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Neotropical Species of *Metaphycus* (Hymenoptera, Encyrtidae) Parasitoids of *Ceroplastes* (Hemiptera, Coccidae): New Species, Interaction Records, and a Checklist

MT TAVARES¹, MEV SANTOS², A DAL MOLIN¹, ALBG PERONTI³, CR SOUSA-SILVA⁴

¹Depto de Ciências Biológicas, Univ Federal do Espírito Santo, Vitória, ES, Brasil

²Programa de Pós-graduação em Biologia Animal, Univ Federal do Espírito Santo, Vitória, Brasil

³Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Univ Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, Brasil

⁴In memoriam. Depto de Ecologia e Biologia Evolutiva, Univ Federal de São Carlos, São Carlos, Brasil

Keywords

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Correspondence

MT Tavares, Depto de Ciências Biológicas, Univ Federal do Espírito Santo, Av. Fernando Ferrari 514, 29.075–910, Vitória, ES, Brasil; tavares.mt@gmail.com

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Abstract

Parasitoids of *Ceroplastes* Gray were surveyed in the State of São Paulo, Brazil. Among the parasitoids reared, seven species of *Metaphycus* Mercet (Hymenoptera, Encyrtidae) were obtained. *Metaphycus analuciae* **sp. nov.**, *M. floridensis* **sp. nov.**, and *M. grandis* **sp. nov.**, are described as new species, and *M. ceros* Noyes, *M. comes* Noyes, *M. dardanus* Noyes, *M. opis* Noyes are recorded for the first time from Brazil. Ten new hostparasitoid associations are reported: *M. analuciae* **sp. nov.** parasitizing *C. formicarius* Hempel, *C. glomeratus* Peronti and *C. iheringi* Cockerel; *M. ceros* parasitizing *C. cirripediformis* Comstock; *M. comes* parasitizing *C. formicarius*; *M. dardanus* parasitizing *C. glomeratus*; *M. floridensis* **sp. nov.** parasitizing *C. floridensis*; *M. grandis* **sp. nov.** parasitizing *C. grandis* Hempel; and *M. opis* parasitizing *C. glomeratus* and *C. janeirensis* (Gray). A checklist of *Metaphycus* species associated with *Ceroplastes*, their distribution and host ranges are summarized and discussed.

Introduction

The cosmopolitan genus *Metaphycus* Mercet contains over 450 species, and with 228 recorded from the Neotropical region (Noyes 2017). Until the early 2000s, only 28 species were known from this region (Noyes 2002), and another 12 were reported in the following years (Myartseva 2003, Myartseva & Ruíz-Cancino 2003, Myartseva & Ruíz-Cancino 2005, Noyes & Lozada 2005). This number increased significantly with the publication of the "Encyrtidae of Costa Rica" by Noyes (2004), a remarkable contribution to the knowledge of neotropical fauna, which added another 188 species records, mainly to Costa Rica, of which 182 were described as new. That monograph also provided the first and most comprehensive identification key to neotropical species of this genus.

Metaphycus are mainly primary endoparasitoids of Coccidae and Diaspididae (Hemiptera), although species of this genus have also been reported parasitizing Triozidae, Aleyrodidae, and six other families of Coccoidea (Noyes 2004). Because of their role as parasitoids of scale insects, some species have been used as biological control agents in programs around the world (Noyes & Hayat 1994). About 26 species of *Metaphycus* are known to be associated with *Ceroplastes* Gray wax scales (Noyes 2002, 2017).

Ceroplastes is the largest genus of Coccidae, with approximately 144 described species distributed worldwide, although most are native or endemic to the Neotropics and Afrotropics (78 and 47 species, respectively) (Qin *et al* 1998, Hodgson & Peronti 2012, García Morales et al. 2016). Some wax scales are pests of orchard trees and ornamental plants, including plants used in urban landscaping (Jordão & Silva 2006, Peronti et al 2008, Hodgson & Peronti 2012). They are found mainly on leaves and branches of their host plants, and less frequently on stems and fruits (Peronti et al 2008). Similar to other scale insects, they cause direct damage to plants by sucking their sap, and indirect damage by injecting salivary secretions and secreting sugary honeydew, which can promote the development of sooty mold and attract other insects. Wax scales earned their common name as a reference to the thick waxy layer that covers the body of the adult females (Gimpel et al 1974, Peronti et al. 2008), which protects the individuals from contact insecticides. As a result, most management measures rely on oils, insecticidal soap, and the presence of natural enemies. Biological control is an important method for population management of these pests, and taxonomic studies of natural enemies are an important initial step to develop biological control programs (Zucchi 1990, Schauff & Rossman 1991, Perioto & Tavares 1999, Huber et al 2002).

Throughout the course of a survey of Ceroplastinae from the State of São Paulo (Brazil), new and described species of *Metaphycus* were reared from *Ceroplastes* hosts. These species and their host records are presented here. In addition, we present a synopsis and discussion of *Metaphycus* species associated with *Ceroplastes*.

Material and Methods

Parasitoid wasps were reared from 157 samples of 18 species of *Ceroplastes*, as part of the survey conducted by Peronti *et al* (2008). The samples were collected in 18 localities in the state of São Paulo (Brazil), comprising urban and native vegetation (cerrado) areas, between March 2000 and September 2002.

Wax scales on plant branches and leaves were placed into glass tubes closed with a cotton plug. Mixed series of hosts may introduce erroneous host records (Noyes 1994); therefore, all insects except for the *Ceroplastes* scales were removed from the samples to ensure any emerged parasitoids were associated with them.

The *Ceroplastes* hosts were vouchered, described and identified by ALBGP as reported in Peronti *et al* (2008). Identification of the *Metaphycus* species was based mainly on Noyes (2004), who provided keys and differential diagnoses to most species recorded in the Neotropics. Additionally, specimens were compared with extensive literature on other described species recorded from the New World and other regions, or associated with *Ceroplastes* scales.

Morphological terms and measurements in *Metaphycus* descriptions mainly follow Noyes (2004). Integument sculpture terms follow Harris (1979). Measurements were

taken from critical-point-dried specimens. A URI table with links of morphological terms to corresponding definitions in the Hymenoptera Anatomy Ontology, as described by Seltmann *et al* (2012) is provided as supplementary material (Supplementary Table S1).

Abbreviations applied in descriptions follow Noyes (2004): AOD, largest diameter of anterior ocellus; AOL, minimum distance between posterior ocellus and anterior ocellus; EL, maximum diameter of eye; EW, minimum diameter of eye; F1, F2, etc., funicle segment one, two, etc.; FV, minimum width of frontovertex; FVL, length of frontovertex from occipital margin to top of antennal scrobes as seen in dorsal view; FWL, length of fore wing measured from base of costal cell to apex of wing membrane; FWW, maximum width of fore wing; GL, length of ovipositor sheath (gonostylus); HW, head width measured in frontal view; MT, length of mesotibia; OCL, minimum distance between posterior ocellus and occipital margin; OOL, minimum distance between posterior ocellus and adjacent orbit; POD, largest diameter of posterior ocellus; POL, minimum distance between posterior ocelli; SL, length of scape, excluding radicle; SW, maximum width of scape; T1, T2, etc., gastral tergite one, two, etc.

Voucher specimens (including holotypes and paratypes) are deposited in the following collections: **DCBU**, Department of Evolutionary Biology, Universidade Federal de São Carlos, São Carlos, Brazil; **MZSP**, Museum of Zoology, Universidade de São Paulo, São Paulo, Brazil; **UFES**, Department of Biological Sciences, Universidade Federal do Espírito Santo, Vitória, Brazil. Specimen identifiers are provided, where known, on text (holotypes) and on Supplementary Material (Table S2). Updates to this table are also available via Zenodo (DOI: https://doi.org/10.5281/zenodo.1481912).

All new distributional and host records for previously described *Metaphycus* species are marked with an asterisk (*). Proposed modifications to the key presented by Noyes (2004) to include each new species are presented under "Species Identification," at the end of each new species description.

The checklist of *Metaphycus* species associated with *Ceroplastes* species and related information (distribution, host range, and *Ceroplastes* hosts) was first compiled from the Universal Chalcidoidea Database (Noyes 2002, 2017) and complemented with other sources, mainly using a metasearch engine from the Periodicos CAPES web portal (http://periodicos.capes.gov.br), selecting all available databases in Life Sciences, and specialized databases. That portal queries most basic and applied entomology databases, including Biological Abstracts, CAB Direct, Web of Science, and others. Primary records were checked to the extent it was possible and likely misidentifications were indicated.

Results

Seven species of *Metaphycus* were reared from the *Ceroplastes* samples: 3 described below as new and 4 previously known from Costa Rica: *M. ceros* Noyes, *M. comes* Noyes, *M. dardanus* Noyes, and *M. opis* Noyes. Ten host-parasitoid associations between these species of *Metaphycus* and 7 species of *Ceroplastes* were observed, most previously unrecorded. These are the first host records for the *Metaphycus* species mentioned above and also the first records of *Metaphycus* as parasitoids of *C. formicarius* Hempel, *C. glomeratus* Peronti, *C. grandis* Hempel, *C. iheringi* Cockerel, and *C. janeirensis* (Gray).

The reared species are presented below in alphabetical order, along with their host records, distributional records, and descriptions of the new species. This section is followed by a checklist of *Metaphycus* species recorded as parasitoids of *Ceroplastes* (Table 1) and a discussion of these host-parasitoid relationships.

Reared species

All reared species presented below belong to the *insidiosus* species group of *Metaphycus*, which is characterized by both labial and maxillary palps with 3 segments. Noyes (2004), revising the Costa Rican fauna, keyed or discussed most of the species of this group present in the New World. Besides those, there are records of another 24 species of this species group and 28 species of *Metaphycus* not assigned to species groups for the New World. Comparative notes are provided below regarding at least the other New World species.

Metaphycus analuciae Santos & Tavares **sp. nov.** (Figs 1– 4, 14–15)

Zoobank: URN:LSID:ZOOBANK.ORG:ACT:46D38577-178C-4788-9163-D9B09660475C

<u>Type material</u>. Holotype (dry-mounted, UFES 160458): female, labeled "Brasil, São Paulo, São Carlos, ex. *Ceroplastes iheringi* on *Baccharis dracunculifolia*, sample no. 398, V.2002, A.L.G.Peronti col." (deposited at UFES). Paratypes: São Paulo: 5 $\bigcirc \bigcirc$ (1 on slide) and 1 \bigcirc , same data as holotype (1 \bigcirc DCBU, 1 \bigcirc MZSP, UFES); 1 \bigcirc , Gália, Estação Ecológica de Caetetus, ex. *C. glomeratus* on *Acacia plumosa*, sample no. 390, 31.IV.2002, A.L.G.Peronti col. (UFES); 2 $\bigcirc \bigcirc$ and 1 \bigcirc , São Carlos, EMBRAPA, ex. *C. formicarius* on *Banisteriopsis oxyclada*, sample no. 281, 16. VIII.2001, A.L.G.Peronti col., (1 \bigcirc DCBU, 1 \bigcirc MZSP, UFES).

<u>Etymology</u>. The name of this species is dedicated to Dr. Ana Lúcia Benfati Gonzalez Peronti, for her efforts on the survey of Ceroplastinae and their parasitoids.

<u>Description</u>. FEMALE. Body length 1.47 mm (including ovipositor sheath 1.80 mm).

Color. Head mostly black, with interantennal prominence white; upper face, vertex and outer orbits yellow to orange (Figs 1–2); antenna with scape dark brown, yellow on apical quarter, pedicel with anterior half dark brown and posterior half yellow, F1–F4 brown, clava dark brown (Fig 4); pronotal neck black, collar white with a small brown dorso-lateral spot; mesonotum orange-brown with darkened margins (Figs 2–3); tegula white with a brown median stripe; metanotum black, yellow anteriorly and medially; propodeum black, yellowish medially; prepectus white; mesopleuron yellow with white margins (Fig 3); legs (Fig 3) mostly yellow, except for the base of pro- and metacoxa, apex of pro- and mesofemur, longitudinal stripe on metafemur, middle and apex of protibia, two anellar spots on meso- and metatibia, brown; wings hyaline; gaster dorsally and ventrally dark brown.

Head. foveate-imbricate; 4.0 times as wide as frontovertex; ocelli forming an angle of about 60°; eye reaching occipital margin; frontovertex narrowest at level of dorsal margin of scrobes; scrobe -shaped, slightly deep; interantennal prominence protruding and dorsally acuminate; lateral antennal groove present; torulus separated from mouth margin by about 0.5 times its own length; gena glabrous; palp formula 3:3; mandibles 3-toothed; scape 4.0 times as long as broad (Figs 4, 14); funicle with linear sensilla on F2–F6; clava as long as F4–F6 combined; apex truncate. Relative measurements: AO 6, AOL 9, EL 42, EW 23, FV 19, FVL 23, HW 75, OCL 4, OOL 2, POD 5, POL 10.

Mesosoma. Mesoscutum imbricate-alveolate, 1.5 times as long as broad; notaular line inconspicuous; side of propodeum with several scattered setae below spiracle, with a carina bordering the postspiracular sulcus; mesopleuron without apical-posterior carina; fore wing with *linea calva* interrupted by two lines of setae (Fig 15); mesotibial spur as long as mesobasitarsus. Relative measurements: FWL 193, FWW 85, MT 45.

Metasoma. Gaster dorsally coriaceous; T6 with spiracle not on a protuberance; apex of epipygium rounded; hypopygium reaching about 0.4 times along gaster; ovipositor sheath about 2.4 times as long as mesotibial spur and 0.75 times as long as metatibia (Fig 3). Relative measurements: GL 50.

MALE: Unknown.

Variation. No significant variation observed.

<u>Hosts</u>. Ceroplastes formicarius, C. glomeratus and C. iheringi.

Distribution. Brazil (State of São Paulo).

<u>Comments</u>. *Metaphycus analuciae* **sp. nov.** differs from other New World species by the following combination of characters: palp formula 3:3; most of the gena and mouth margin dark (Figs. 1–3); scape 4.0 times as long as broad (Figs. 4, 14); funicle with linear sensilla on F2–F6; clava as long as F4–F6 combined, apex truncate; hypopygium reaching about 0.4 length of gaster; ovipositor sheath long, about 2.4 times as long as mesotibial spur and 0.75 times as long as

Table 1 Checklist of *Metaphycus* species recorded as parasitoids of *Ceroplastes*, including distribution, host range, verbatim *Ceroplastes* hosts, record reference, and country. Distribution and hosts follow mainly Noyes (2002, 2017). Except where noted, host-parasitoid record references are primary records. A question mark (?) indicates that taxon identification was not confirmed. Coccoidea families are abbreviated as: **As**, Asterolecaniidae; **Ce**, Cerococcidae; **Co**, Coccidae; **Er**, Eriococcidae; **Ke**, Kerridae; **Ps**, Pseudococcidae.

Metaphycus species	Distribution (country or region)	Host range (family (genera/species))	Ceroplastes hosts	Reference and country of primary host-parasitoid association record
africanus Compere	Egypt, South Africa	Co (5/6), Ke (1/1)	Ceroplastes sp.	Annecke & Mynhardt (1981), South Africa
agrestis Annecke & Mynhardt	South Africa	Co (1/1)	Ceroplastes sp.	Annecke & Mynhardt (1972), South Africa
alberti ¹ (Howard)	USA (California, Hawai), Costa Rica, South Africa, China, Australia, New Zealand	Co (2,4)	Ceroplastes sp.	Annecke & Mynhardt (1981), South Africa
albiventris Compere	Congo, Eritrea	Co (1/1)	C. destructor Newstead	Ghesquière (1955), Congo
alienus Wang	China	Co (1/1)	C. rusci (Linnaeus)	Wang et al (2014a), China
analuciae ¹ Santos & Tavares sp. nov.	Brazil	Co (1/3)	C. formicarius C. glomeratus	present study, Brazil present study, Brazil
			C. iheringi Cockerel	present study, Brazil
aquilus Annecke & Mynhardt	South Africa	Co (3/4), Ke (1/1)	<i>C. deceptrix</i> (De Lotto)	Annecke & Mynhardt (1981), South Africa
			C. elytropappi (Brain)	Annecke & Mynhardt (1981), South Africa
bolangerae Hayat	India	Co (2/2)	C. actiniformis Green	Hayat et al (2003), India
powensis (Girault)	Australia	Co (1/1)	C. rubens Marskell	Dahms (1983), Australia
ceroplastis ² (Howard)	USA (Florida, New Mexico)	Co (1/2)	C. floridensis Comstock	Hubbard (1885), as <i>M</i> .? ceroplastis in USA (Florida)
			C. irregularis Cockerell	Thompson (1955) ³ , USA
capensis Annecke & Mynhardt	South Africa	Co (2/3), Ps (1/1)	C. rufus De Lotto	Annecke & Mynhardt (1971), South Africa
			C. tachardiaformis Brain	Annecke & Mynhardt (1971), South Africa
teros ¹ Noyes	Central America (Trinidad & Tobago, Costa Rica), Brazil, Galapagos	Co (1/1)	C. cirripediformis Comstock	present study, Brazil
<i>claviger</i> (Timberlake)	Iran, China, New Zealand	Co (2/3)	C. floridensis	Xu & Huang (2004), China
			C. japonicus Cockerell	Xu & Huang (2004), China
comes ¹ Noyes	Costa Rica, Brazil	Co (1/1)	C. formicarius Hempel	present study, Brazil
confusus Compere	South Africa	Co (1/1)	Ceroplastes sp.	Annecke & Mynhardt (1972), South Africa
dardanus ¹ Noyes	Costa Rica, Brazil	Co (1/1)	C. glomeratus Peronti	present study, Brazil
<i>decorus</i> Annecke & Mynhardt <i>discolor</i> ¹ (De Santis)	South Africa Brazil	Co (1/1) Co (1/1)	Ceroplastes sp. Ceroplastes sp.	Annecke and Mynhardt (1972), South Africa
dispar ² (Mercet)	USA (California), Palearctic	Co (9/15)	Ceropiusies sp. C. rusci	De De Santis (1970), Brazil Öncüer (1991), Türkey
eruptor ¹ (Howard)	USA, Mexico, Caribbean, Costa Rica, Israel, Hawaii	Co (3/6)	C. cirripediformis	Dozier (1936), as <i>M. ceroplastae</i> in Haiti; Thompson (1955), as <i>M. mexicanus</i> in USA; De Santis (1979), Puerto Rico
			C. floridensis	Dozier (1936), Haiti; Muma <i>et al</i> (1961), USA (Florida); Argov <i>et al</i> (1992), Israel
			C. giganteus Dozier	Dozier (1936), Haiti
<i>farfani</i> ¹ Myartseva <i>ferinus</i> Annecke &	Mexico South Africa	Co (1/1) Co (1/2)	Ceroplastes sp. C. elytropappi	Myartseva (2003), Mexico Annecke & Mynhardt (1972),
Mynhardt			C. tachardiaformis	South Africa Annecke & Mynhardt (1972), South Africa
<i>ferrierei</i> Compere	Uganda	Co (1/1)	C. destructor	South Africa Annecke & Insley (1971), Uganda
flavus ¹ (Howard)	~ cosmopolitan	Ce (1/1), Co (16/42), Di (2/6), Er (1/1), Ke (2/2)	C. cirripediformis	Bennett & Alam (1985), Barbados
		2. (2, 0), 2. (1, 1), 10 (2) 2)	C. floridensis	Muma et al (1961), USA (Florida); Cohen & Kamburov (1968), Trinidad & Tobago; Hafez et al (198 Egypt [as <i>M. subflavus</i> in Noyes 20 Argov et al (1992), Israel
floridensis ¹ Santos & Tavares	Brazil	Co (1/1)	C. floridensis	present study, Brazil

Table 1 (continued)

Metaphycus species	Distribution (country or region)	Host range (family (genera/species))	Ceroplastes hosts	Reference and country of primary host-parasitoid association record
fusiscapus Wang, Li & Zhang	China	Co (1/1)	C. floridensis	Wang et al (2013), China
galbus Annecke	Spain, Africa	Co (5/5)	Ceroplastes sp.	Annecke & Mynhardt (1981), South Africa
gerardi Sugonjaev	China, Vietnam	Co (1/3)	C. ceriferus (Fabricius)	Sugonjaev (1996), Vietnan
			C. rubens	Wang et al (2014b), China
			C. rusci	Wang et al (2014b), China
grandis ¹ Santos & Tavares sp. nov.	Brazil	Co (1/1)	C. grandis Hempel	present study, Brazil
helvolus ¹ (Compere)	~ Cosmopolitan	Co (13/33), Di (3/3), Ps (1/1)	C. destructor	Snowball (1970), Australia
			C. pseudoceriferus Green	Bhuiya et al (1997), Bangladesh
			C. sinensis Del Guercio	Snowball (1970), Autralia; Martinez- Ferrer <i>et al</i> (2015), Mediterran region
			C. sinoiae Hall	Annecke & Mynhardt (1981), South Africa
limuruensis Compere	Kenya	Co (1/1)	Ceroplastes sp.	Compere (1940), Kenya
lineascapus Compere	South Africa	Co (3/3)	Ceroplastes sp.	Compere (1940), South Africa
<i>lounsburyi</i> ¹ (Howard)	~ Cosmopolitan	As (1/1), Co (7/11), Di (1/1)	C. floridensis	Trjapitzin (1989) ³ , as <i>M. bartletti</i> in Israel
lutus Annecke & Mynhardt	South Africa	Co (1/1)	Ceroplastes sp.	Annecke & Mynhardt (1972), South Africa
maculatus Agarwal	India	Co (4/4), Er (1/1)	Ceroplastes sp.	Agarwal (1965), India
malgacinus (Risbec)	Madagascar	Co (1/1)	C. madagascariensis (Tordioni-Tozzetti)	Risbec (1957), Madagascar [after Herting 1972, cited under <i>M. praevidens</i>]
<i>niger</i> ¹ (Brèthes)	Chile, Argentina	Co (3/6), Di (1/1), Er (1/1)	C. ceriferus	De Santis (1979) ³ , Argentina & Chile
opis ¹ Noyes	Costa Rica, Brazil	Co (1/1)	C. glomeratus	present study, Brazil
			C. janeirensis (Gray)	present study, Brazil
solatus Annecke & Mynhardt	South Africa	Co (1/2)	C. destructor	Annecke & Mynhardt (1971), South Africa
			C. rusticus De Lotto	Annecke & Mynhardt (1971), South Africa
stanleyi ¹ Compere	New World, Europe, Africa	Co (9/21)	C. brevicauda Hall	Guerrieri & Noyes (2000) ³ , Europe
			Ceroplastes sp.	Annecke & Mynhardt (1972), South Africa; Cave & Márquez (1994), Honduras
utibilis Annecke & Mynhardt	South Africa	Co (1/1)	C. brevicauda	Annecke & Mynhardt (1971), South Africa
vanderplanki Annecke & Mynhardt	South Africa, Swaziland	Co (1/2)	C. brevicauda	Annecke & Mynhardt (1972), South Africa
			C. destructor	Annecke & Mynhardt (1972), South Africa
varius (Girault)	Australia	Co (1/1)	C. rubens	Fry (1989) ³ , Australia
vernus Annecke & Mynhardt	South Africa	Co (3/3)	Ceroplastes sp.	Annecke & Mynhardt (1972), South Africa
wui Wang, Li & Zhang	Iran, China, New Zealand	Co (1/1)	Ceroplastes sp.	Wang et al (2013), China
zebratus ² (Mercet)	USA (California), Palearctic, India	As (2/2), Co (11/23), Di (1/1), Er (2/2), Ps (3/3)	C. floridensis	Rosen (1962) ³ , Israel
		(,,,, (-,-,, - (3)))	C. rusci	Abd-Rabou (2011), Egypt
Metaphycus sp. undetermined			C. Rubens	Konar & Saha (2002), as <i>M. nr.</i> <i>lichtensiae</i> in India [as <i>M. lichtensiae</i> in Noyes 2017]

¹Species found in Neotropics; ² likely to be found in Neotropics; ³ secondary record.

metatibia (Fig. 3); and general color of dorsum of meso- and metasoma. In Noyes (2004), *M. analuciae* **sp. nov.** keys out close to *M. comes,* sharing the dark gena and mouth margin (Figs. 1–3). These species also share a host, *C. formicarius*. They differ in that *M. analuciae* **sp. nov.** has linear sensilla on F2–F6 (*M. comes* only on F3–F6) (Fig. 14), mesopleuron

yellow with white margins (Fig. 3), legs with dark spots (*M. comes* mesopleuron is dark brown and the legs are pale orange), and ovipositor sheath 2.4 times as long as mesotibial spur (*M. comes* 0.25 times).

<u>Species Identification</u>: In the key provided by Noyes (2004), this species runs to couplet 11, through the second

option, and could be separated by an additional couplet as follows:

11a (11) Linear sensilla on F2–F6	analuciae
Santos & Tavares sp. nov.	
Linear sensilla only on F6	12

Metaphycus ceros Noyes

Metaphycus ceros Noyes 2004: 67-68.

<u>Material examined.</u> BRAZIL. *State of São Paulo*. São Carlos: 19 $\bigcirc \bigcirc$ and 2 $\bigcirc \bigcirc$, ex. *C. cirripediformis* on *Duranta repens* v. *aurea*, sample no. 37, 21.III.2001, A.L.G. Peronti col., (4 $\bigcirc \bigcirc$ DCBU, 4 $\bigcirc \bigcirc$ 1 \bigcirc MZSP, UFES). São Paulo: 5 $\bigcirc \bigcirc$ and 1 \bigcirc , ex. *C. cirripediformis* on *Duranta repens* v. *aurea*, sample no. 41, 22.IV.2001, A.L.G. Peronti col. (UFES).

<u>Distribution.</u> Costa Rica, Trinidad & Tobago, Ecuador (Galapagos) (Noyes 2004) and Brazil (State of São Paulo)*.

Hosts. Ceroplastes cirripediformis*.

<u>Comments.</u> Females of *M. ceros* are very distinctive from other species, due to their mainly orange body color, with the mesopleuron dark brown on posterior two-thirds. Males are similar to females, except by the body dorsum, dark brown.

Metaphycus comes Noyes

Metaphycus comes Noyes 2004: 65-66.

Material examined. BRAZIL. *State of São Paulo*. São Carlos: 4 $\bigcirc \bigcirc$ (1 on slide), UFSCar campus, Trilha da Natureza, ex. *C. formicarius*, sample no. 302, 18.IX.2001, A.L.G. Peronti col. (1 \bigcirc MZSP, UFES).

Distribution. Costa Rica (Noyes 2004) and Brazil (State of São Paulo)*.

Hosts. Ceroplastes formicarius*.

<u>Comments</u>. *Metaphycus comes* and *M. analuciae* **sp. nov.** are parasitoids of *C. formicarius* and they share some features, as indicated under comments for *M. analuciae* **sp. nov.**

Metaphycus dardanus Noyes.

Metaphycus dardanus Noyes 2004: 205–206.

<u>Material examined</u>. BRAZIL. *State of São Paulo*. Gália: 10 $\bigcirc \bigcirc$ (1 on slide) and 1 \bigcirc , ex. *C. glomeratus* on *Acacia plumosa*, sample no. 392, 31.IV.2002, A.L.G. Peronti col. (2 $\bigcirc \bigcirc$ DCBU, 3 $\bigcirc \bigcirc$ MZSP, UFES).

Hosts. Ceroplastes glomeratus*.

<u>Distribution</u>. Costa Rica (Noyes 2004) and Brazil (State of São Paulo)*.

<u>Comments</u>. The females of *M. dardanus* examined here present F1–F3 brown and head 3.5 times as wide as frontovertex. *Metaphycus dardanus* and *M. floridensis* **sp. nov.** share features, as indicated under comments for *M. floridensis* **sp. nov.**

Metaphycus floridensis Santos & Tavares sp. nov.

Zoobank: URN:LSID:ZOOBANK.ORG:ACT:FDE29EDD-376B-4E23-A116-6BB9B0D791AE <u>Type material</u>. Holotype (dry-mounted, UFES 160488): female, labeled "BRASIL, São Paulo, São Paulo, ex. *C. floridensis* on *Eriobotrya japonica* (as *Prunus domestica* in *Peronti et al* 2008), sample no. 228, 08.III.2001, A.L.G. Peronti col." (deposited at UFES). Paratypes: 11 QQ (1 on slide) and 2 dd, same data as holotype (2 QQ DCBU, 2 QQ 1 dd MZSP, UFES).

Etymology. Named after the host species, Ceroplastes floridensis.

<u>Description</u>. FEMALE. Body length 1.27 mm (including ovipositor sheath 1.33 mm).

Color. Body predominantly pale yellow (Figs 6–7), except as follows: orange-yellow on mesonotum; yellow on antennal radicle, base and apex of scape, and base of pedicel; pale yellow on F5 and base of F6; dark brown on outer face and a ventral strip on inner face of scape, apex of pedicel, apex of F6, and clava (Figs 5, 8); F1–F3 brown; black on occiput, pronotal neck, a small dorso-lateral spot on collar; brown on transscutal line, lateral portion of propodeum and dorsal surface of gaster. Wings hyaline.

Head. Foveate-imbricate, 4.5 times as wide as frontovertex; ocelli forming an angle of about 45°; eyes reaching occipital margin; frontovertex parallel-sided, wider at level of posterior ocelli; scrobe -shaped; moderately deep; interantennal prominence slightly protruding and dorsally acuminate; lateral antennal groove present; torulus separated from mouth margin by about half of its own length; gena pilose; palp formula 3:3; mandibles 3-thoothed; scape 3.0 times as long as broad; funicle with linear sensilla on F5–F6 (Fig 16); clava as long as F3–F6 together; apex not truncate. Relative measurements: AOD 4, AOL 9, EL 34, EW 22, FV 12, FVL 24, HW 56, OCL 6, OOL 1, POD 6, POL 8.

Mesosoma. Mesoscutum imbricate-alveolate, 1.5 times as long as broad; notaular line reaching 0.25 times across mesoscutum; side of propodeum more or less naked below spiracle, but with a line of about 10 setae on posterior margin above metacoxae; propodeum with one carina bordering the postspiracular sulcus; mesopleuron without apical-posterior carina; fore wing (Fig 17) with *linea calva* interrupted by three lines of setae; mesotibial spur 0.8 times as long as mesobasitarsus. Relative measurements: FWL 145, FWW 58, MT 52.

Metasoma. Gaster dorsally imbricate; T6 with spiracle not on a protuberance; apex of epipygium rounded; hypopygium reaching about 0.4 times along gaster; ovipositor sheath 1.2 times as long as mesotibial spur and 0.30 times as long as metatibia (Fig 3). Relative measurements: GL 8.

MALE: Body length 1.00 mm. Similar to female, except by the body color (dorsum mainly dark brown, ventral and lateral surfaces pale yellow; antenna predominantly brown; inner margin of radicle, scape and funicle pale yellow; pronotal collar white).

<u>Variation</u>. No significant variation observed. <u>Hosts</u>. *Ceroplastes floridensis*. <u>Distribution</u>. Brazil (State of São Paulo).

Comments. The predominantly pale yellow color on the head, sides of meso and metasoma, and the short ovipositor sheath are characteristics of many species of Metaphycus. M. floridensis sp. nov. differs from other New World species by the following combination of characters: palp formula 3:3; scape 3.0 times as long as broad (Figs 4, 14); funicle with linear sensilla on F5-F6; clava as long as F3-F6 combined, apex not truncate; interantennal prominence dorsally acute; notaular line reaching about 0.2 times across mesoscutum; hypopygium reaching about 0.4 times along gaster; ovipositor short, about 1.2 times as long as mesotibial spur and 0.30 times as long as metatibia (Fig 3); legs without dark spots; dorsum of gaster brown; and color of antennal segments (Fig 8). In Noyes (2004), M. floridensis sp. nov. keys close to M. medio Noyes mainly due to the body color, antennal shape and antennal sensilla, but M. floridensis sp. nov. has a dorsally acuminate interantennal prominence and notaular line reaching extending about 0.25 length of mesoscutum (M. medio with interantennal prominence dorsally rounded and notaular line extending about 0.4 length of mesoscutum).

Species Identification: In the key provided by Noyes (2004), this species runs to couplet 179, which is adapted as follows:

179 (178) Outer face of scape with a more or less complete medium band (Figs. 692, 693)......179a

Outer face of scape with only an elongate, slender, brown spot along the dorsal margin (Figs. 696, 699)......180 179a (179) Interantennal prominence dorsally roundedmedio Noyes (p. 297) Interantennal prominence dorsally acute

......floridensis Santos & Tavares sp. nov.

Metaphycus grandis Santos & Tavares **sp. nov**. (Figs 9–13, Figs. 18–19)

Zoobank: URN:LSID:ZOOBANK.ORG:ACT:481F870F-BAC2-4044-951D-5344E308315C

<u>Type material</u>. Holotype (dry-mounted, UFES 160495): female, labeled "BRASIL, São Paulo, Campos do Jordão, ex. *Ceroplastes grandis* on *Platanus* sp., sample no. 316, 25.XI.2001, A.L.G. Peronti col." (deposited at UFES). Paratypes: 8 \bigcirc (1 on slide) and 2 \bigcirc , same data as holotype (1 \bigcirc DCBU, 2 \bigcirc \bigcirc 1 \bigcirc MZSP, UFES).

<u>Etymology</u>. Named after the host species, *Ceroplastes grandis*.

<u>Description</u>. FEMALE. Body length 2.40 mm (including ovipositor sheath 3.60 mm).

Color. Body mainly lemon yellow (Fig 10–11), except as following: upper face light orange; mesonotum orange; gaster dorsally brown; medial portion of scape, distal half of pedicel, F1 and clava (Fig 12), most of occiput, pronotal neck, dorso-lateral spot on collar, sides of metanotum and propodeum dark brown. Wings hyaline.

Head. Coriaceous; 4.0 times as wide as frontovertex; ocelli forming an angle of about 45°; eye reaching occipital margin; frontovertex parallel-sided, becoming wider at level of posterior ocelli; scrobe \cap – shaped; moderately deep; interantennal prominence slightly protruding and dorsally rounded (Fig 13); lateral antennal groove present; torulus separated from mouth margin by about half its own length; gena pilose; palp formula 3:3; mandibles 3-toothed; scape 3.5 times as long as broad; funicle with linear sensilla on F4–F6 (Fig 18); clava as long as F3–F6 together, apex truncate. Relative measurements: AOD 6, AOL 12, EL 49, EW 31, FV 20, FVL 30, HW 75, OCL 8, OOL 3, POD 6, POL 7.

Mesosoma. Mesoscutum coriaceous, 1.5 times as long as broad; notaular line reaching 0.25 times across mesoscutum; side of propodeum more or less naked below spiracle, but with a line of about 10 setae on posterior margin above metacoxae; propodeum with pair of carinae bordering the postspiracular sulcus; mesopleuron with apical-posterior carina; fore wing with *linea calva* interrupted by one line of setae (Fig 19); mesotibial spur 0.8 times as long as mesobasitarsus. Relative measurements: FWL 250, FWW 100, MT 90.

Metasoma. Gaster dorsally coriaceous; T6 with spiracle on a tubercle; apex of epipygium acuminate (Fig 9); hypopygium reaching about 0.3 times along gaster; ovipositor sheath about 6.5 times as long as mesotibial spur and 2.0 times as long as metatibia (Fig 11). Relative measurements: GL 145.

MALE: Body length, 1.32 mm. Similar to female, except: inner margin of scape, pedicel and funicle, yellow; clava light brownish.

Variation. Linea calva may be not interrupted.

Distribution. Brazil (State of São Paulo).

Hosts. Ceroplastes grandis Hempel.

<u>Comments</u>. *Metaphycus grandis* **sp. nov.** differs from other species of the genus by the long ovipositor sheath, 1.5 times as long as length of gaster (Fig 7), by the spiracle of T6 on a tubercle (Fig 9), and by the presence of linear sensilla only on F4–F5 (Fig 18). It is similar to *M. discolor* (De Santis), a species described from Brazil and associated with an undetermined *Ceroplastes*. Both species share most of body color, long ovipositor sheath, and *linea clava* interrupted by one line of setate (Fig 19), but *M. grandis* **sp. nov.** has the occiput entirely brown (*M. discolor* with two spots on occiput), the ovipositor sheath 2.0 times as long as metatibia (Fig 11) (*M. discolor* with 1.3 times), besides the characters mentioned above.

Species Identification: In the key provided by Noyes (2004), this species runs to couplet 127, which is adapted as follows:

127a (127) Palp formula 2-2; ovipositor sheath 6	5.5 times as
long as mesotibial spur	<i>clio</i> Noyes

l



Fig. 1–13 1–4, *Metaphycus analuciae* sp. nov., female: 1, holotype, head, frontal; 2, holotype, habitus, dorsal; 3, holotype, habitus, lateral; 4, paratype, antenna. 5–8, *Metaphycus floridensis* sp. nov., female: 5, holotype, head, frontal; 6, holotype, habitus, dorsal; 7, holotype, habitus, lateral; 8, paratype, antenna. 9–13, *Metaphycus grandis* sp. nov., holotype, female: 9, gaster, spiracle on a tubercle; 10, habitus, dorsal; 11, habitus, lateral; 12, antenna; 13, head, frontal.

Palp formula 3-3; ovipositor sheath 0.6 times as long as mesotibial spur.....grandis Santos & Tavares **sp. nov.**

Metaphycus opis Noyes *Metaphycus opis* Noyes 2004: 266–267.



Fig. 14–19 14–15, *Metaphycus analuciae* sp. nov., female: 14, paratype, antenna; 15, paratype, fore wing. 16–17, *Metaphycus floridensis* sp. nov., female: 16, paratype, antenna; 17, paratype, fore wing. 18–19, *M. grandis* sp. nov., female: 18, paratype, antenna; 19, paratype, fore wing.

<u>Material examined</u>. BRAZIL. State of São Paulo. São Carlos: 3 $\bigcirc \bigcirc$ (1 on slide) and 1 \circlearrowleft , ex. *C. janeirensis* on *Stenocalyx pitanga*, 22.VI.2001, sample no. 264, A.L.G.Peronti col. (UFES). Gália: 8 $\bigcirc \bigcirc$, ex. *C. glomeratus* on *Acacia plumosa*, 24.II.2002 (4 $\bigcirc \bigcirc$) and 31.IV.2002 (4 $\bigcirc \bigcirc$), A.L.G. Peronti, col. (2 \bigcirc DCBU, 2 \bigcirc MZSP, UFES).

Distribution. Costa Rica (Noyes 2004) and Brazil (State of São Paulo)*.

Hosts. Ceroplastes glomeratus* e C. janeirensis*.

<u>Comments</u>. *Metaphycus opis* is similar to *M. floridensis* **sp. nov.** as pointed in comments to the latter.

Metaphycus-Ceroplastes relationships

A checklist of the *Metaphycus* attacking *Ceroplastes*, their geographical distribution, host range, and source references are presented in Table 1. A total of 47 species of *Metaphycus* are known as parasitoids of at least 25 species of *Ceroplastes* (in 15 additional records, hosts are identified only at the genus level) in 67 host-parasitoid interactions, including the new records presented here.

About one-third (16) of these species occur in the Neotropics, 10 of them restricted to this region and 6 cosmopolitans. The neotropical species are associated with at least 14 species of *Ceroplastes* (in four additional records, hosts are identified only at the genus level) in 26 host-parasitoid associations. *Metaphycus ceroplastis* (Howard), *M. dispar* (Mercet), and *M. zebratus* (Mercet) occur in southern areas of the USA (California, Florida, and New Mexico) and they attack *Ceroplastes* species recorded for the neotropics, but there has been no formal record of these parasitoids in this region so far.

Most species of *Metaphycus* (23) attacking *Ceroplastes* were recorded from the Afrotropical region, 17 of them exclusively found in the afrotropics. Most of those records are based on the studies by Annecke and Mynhardt (1971, 1972, 1981) describing the *Metaphycus* fauna mainly from South Africa. Few species seem to be restricted in distribution to Australia (2), China (2), and India (2). The remaining species are cosmopolitan.

Some *Metaphycus* species were introduced to different parts of the world as biological control agents against *Ceroplastes* or soft scales, such as *M. eruptor*, *M. flavus*, *M. helvolus*, *M. lounsburyi*, *M. stanleyi*, and *M. zebratus* (Noyes & Hayat 1994, Noyes 2004). *Metaphycus helvolus* and *M. stanleyi* were also introduced to the Caribbean along with their hosts (Trjapitzin *et al* 2004), which indicates some *Metaphycus* may be cosmopolitan due to the distribution of their hosts, such as *Coccus viridis*, *Protopulvinaria pyriformis*, *Saissetia olea*, *S. nigra*, and others.

Most *Metaphycus* species associated with *Ceroplastes* (28) have been recorded attacking one or more host species within the genus, suggesting host specificity at the genus level. Nine species show host specificity at the family level and attack

species of other coccid genera (2 to 9). The remaining species attack hosts belonging to 2 or more families of Neococcoidea (sensu Gullan & Cook 2007), a clade within Coccoidea. The extreme cases are *C. flavus* and *C. zebratus*, which are associated with hosts of 5 families, 19 or more genera, and dozens of species. However, even among the generalist *Metaphycus*, the main hosts are often Coccidae.

Lampson *et al* (1996) stated that *M. helvolus* acts as an idiobiont parasitoid. If this is in fact the life strategy of the other species of the genus, it would be expected that they could attack at least a few different species of hosts. While Neococcoidea is a monophyletic group in Coccoidea, it is likely that species of *Metaphycus* associated with *Ceroplastes* present some degree of specificity, although some seem to be more specific than others.

As wax scales, females of *Ceroplastes* secrete a thick waxy layer (test) that covers their dorsum, varying on hardness and thickness (Hodgson and Peronti 2012) and seems to act as a barrier to parasitoid oviposition. The wax starts to be secreted in the 1st instar as isolated filaments and it covers the entire dorsum of specimen, as a test, only when it reaches the 3rd or 4th (adult female) instars (Hodgson & Peronti 2012). With the exception of *M. analuciae* **sp. nov.**, *M. discolor*, and *M. grandis* **sp. nov.** (ovipositor about 1.2 mm long, Fig. 11), *Metaphycus* species have short ovipositors that do not seem to be long enough to penetrate the entire thickness of the host test. This probably indicates these species attack hosts at the 1st and 2nd instars, when their body surface is partly exposed.

Martinez-Ferrer *et al* (2015) found *M. helvolus* attacking *C. sinensis* almost exclusively in the 3rd and 4th instars; however, this parasitoid seems to have preference for nymphs of 2nd and early 3rd instar of *Saissetia olea* (Lampson *et al* 1996), a coccid which does not develop a waxy test. Lampson *et al* (1996) also stated that older 3rd instar nymphs are refused by parasitoid females, once the host epidermis is thicker and apparently too hard to be punctured by the parasitoid ovipositor. Therefore, if the epidermis of 3rd instar nymphs of *S. olea* is a barrier to oviposition, it is expected that the test is also a barrier.

From the above, the test does not prevent all parasitoid attacks, since *Metaphycus* females are able to attack hosts before the test completely covers the scale. Therefore, in spite of the development of this structure in their hosts, the associated *Metaphycus* species do not present evident morphological adaptations for oviposition.

It is expected that some parasitoids of *Ceroplastes* will also be found on the other genus in Ceroplastinae, *Waxiella* DeLotto. Almost all species of this genus were previously classified as *Ceroplastes*, and 2 of them are known hosts of *Metaphycus* species not listed in Table 1, as follows: *W. mimosa* (Signoret) host of *M. amiculus* Annecke & Mynhardt and *M. anneckei* Guerrieri & Noyes; *W. subsphaerica* (Newstead) (= *berlinae* Hall) host of *M. sylvaticus* Annecke & Mynhardt. The new records presented in this paper (seven *Metaphycus* species, five *Ceroplastes*, and 10 hostparasitoid interactions) improved the knowledge of *Metaphycus-Ceroplastes* associations. But, considering that there are about 450 (228 in neotropics) species of *Metaphycus* and 144 (77 in neotropics) species of *Ceroplastes*, we can expect many more host-parasitoid interactions to be described.

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Author Contribution ALBGP and CRSS planed, designed, and executed experimental work; ALBGP identified the species of *Ceroplastes*; MTT and MEVS identified and described the *Metaphycus* species; MEVS produced the illustrations and plates; MTT, MEVS, ADM, and ALBGP wrote the manuscript; MTT and ADM compiled the checklist of *Metaphycus* species recorded as parasitoids of *Ceroplastes* (Table 1); ADM prepared the supplementary files (morphology and material data sheets) and revised the English version of the manuscript.

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