhais, Paraná, Brazil. The crop was sampled during the 2016/2017 season (from January 4 to February 15, 2017), in an area of approximately 12 ha (25°36’44.68” S 49°08’17.31” W) planted with the cultivar Syn13671 IPRO expressing the Cry1Ac toxin from B. thuringiensis. Samplings were performed in ten random points using the beat cloth method (Shepard et al., 1974). The collected caterpillars were individualized in 3 x 7 cm polyethylene containers and fed with soybean leaves from the same cultivar in which they were collected. A total of 37 Spodoptera caterpillars were collected; nine S. cosmioides, of which seven were parasitized, and 28 S. eridania, 20 of which were parasitized. From the S. eridania larvae collected, a single one was parasitized by C. scotti.

Adult parasitoids were preserved in 96% alcohol for identification. Part of the material was mounted and morphologically identified using keys to genera (Whitfield, 1997) and species (Muesebeck, 1921; Papp, 1987; Sharkey et al., 2005; Valerio et al., 2009). For comparisons with most similar species in the genus Cotesia, original descriptions and authenticated specimens (i.e. specimens identified and compared with holotype) were examined for all specimens cited in the discussion. Authenticated specimens are deposited at the Canadian National Collection of Insects and Arachnids (CNC), Ottawa, Canada. Five females were used for DNA extraction and then mounted. The studied material was deposited at Coleção Entomológica do Departamento de Ecologia e Biologia da UFSCar (DCBU), São Carlos, SP, and Museu de Entomologia ESALQ (ESALQ), Piracicaba, SP, both in Brazil. Color image of the adult parasitoid was captured with a 3MP Leica video camera and a Leica M205C stereomicroscope running Leica Application Suite (LAS) software, and focus-stacked using the same software. Greyscale image was taken using a Scanning Electronic Microscope FEI Quanta 250. Caterpillars were chosen from a mass rearing, at the Laboratório de Controle Integrado de Insetos in the Universidade Federal do Paraná, and photographed using Sony Cyber Shot 30x optical zoom. Pictures of cocoon masses before parasitoid emergence were taken, at the rearing site (Fig. 4); the cocoon mass reared from S. eridania (Fig. 2) was photographed using a Leica™ DMC 4500 video camera attached to a Leica M205C with a Planapo 1.0× objective, using Leica LAS (Leica Application Suite™ version 3.7) Microsystems and the software Syncroscope™ Auto-montage Pro™ version 5.03.0040. Minor adjustments in images and plate preparation were performed in Adobe Photoshop version CS4.
Laboratory procedures

Genomic DNA was extracted from whole wasp using DNeasy Blood & Tissue Kit (QIAGEN Inc, Valencia, California) following manufacturer’s instructions. Subsequent DNA purification was performed by Ethanol Precipitation (Sambrook and Russell, 2001) and then DNA extracts were resuspended in 50 μL of TE Buffer. COI fragment belonging to the barcoding locus was amplified using the universal LCO 1490 and HCO 2198 primers (Folmer et al., 1994). Polymerase Chain Reaction was carried out in 25 μL final volume (2.5 mM MgCl₂, 2.5 mM dNTP Mix, 0.2 μM each primer, 1× HOT FIREPol® Buffer B1 and 1 U HOT FIREPol® DNA Polymerase, Solis Biodyne) and followed a cycling process of initial denaturation at 95°C for 15 min; 35 cycles of denaturation at 95°C for 45 s, annealing at 56°C for 30 s and extension at 72°C for 1 min; and finally a final extension at 72°C for 5 min. PCR products were purified by PEG (Polyethylene Glycol) precipitation (Lis and Schleif, 1975) and sequenced at Centro de Pesquisa sobre o Genoma Humano e Células-Tronco in Universidade de São Paulo, São Paulo, Brazil.

Sequence analyses

Sequences were edited using the program Sequencher 4.1.4, and then aligned and analyzed with all sequences of Cotesia and Parapanteles which were identified at species level found on BOLD (Barcode of Life Data System, http://v4.boldsystems.org/). The alignment was made using MAFFT version 7 and the phylogenetic analysis using the program Mega X (Kumar et al., 2018). A maximum likelihood (ML) tree was reconstructed with these data using the General Time Reversible (GTR, Nei and Kumar, 2000) evolutionary model, which was found as the most appropriate model of nucleotide substitution for our dataset by hierarchic likelihood ratio test in MEGA X (−LnL = 10642.625, BIC = 27059.765, AIC = 22258.441). A genetic distances table was generated based on the p-distance model with the program MEGA X (Kumar et al., 2018) for better species comparison. We also refer to the Barcode Index Number (BIN) System to discuss species limits, following the BIN concept detailed by Ratnasingham and Hebert (2013).

Results and discussion

Parasitoids were observed in 98 last instar caterpillars of S. cosmoides (Fig. 3) feeding on tomato, which died after the parasitoid larvae left their body. Soon after leaving the body of the host, the larvae began to form cocoon masses involving the body of each caterpillar (Fig. 4). The pupal phase of the parasitoid lasted on average five days and an average of 94.67 ± 4.46 pupae (varying from 20 to 272) were formed in each cocoon mass of the parasitoid per caterpillar, with 98% emergence. From the emerged adults, 81% were females and 19% males.

The single individual of S. eridania (Fig. 1) parasitized was collected from a soybean crop and yielded 25 cocoons (Fig. 2), with emergence of 24 females and 1 male.

A comparison of COI sequences, using the BLAST tool on GenBank, retrieved a 99% similarity with Parapanteles scotti Valerio et al., 2009 (Hymenoptera, Braconidae) (Fig. 7). The identification tool in BOLD Systems, also confirmed the identity of our specimens as P. scotti, with 99.64–99.66% similarity with other public sequences with at least 500 bp. Our sequences are clearly within a BIN cluster (BIN BOLD:AA9141) formed exclusively by P. scotti (31 sequences from specimens from Costa Rica and Argentina). Tree based identification tool also shows that all closer sequences belongs to species of Cotesia. The closest BIN with sequences deposited in BOLD (BIN BOLD:AB20768) corresponds to Cotesia orobeneae (Forbes, 1883) (2.84% distance), a gregarious parasitoid which attacks Evergestis rimosalis (Guenée, 1854) (Pyralidae) in U.S.A. (Yu et al., 2016).

Results of the analyses of the five barcoding sequences of specimens parasitizing S. cosmoides (Genbank accession numbers: MK239190, MK239191, MK239192, MK239193, MK239194), plus one barcoding sequence from specimens on S. eridania (Genbank accession number: MK239195) retrieved virtually identical sequences which are only 0.3-0.4% different from the COI sequence of P. scotti (Table 1 – Supplementary Appendix). The maximum likelihood (ML) tree including all generated sequences is showed in Fig. 5, while the Estimates of Evolutionary Divergence between Sequences (Tamura et al., 2011) with same data is shown in Table 1.

A morphological identification, following Valerio et al. (2009) was performed and the identity of the species confirmed as P. scotti. Morphology of cocoon masses compared with photos in ACG Database (Janzén and Hallwachs, access July 2018) also confirmed identity of the newly reared specimens. Confirmation of the species, however, raised the question of the generic boundaries for Parapanteles and Cotesia. This question was acknowledged and briefly commented by the authors of P. scotti, mainly because that same species was previously treated as Cotesia sp. ‘whitfieldi4’ by Smith et al. (2008). Unfortunately, Valerio et al. (2009) refrained to discuss their decision to describe this species in a different genus. It also happens to be the case for Parapanteles mariae (Valerio et al., 2009), evidencing the difficulty in separating the aforementioned two genera. Our molecular results, as well as those presented by Smith et al. (2008), corroborate the placement of P. scotti in Cotesia.

The genus Parapanteles is poorly defined, and the only available phylogeny does not test its monophyly (Whitfield et al., 2002). Nevertheless, the genus is repeatedly recovered as closely related to Hypomicrogaster, rather than Cotesia, in molecular and morphological analyses (Banks and Whitfield, 2006; Whitfield et al., 2002). Contradicting results of these papers, P. scotti is hardly different from Cotesia species, but easily distinguished from any Hypomicrogaster. Based on the definition of Parapanteles presented by Valerio et al. (2009), and on the morphology matrix in Whitfield et al. (2002), there are up to 13 characters separating Parapanteles and Cotesia. For P. scotti, states of at least six of those characters are the ones found in Cotesia (e.g.: absence of sublateral hairs on metastomum – Fig. 6), and four characters are unknown or intermediate. Only three characters could justify the placement of P. scotti in Parapanteles, all of them on the propodeum: presence of propodeal areola, presence of transverse carina, and absence of median longitudinal carina (Fig. 6). In fact, all keys for genera separate Parapanteles from Cotesia by the presence of a well-defined areola on propodeum (e.g. Austin and Dangerfield, 1992; Whitfield, 1997). However, areola and transverse carina on propodeum are difficult to see in P. scotti due to the strong sculpturing on this sclerite. Morphology of the cocoon masses, which are relatively large and covered by a fluffy silk in P. scotti (Figs. 2,4), unlike usual masses produced by Cotesia, could be an informative character for distinction. The possibility of misidentification is an important taxonomic issue that needs attention, especially regarding the Neotropical fauna. Here, based on morphology of adults and molecular data (Fig. 7) we transfer Parapanteles scotti to Cotesia scotti (Valerio et al., 2009) comb. nov.

All sequences in BOLD cluster as a unique BIN (BIN BOLD:AA9141; average distance: 0.16% p-dist), which translates as a putative species according to a clustering algorithm (Ratnasingham and Hebert, 2013), and therefore corroborates the hypothesis of this species as a distinct entity within Cotesia. This alone is not sufficient evidence to separate C. scotti comb. nov. from other Cotesia species, since not all of them have sequences deposited in this database. Cotesia orobeneae is the closest match for C. scotti, but the two species differ significantly in their barcodes.
(2.84% distance). These two species are readily distinguished by the color of metacoxa, black to dark brown in *C. orobena* and mostly yellow with dark base in *C. scotti*, and the sculpturing of tergite 3, smooth in *C. orobena* but basally striate in *C. scotti*.

Morphology of the species herein transferred is similar to *Cotesia delicata* (Howard, 1897), in having a rugose propodeum, metasomal terga 1–2 and base of tergite 3 rugose striate, inner spur of metatibia less than 0.5× the metabasitarsus and barely longer than outer spur, and mesoscutum strongly punctate with larger/coarser punctuation at notaui. The two species differ mainly in color features, *C. scotti* having more extensive yellow (pale) coloration as compared with *C. delicata*, including all coxa, trochanter, trochantellus, tegula and humeral complex, and base of antenna, all of which are dark brown in *C. delicata*. In *C. scotti*, the propodeum has a poorly defined carina on top of a strongly rugose background, and no median carina, while in *C. delicata* the propodeum has no indication of an areola and the median carina is present at least partially, and tergite 1 is nearly parallel sided in *C. scotti*, but distinctly widening toward apex in *C. delicata*, with apical width about 1.5 times basal width. Additionally, *C. scotti* has a Neotropical distribution (Costa Rica, Brazil and Argentina), and parasitizes Noctuidae, in comparison to a Nearctic distribution, and Erebidae hosts for *C. delicata*. Regarding the Neotropical fauna, *C. scotti* resembles *Cotesia ornatrix* (Muesebeck, 1958), which is also a gregarious parasitoid, however associated with *Ulethesia ornatrix* (L., 1758) (Erebidae: Arctiinae) (Muesebeck, 1958). Although both species have a rugose propodeum, in *C. ornatrix* the median carina is more or less distinct and there is no indication of an areola, but in *C. scotti* the median carina is absent and the areola is more or less distinct. These two species also differ in the sculpturing of tergite 3, smooth in *C. ornatrix*, but rugose-striate basally in *C. scotti*, and in the color of tegula and humeral complex, dark brown in *C. ornatrix*, but yellow in *C. scotti*.

For species with economic importance as natural enemies of pests, covered in the interactive key to species by Sharkey (2005). *C. scotti* is similar to *Cotesia marginiventris*, a species with widespread distribution in the Americas and known to parasitize several species of agricultural pests. Besides morphological resemblance, both species are known to parasitize *Spodoptera* spp. (three and seven species respectively), and the available DNA barcodes for both species are relatively similar (4.7% distance) (Table 1 – Supplementary Appendix). Biology is useful for separating the two species,
since C. scotti is gregarious, and C. marginiventris is mainly solitary (Harris et al., 2012), with only occasional reports, in laboratory conditions, of two adults emerging from one host (Kunnalaca and Mueller, 1979; Riddick, 2002). Color in these two species is very similar, including the mostly yellow metaca with dark brown base (not shared with the other mentioned species), although the antennae of C. marginiventris is entirely dark brown (pale basally in C. scotti) and its metasoma usually with some degree of orange-yellow on terga, usually varying from entire metasoma to tegrite 1–3. Few specimens examined have the entire metasomal tegra black as in C. scotti. Besides color, the main difference is the length of the inner spur of metatibia, less than 0.5 times basitarsus and nearly as long as outer spur in C. scotti, compared to more than 0.5 times basitarsus and distinctly longer than outer spur in C. marginiventris.

Both species, *S. cosmioides* and *S. eridania*, are gaining importance as pests in recent years (Silva et al., 2017; Teodoro et al., 2013). For instance, *S. cosmioides* was used to be considered a sporadic pest, with economic damage registered in different crops during outbreaks (Habib et al., 1983). The same was true for *S. eridania*, considered an incidental pest in soybean (Sosa-Gómez et al., 1993). The frequency of outbreaks of *S. cosmioides* and *S. eridania* is increasing, and currently they are considered as key pests in some grain production systems, especially soybean, and cotton (Bueno et al., 2011; Favetti et al., 2015; Link, 2010; Quintela et al., 2007; Santos et al., 2005, 2010), as well as tomato, in which *S. eridania* is already considered the main fruit borer pest for some regions in Brazil (Miranda et al., 2005). The voracity of these species (Bueno et al., 2011), and the widespread use of Bt crops as the main tactic for pest control, for which these two species are important (Bernardi et al., 2014) are likely the main reasons for their increasing importance as pests (Chilcutt et al., 2007; Silva et al., 2016). The parasitoid *Cotesia scotti comb. nov.* is recorded for the first time on these two species, and from agricultural systems (i.e. organic tomato and Bt soybean), suggesting the importance of this species as a natural enemy of the black arm-worm and the southern arm-worm. Research on natural enemies for species of *Spodoptera* is particularly relevant considering their demonstrated natural tolerance to transgenic Bt crops (Bernardi et al., 2014), a technology which may suppress herbivory for most defoliators, without compromising the ability of parasitoids to find (Liu et al., 2015) and parasitize their host caterpillar.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rbe.2019.05.001.