



A new species of *Doryctobracon* Enderlein (Hymenoptera, Braconidae) parasitizing larvae of *Anastrepha* Schiner (Diptera, Tephritidae), with illustrated key to species of *Doryctobracon* that parasitize fruit-infesting tephritids in Brazil

CLÁUDIA F. MARINHO¹, MIGUEL F. DE SOUZA-FILHO^{2,5}, ADALTON RAGA^{2,6},WYRATAN DA S. SANTOS³ & ROBERTO A. ZUCCHI^{4*}¹Centro de Investigaciones y Transferencia de Catamarca (CITCA—CONICET/UNCA), Prado 366, K4700AAP. San Fernando del Valle de Catamarca, Catamarca, Argentina.✉ cfmarinho@gmail.com; <https://orcid.org/0000-0003-3786-6943>²Instituto Biológico, Centro Avançado de Pesquisa em Proteção de Plantas e Saúde Animal, 13101-680, Campinas, São Paulo, Brazil³Instituto Federal de Educação, Ciência e Tecnologia do Tocantins, Campus Araguatins, Povoado Santa Teresa, km 5 Zona Rural, Araguatins, Tocantins, Brazil✉ wyratan@ifto.edu.br; <https://orcid.org/0000-0002-5257-9152>⁴Departamento de Entomologia e Acarologia, ESALQ, Universidade de São Paulo (USP), Caixa Postal 9, 13418-900, Piracicaba, São Paulo, Brazil.✉ razucchi@usp.br; <https://orcid.org/0000-0001-9861-7460>⁵✉ miguel.souza@sp.gov.br; <https://orcid.org/0000-0001-7838-1489>⁶✉ adalton@sp.gov.br; <https://orcid.org/0000-0002-4562-2562>

*Corresponding author

Abstract

Doryctobracon maculatus Marinho, a new species of Braconidae (Opiinae) collected in the municipalities of Piracicaba and São Roque, state of São Paulo, Brazil, is described and illustrated. This new species is placed in group of species with areolate propodeum, but is easily distinguished from other species of this group, and other members of the genus by the noticeable black to dark-brown spots on the head, mesosoma and metasoma. This new species was reared in larvae of *Anastrepha pseudoparallela* (Diptera, Tephritidae) in passion fruits, *Passiflora alata* Curtis (Passifloraceae). An illustrated key to species of *Doryctobracon* recorded in Brazil is presented.

Key words: Opiinae, parasitoid, fruit fly, *Anastrepha pseudoparallela*, *Passiflora alata*, sweet passion fruit

Introduction

The 17 species of the genus *Doryctobracon* Enderlein, 1920 occur exclusively in the Americas (Wharton 1997; Ovruski 2000). Most of them (12 species) are koinobiont parasitoids of fruit-infesting tephritids (Fischer 1977; Wharton & Marsh 1978; Wharton 1997; Yu *et al.* 2012; Marinho *et al.* 2017). A single species parasitizes tephritid larvae in flowers (Wharton & Norrbom 2013), and no host is known for four species (Yu *et al.* 2012; Wharton & Yoder n.d.). The genus is divided into two readily recognizable species groups, based on the propodeal sculpturing. In group 1, the propodeum is areolate, and in group 2, the propodeal sculpture is reduced to two apical ridges (Wharton & Yoder n.d.). The new species described here belongs to group 1.

From the pioneering studies of Lima (1937) until approximately the first decade of this century, only *D. areolatus* (Szépligeti), *D. brasiliensis* (Szépligeti) and *D. fluminensis* (Lima) were known in Brazil. In the current decade, three additional species have been found in Brazil: *D. crawfordi* (Viereck) (Zucchi *et al.* 2011), *D. adamei* Marinho & Pentead-Dias and *D. whartoni* Marinho & Pentead-Dias (Marinho *et al.* 2017). Information for all these *Doryctobracon* species recorded in Brazil, emphasizing mainly taxonomic aspects, has recently been discussed (Marinho *et al.* 2018). Including the new species described herein, seven species are currently known in Brazil. An illustrated key is presented to separate all *Doryctobracon* species that parasitize fruit-infesting tephritids in Brazil.

Material and methods

Doryctobracon species, including type-material, from Museum of Entomology “Luiz de Queiroz” (MELQ/ESALQ) were examined in order to identify braconids sampled from *Anastrepha* larvae in passion fruit. Also, keys to the species of *Doryctobracon* (Fischer 1965; Wharton & Yoder n.d.; Marinho *et al.* 2018) were used in the attempt to identify these specimens.

Passion fruit (*Passiflora alata* Curtis) was sampled in two municipalities of the state of São Paulo: (1) Piracicaba, on the campus “Luiz de Queiroz”, Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ), and (2) São Roque, in the “Unidade de Pesquisa e Desenvolvimento em Agricultura Ecológica (UPD AE)”. The distance between these municipalities is approximately 130 km. The tritrophic relationship among parasitoid, fruit fly and host plant was established following the methodology described by Leonel Junior *et al.* (1996).

The terminology for body morphology and wing veins broadly follows Sharkey & Wharton (1997) and Wharton (1997). Yoder *et al.* (2010) and on the description of Opiinae by Karlsson & Ronquist (2012). Wharton (1997) was followed for the description of the ovipositor. For clarity, the first metasomal tergum, referred to as the petiole, is termed T1, and the following median ridges are termed T2 and T3 (Walker & Wharton 2011). The measurements broadly follow Ovruski (2003) and Walker & Wharton (2011). Electron microscopy images were obtained using Zeiss DSM940A and LEO 435VP scanning electron microscopes at the Research Support Center, Electron Microscopy Applied to Agricultural Research (NAP/MEPA), ESALQ/USP. Digital images were produced using a Motic® 2000 2.0MP digital camera coupled to a Nikon® E200 stereoscopic light microscope. In some cases, specimens were photographed with a Leica DFC 420 digital camera installed on a Leica M165C stereomicroscope; photographs were combined with the Leica Application Suite v3.8 to produce the final extended-focus images. Measurements were performed using the software Motic Image Plus 2.0, coupled to a light microscope (Nikon® E200). Type-material is deposited at the Luiz de Queiroz Museum of Entomology (MELQ), Department of Entomology and Acarology, ESALQ, Piracicaba, SP, Brazil.

Results

The specimens obtained from larvae of *Anastrepha pseudoparallela* (Loew) from sweet passion fruit (*Passiflora alata* Curtis) sampled in the municipalities of Piracicaba and São Roque belong to the genus *Doryctobracon*, based on the following morphological characters: small second submarginal cell, vein 3RSa smaller than 2RS, m-cu directly in line with 2RS, occipital carina absent, and propodeum areolate. The specimens collected in both municipalities are conspecific, and do not fit the identification keys to the species of *Doryctobracon*, associated or not with fruit-infesting tephritids (Fischer 1965; Wharton & Yoder n.d.). Therefore, the specimens comprise a new species of *Doryctobracon*, belonging to group 1 (areolate propodeum) of Wharton & Yoder (n.d.).

Description

Doryctobracon Enderlein

See <http://species-id.net/wiki/Doryctobracon>

Doryctobracon Enderlein, 1920: 144. Type species: *Doryctobracon conjungens* Enderlein, 1920 [junior subjective synonym of *Doryctobracon crawfordi* (Viereck, 1911)]. Original designation.

Doryctobracon maculatus Marinho sp. nov.

<http://zoobank.org/DB81D709-822E-4946-9BC0-28F8F6EA6B70>

(Figs 1 A–H, 2 A–E)

Diagnosis. *Doryctobracon maculatus* sp. nov. is readily separated from the other species of *Doryctobracon*, as it is the only species with dark-brown to black spots on the head, lobe of mesoscutum, mesopleura, mesosternum and

dorsal metasoma (Fig 1A–G). Besides the dark spots on the body, *D. maculatus* is also distinguished from the species of group 1 (areolate propodeum), namely *D. areolatus*, *D. adamei*, *D. fluminensis* and *D. whartoni*.

Type material. Holotype. Female (ESALQENT0261), Brazil, São Paulo, Piracicaba, 23-III-2006, reared from larva of *Anastrepha pseudoparallela* (Loew) in sweet passion fruit (*Passiflora alata* Curtis), coll. Wyratan S. Santos. Paratype with same data as holotype, 1 female (ESALQENT0262); 2 females (ESALQENT0263 and 581), São Roque, SP, 31.III.2000, reared from larva of *Anastrepha pseudoparallela* (Loew) in sweet passion fruit (*Passiflora alata* Curtis), colls. Miguel F. Souza-Filho and Adalton Raga.

Female. Length of body, excluding ovipositor, 5.7–6.7 mm.

Head. 1.4–1.5 × wider than long; 1.2–1.3 × wider than width of mesoscutum; face polished and shiny, distinctly setose, weakly punctate; midridge smooth, weakly developed (Fig 2A); distance between toruli equal to distance from torulus to eye. Antenna longer than body, 7.7–9.7 mm in length, with 58 to 60 flagellomeres; first flagellomere about 0.9–1.0 × length of second flagellomere, 1.4–1.8 × longer than wide. Eyes large, 1.2–1.3 × wider than high (Fig 2A); in dorsal view, eye 1.7–1.8 × wider than temple width; in lateral view 2.1–2.2 × longer than temple length; malar space 0.4–0.5 × eye height. Clypeus 2.7–4.0 × wider than high, sinuate with median lobe on ventral margin, polished, obscuring labrum, with sparse setae, two to three times longer than setae on face (Fig 2A).

Mesosoma. 1.3–1.5 × longer than high; 1.8–1.9 × longer than wide; 1.2–1.4 × higher than wide. Pronotum not visible dorsally; median lobe and lateral lobes of mesoscutum polished, shiny, with few sparse bristle-like setae; notaulus smooth, complete, deeper anteriorly and shallower posteriorly, converging in large impression without midpit (Fig 2B); scutellar sulcus divided into two large pits by median longitudinal carina; scutellum smooth with small punctures, margins setose (Fig 2B); mesopleuron shiny, smooth, without longitudinal impression; propodeum with anterior median longitudinal carina (0.11–0.14 mm) followed by complete posterior areola; anteriorly, on both sides of areola, small transverse carinae radiate without reaching lateral longitudinal carina (only one, which appears at midpoint of areola, reaches lateral longitudinal carina). Many bristle-like setae coincide with the carinae, from center, anteroposterior region and base of propodeum and metapleura (Figs 1F, 2D).

Metasoma. 1.9–2.2 × longer than wide and 1.0–1.6 × wider than high; length of T1 0.8–1.0 × greater than width at apex; T1 apex about 1.3–1.5 × width at base; tergum completely smooth, polished and shiny (Figs 1G, 2C); T1 with two parallel dorsal keels, just above spiracles, well developed at base but indistinct posteriorly (Figs 1G, 2C); spiracles just before midpoint of T1; ovipositor about 5.6–6.4 mm long; ovipositor sheath twice as large as metasoma; ovipositor with one subapical dorsal node and ventral serrations, similar to *D. areolatus* (Fig 2E).

Wings. Fore wing infumate, stigma, veins and setae dark brown, 5.9–6.6 mm long; stigma 3.5–3.7 × longer than wide, with vein r slightly projecting from its midpoint; (RS+M)a 1.2–1.4 × longer than 3RSa; 2RS 1.1–1.2 × longer than 3RSa, 1.3–1.4 × longer than 1m-cu and 2.0–2.2 × longer than r-m; 1m-cu directly in line with 2RS; 3RSa 2.0–2.5 × longer than vein r; 2M 1.7–1.8 × longer than 3RSa (Fig 1H). Hind wing 3.8–4.8 mm long, m-cu present and distinctly pigmented (Fig 1H).

General coloration. Head and mesosoma orange or reddish orange. Head with black to dark reddish-brown spot resembling dorsomedian band posterior to toruli, which widens as band surrounding the ocellar triangle, vertex, and reaching occiput (Fig 1B); mandibles orange with black apex; pronotum with dark red-brown spots on anterolateral margin, close to propleura, and posteriorly close to scutellum, which gradually fade to orange-red tones and, finally, orange in the center (Figs 1A, 1D); mesoscutum with median mesonotal lobe with semi-oval black spot; lateral mesonotal lobes with broad black bands that reach anterolateral region of scutellum (Fig 1C); mesopleuron with anterior dorsoventral third reddish orange, and posterior margin light orange, remaining part completely black, reaching and involving mesosternum (Figs 1D, 1F); propodeum, metapleura, labial and maxillary palps light yellow; front and median legs with apical tarsomere black (Fig 1A); hind legs dark-brown to black except coxa yellow with small smoky dark-brown spots, posterior third of femur orange to orange-red, external anterior region of tibia with small round orange area, posterior extremities of trochanter and tarsi red-orange (Figs 1A, 1E); metasoma T1, T2 and sternum yellow, terga T3 to T6 with dark-brown to black crossbands (Fig 1G) (only one specimen, from São Roque, showed color variation, i.e., dark mark between dorsal carinae of pronotum laterally and on T1 posteriorly); ovipositor sheath dark brown, ovipositor red-orange (Fig 1A); scape, pedicel and flagellomeres dark brown, anellus red-orange (Fig 1A); and tegulae reddish-orange (Fig 1C, 1D). Fore wing and hind wing infumate; stigma, veins and setae dark brown (Fig 1H).

Male. Unknown.

Etymology. From the Latin, past participle of *maculare*, meaning spotted, referring to the dark spots on the head and body.

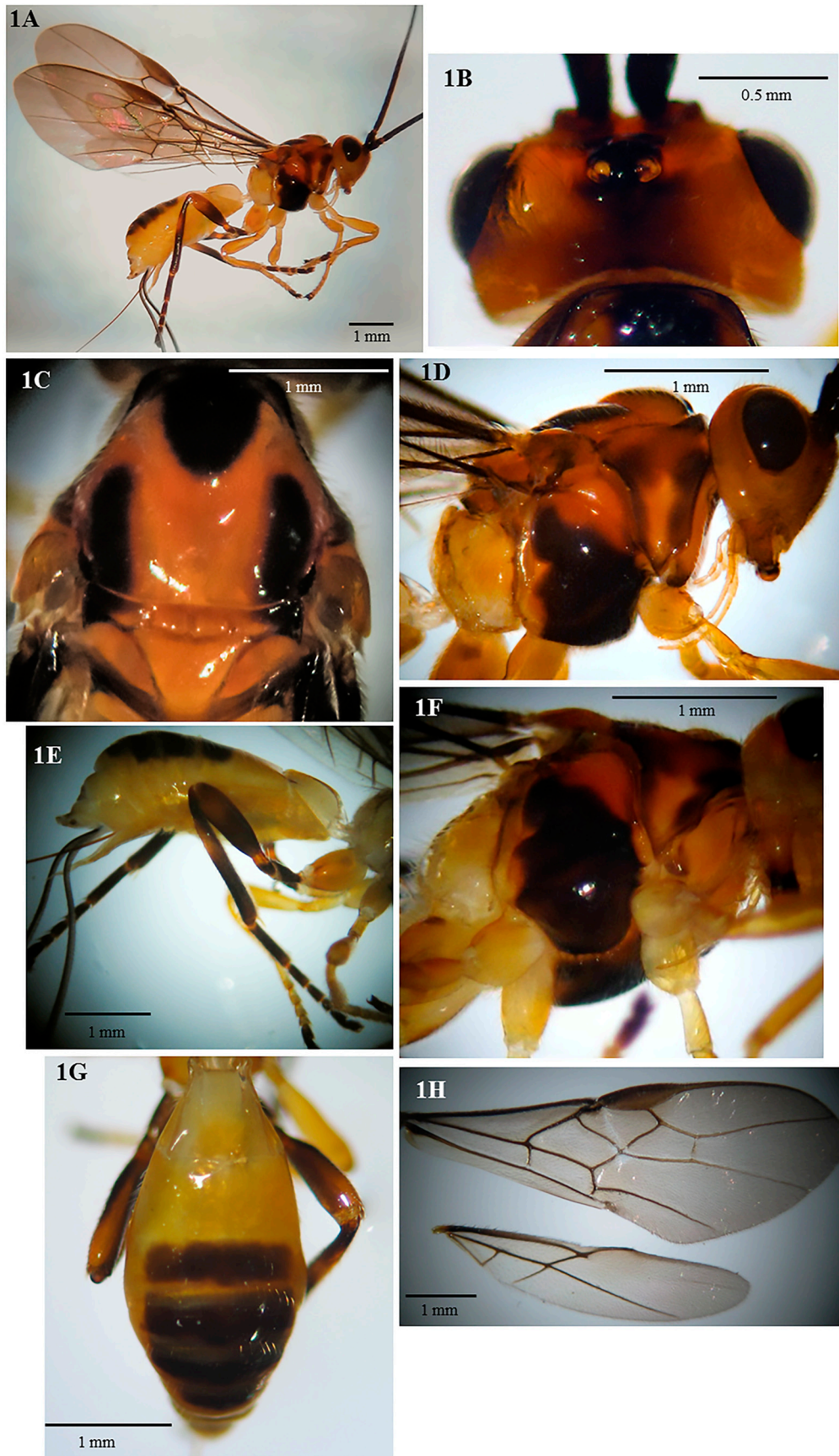


FIGURE 1. *Doryctobracon maculatus* Marinho **sp. nov.** habitus (A), head, dorsal view (B), mesonotum (C), mesosoma, lateral view (D), metasoma and hind legs, lateral view (E), mesosoma, lateroventral view (F), metasoma, dorsal view (G), Fore wing and hind wing (H). (Photos: C. F. Marinho).

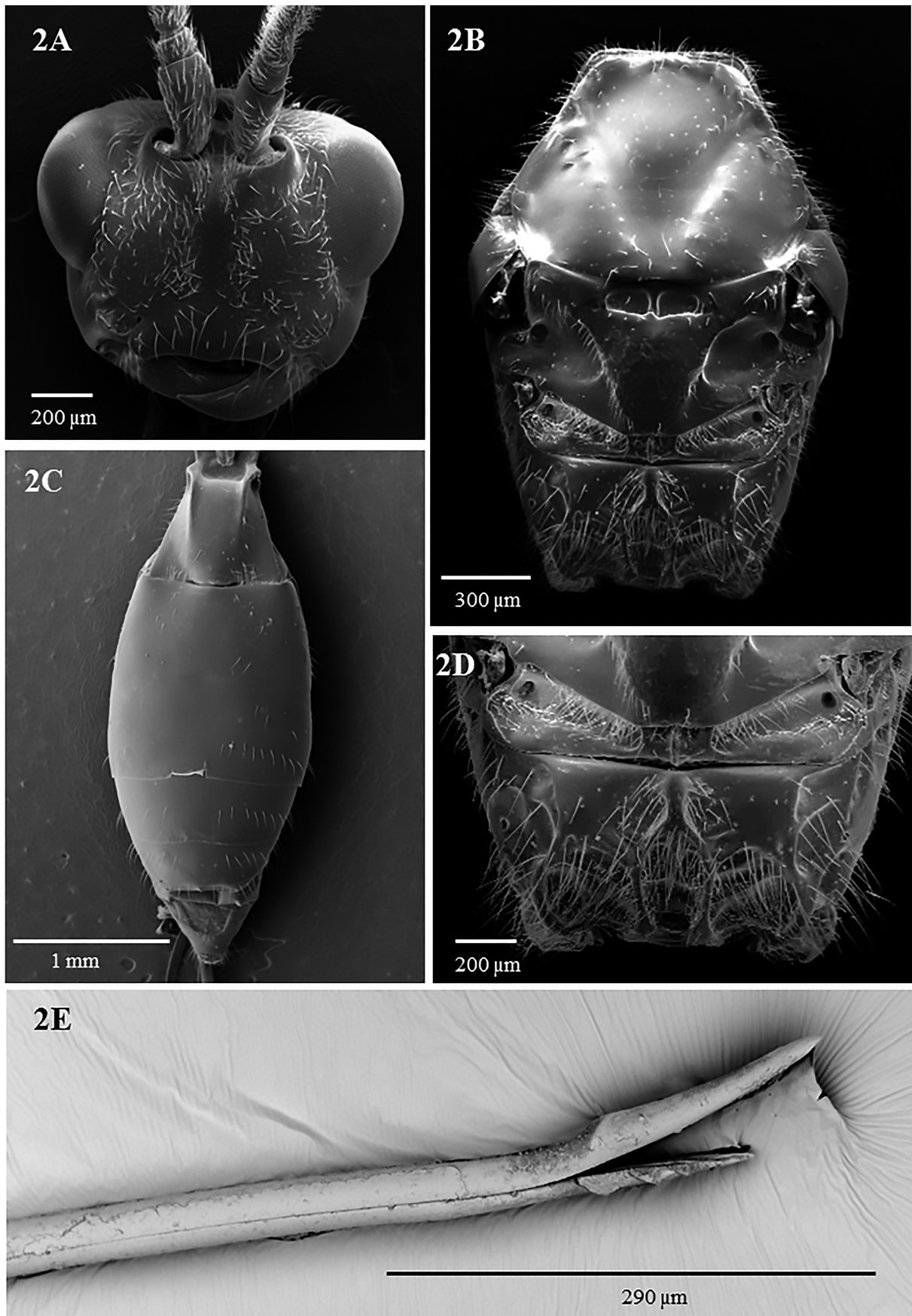
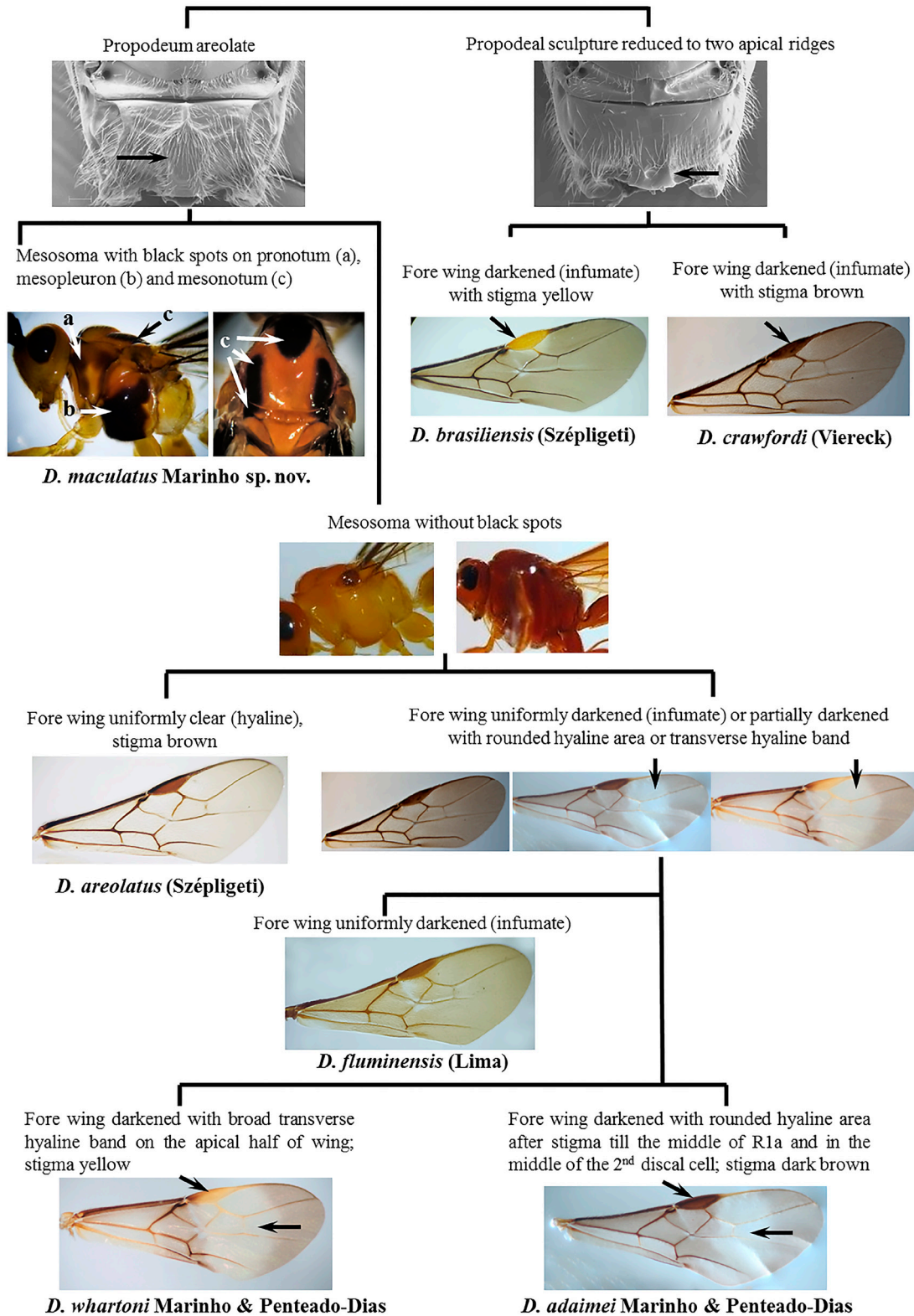


FIGURE 2. *Doryctobracon maculatus* Marinho **sp. nov.** head, front view (A), mesosoma, dorsal view (B), metasoma, dorsal view (C), propodeum, dorsal view (D), ovipositor apex, lateral view (E). (Photos: C. F. Marinho).

Illustrated key

The key is intended for applied entomologists, so it was based on easily recognized morphological characters. However, to avoid misidentification using the key, we recommend that identification be confirmed by consulting the species diagnosis (see Marinho *et al.* 2018).

Illustrated key to the species of *Doryctobracon* from Brazil



Tritrophic relationship

Species of *Passiflora* (Passifloraceae) are infested by nine species of *Anastrepha*, but there are records of braconids only for *A. pseudoparallela* associated with passion fruit (Zucchi & Moraes 2008). Although the fruit fly *A. pseudoparallela* is frequently associated with passion fruit, it is not commonly parasitized by braconids in this host plant. Probably some passion-fruit characteristics, such as the thick mesocarp and bulky endocarp, hinder parasitism of *Anastrepha* larvae by braconids. This observation is supported by data from São Roque, where *D. maculatus* was collected. In this municipality, 218 passion fruits (17.04 kg) were sampled by the coauthors M.F. Souza Filho and A. Raga, and from 520 puparia, 120 adults of *A. pseudoparallela* merged compared with only two females of *D. maculatus*.

Only two records of braconids parasitizing *A. pseudoparallela* in passion fruit were known previously. In the earlier record, Gonçalves (1938) reported *D. fluminensis* parasitizing larvae of *A. pseudoparallela* in *Passiflora edulis* Sims (sour passion fruit). Approximately 60 years later, Leonel Junior *et al.* (1996) recorded *D. areolatus* as a parasitoid of *A. pseudoparallela* in *Passiflora* fruit. Some 25 years after that, the present finding of *D. maculatus* constitutes the third record of a species of *Doryctobracon* that parasitizes larvae of *A. pseudoparallela* in Passifloraceae, and for the first time records *P. alata* Curtis (sweet passion fruit) as the associated host plant.

Acknowledgements

We thank the National Council for Scientific and Technological Development (CNPq) for the financial support to carry out research on fruit flies and their parasitoids in Brazil (RAZ) (grant 306354 / 2014-4). RAZ is a fellow of the CNPq. We also thank the Research Support Center, Electron Microscopy Applied to Agricultural Research at ESALQ/USP, for allowing us to use the SEM. We are thankful to two anonymous reviewers and the subject editor, John T. Jennings, for their valuable comments in the final version of the manuscript.

References

- Enderlein, G. (1920) Zur Kenntnis außereuropäischer Braconiden. *Archiv für Naturgeschichte*, A, 11, 51–224.
<https://doi.org/10.5962/bhl.part.13627>
- Fischer, M. (1965) Über Neotropische Opiinae (Hymenoptera: Braconidae). *Annalen de Naturhistorischen Museums in Wien*, 68, 407–441.
<http://www.jstor.org/stable/41764854>
- Fischer, M. (1977) Hymenoptera Braconidae (Opiinae II-Amerika). *Das Tierreich*, 96, 1–1001.
- Gonçalves, C.R. (1938) As moscas de frutas e seu combate. Experiências e estudos realizados em 1936. *Publicação do Ministério da Agricultura, Departamento Nacional de Produção Vegetal Serviço de Defesa Sanitária Vegetal 12*. Ministério da Agricultura, Rio de Janeiro, 43 pp.
- Karlsson, D. & Ronquist, F. (2012) Skeletal Morphology of *Opius dissitus* and *Biosteres carbonarius* (Hymenoptera: Braconidae), with a Discussion of Terminology. *PLoS ONE*, 7 (4), e32573.
<https://doi.org/10.1371/journal.pone.0032573>
- Leonel Junior, F.L., Zucchi, R.A. & Canal Daza, N.A. (1996) Parasitismo de moscas-das-frutas (Diptera: Tephritidae) por Braconidae (Hymenoptera) em duas localidades do estado de São Paulo. *Anais da Sociedade Entomológica do Brasil*, 25, 199–206.
- Lima, A.C. (1937) Vespas do gênero *Opius*, parasitas de larvas de moscas das frutas (Hymenoptera: Braconidae). *O Campo*, 8, 29–32.
- Marinho, C.F., Cõnsoli, F.L., Pentead-Dias, A.M. & Zucchi, R.A. (2017) Description of two new species closely related to *Doryctobracon areolatus* (Szépligeti, 1911) (Hymenoptera, Braconidae), based on morphometric and molecular analyses. *Zootaxa*, 4353 (3), 467–484.
<https://doi.org/10.11646/zootaxa.4353.3.4>
- Marinho, C.F., Costa, V.A. & Zucchi, R.A. (2018) Annotated checklist and illustrated key to braconid parasitoids (Hymenoptera, Braconidae) of economically important fruit flies (Diptera: Tephritidae) in Brazil. *Zootaxa*, 4527, 21–36.
<https://doi.org/10.11646/zootaxa.4527.1.2>
- Ovruski, S.M. (2003) Nuevos aportes a la taxonomía de las especies de Opiinae (Hymenoptera: Braconidae), parasitoides de *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) en la provincia de Tucumán, Argentina. *Acta Zoológica Lilloana*, 47, 15–44.

- Ovruski, S., Aluja, M., Sivinski, J. & Wharton, R. (2000) Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: Diversity, distribution, taxonomic status and their use in fruit fly biological control. *Integrated Pest Management Reviews*, 5, 81–107.
<https://doi.org/10.1023/A:1009652431251>
- Sharkey, M.J. & Wharton, R.A. (1997) Morphology and Terminology. In: Wharton, R.A., Marsh, P.M. & Sharkey, M.J. (Eds.), *Manual of the New World Genera of the Family Braconidae (Hymenoptera)*. International Society of Hymenopterists Special Publication No. 1. International Society of Hymenopterists, Washington, D.C., pp. 19–37.
- Szépligeti, G. (1911) Zwei neue Braconiden aus Brasilien. *Bollettino del Laboratorio di Zoologia Generale e Agraria della R. Scuola Superiore d'Agricoltura in Portici*, 5, 285–286.
- Viereck, H.L. (1911) Descriptions of six new genera and thirty-one new species of Ichneumon flies. *Proceedings of the United States National Museum*, 40 (1812), 173–196.
<https://doi.org/10.5479/si.00963801.1812.173>
- Walker, A.K. & Wharton, R.A. (2011) A review of New World *Eurytenes* s. str. (Hymenoptera, Braconidae, Opiinae). *Journal of Hymenoptera Research*, 20, 23–46.
<https://doi.org/10.3897/jhr.29.877>
- Wharton, R.A. (1997) Generic relationships of opiine Braconidae (Hymenoptera) parasitic on fruit-infesting Tephritidae (Diptera). *Contributions of the American Entomological Institute, Ann Arbor*, 30 (3), 1–53.
- Wharton, R.A. & Marsh, P.M. (1978) New World Opiinae (Hymenoptera: Braconidae) parasitic on Tephritidae (Diptera). *Journal of the Washington Academy of Sciences*, 68 (4), 147–167. [<http://www.jstor.org/stable/24536576>]
- Wharton, R. & Norrbom, A.L. (2013) New species and host records of New World, mostly Neotropical, opiine Braconidae (Hymenoptera) reared from flower-infesting, stem-galling, and stem-mining Tephritidae (Diptera). *ZooKeys*, 349, 11–72.
<https://doi.org/10.3897/zookeys.349.5914>
- Wharton, R.A. & Yoder, M.J. (2020) Parasitoids of fruit-infesting Tephritidae. <http://paroffit.org> (accessed 20 October 2020)
- Wiegmann, G. von A.F.A. (1918) *Archiv für Naturgeschichte. Bd. 84. Heft. 9–12*. Nicolai, Berlin, 200 pp.
- Yoder, M.J., Mikó, I., Seltmann, K., Bertone, M.A. & Deans, A.R. (2010) A gross anatomy ontology for Hymenoptera. *PLoS ONE*, 5 (12), e15991.
<https://doi.org/10.1371/journal.pone.0015991>
- Yu, D.S.K., van Achterberg, C. & Horstmann, K. (2012) Taxapad 2012. World Ichneumonoidea 2011. Taxonomy, biology, morphology and distribution. On USB Flash drive. www.taxapad.com, Ottawa, Canada
- Zucchi, R.A. & Moraes, R.C.B. (2008) Fruit flies in Brazil: *Anastrepha* species their host plants and parasitoids. Updated 10 November 2020. Available from: <http://www.lea.esalq.usp.br/anastrepha/> (accessed 3 December 2020)
- Zucchi, R.A., Marinho, C.F. & Adaime, R. (2011) First record of the fruit fly parasitoid *Doryctobracon crawfordi* (Viereck) (Hymenoptera: Braconidae) in Brazil. *Neotropical Entomology*, 40 (6), 711–712.
<https://doi.org/10.1590/S1519-566X2011000600014>