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The *Anagyrus* (Hymenoptera: Encyrtidae) parasitoids of the obscure mealybug *Pseudococcus viburni* (Hemiptera: Pseudococcidae) in Spain, with description of a new species

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Abstract

The presence of three species of the genus *Anagyrus* Howard (Hymenoptera: Encyrtidae) parasitizing the obscure mealybug *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae) in agricultural ecosystems of citrus, persimmon and ornamental plants from Eastern Spain is recorded. *Anagyrus aligarhensis* Agarwal & Alam is recorded for the first time as parasitoid on this mealybug. *Anagyrus fusciventris* (Girault) is a new record for Spain. *Anagyrus borrianensis* **sp. nov.**, reared as a specific primary parasitoid of this mealybug, is described and illustrated. The joint presence of *Anagyrus pseudococci* (Girault) and *Anagyrus vladimiri* Triapitsyn on citrus crops is confirmed. A key to species of *Anagyrus* parasitoids of *Planococcus* and *Pseudococcus* mealybugs in Eastern Spain is provided

Key words: *Anagyrus borrianensis,* mealybugs, citrus, persimmon, conservative biological control

Resumen

Se cita la presencia de tres especies del género *Anagyrus* Howard (Hymenoptera: Encyrtidae) que parasitan la cochinilla algodonosa oscura *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae) en ecosistemas agrarios de cítricos, caquis y ornamentales del este de España. *Anagyrus aligarhensis* Agarwal & Alam es el primer registro como parasitoide de éste pseudocóccido. *Anagyrus fusciventris* (Girault) es la primera cita de España. Se describe e ilustra la nueva especie *Anagyrus borrianensis* **sp. nov.**, un parasitoide primario específico de este pseudocóccido. Se confirma la presencia conjunta de las especies *Anagyrus pseudococci* (Girault) y *Anagyrus vladimiri* Triapitsyn en cultivos de cítricos. Se incluye una clave de las especies de *Anagyrus* parasitoides de los pseudocóccidos *Planococcus* y *Pseudococcus* del este de España.

Key words: *Anagyrus borrianensis,* pseudocócccidos, cítricos, caqui, control biológico conservativo

Introduction

The abundance and seasonal dynamics of the natural enemies of citrus pests in Eastern Spain has been addressed in multiple studies (Soler 2000, Soler *et al*. 2002, Laborda 2012, Calabuig 2015), along with its evolution in the last 20 years (Soler *et al*. 2015). It has been observed that, since 2013, members of the Encyrtidae family are the most

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abundant parasitoids in the citrus agricultural ecosystem in Eastern Spain, having taken the place of Aphelinidae in importance (Soler *et al*. 2015).

At present, about 4,700 encyrtid species around the world have been described and the family is very abundant in the Palearctic region, where over 1,700 species have been recorded (Noyes 2019). García-Mercet (1921) described a significant number of these species from the Iberian Peninsula. Encyrtids contain a large number of species that attack scale insects (Coccoidea) and jumping plant lice (Psyllidae); members of this family are important agents in the biological control of certain pests (Noyes *et al*. 1997). Over 400 species of Encyrtidae have been or are currently used around the world to suppress diverse crop pests, with enormous success having been achieved in biological control programs (Noyes & Hayat 1994; Noyes 2006; Japoshvili & Noyes 2006; Japoshvili & Hansen 2014).

The family had various taxonomic classifications in the past, but the one most accepted currently is that proposed by Trjapitzin (1973 a, b), which recognizes only two subfamilies: Encyrtinae and Tetracneminae. Most species that belong to the subfamily Tetracneminae are primary koinobiont endoparasitoids of mealybugs (Noyes 2006). The Tetracneminae genus *Anagyrus* Howard, 1896 follows this pattern regarding its hosts. Having cosmopolitan distribution, there are approximately 293 *Anagyrus* species worldwide, and many more species have yet to be described in all regions of the world (Noyes 2019).

The pseudococcids or mealybugs (Hemiptera: Pseudococcidae) are considered the most important citrus pests because of their presence worldwide (Talhouk 1975), and one of the most economically important in Spain due to the increasing problems associated with some of the species in this family, the introduction of other invasive species, and the complexity of controlling them agronomically (Beltrà & Soto 2012).

Five pseudococcids of economic importance are abundant in Eastern Spain (Beltrà & Soto 2012): *Planococcus citri* (Risso, 1813), *Planococcus ficus* (Signoret, 1875), *Planococcus vovae* (Nasonov, 1909), *Pseudococcus longispinus* (Targioni Tozzetti, 1867) and *Pseudococcus viburni* (Signoret, 1875), and three of them are observed in the citrus and persimmon agroecosystem: *P. citri*, *P. longispinus* and *P. viburni*. While *P. citri* is the most important species on citrus fruits in Spain and it is widely distributed across many crops all over the world (García-Marí 2012), *P. longispinus* infests mainly ornamental crops and *P. viburni* is the most abundant species on persimmon in spring and autumn (Vercher *et al*. 2017). However, any of these species can be the most abundant when they specifically colonize local plots of citrus, persimmon or ornamental crops. It should be mentioned that the mealybug *Delottococcus aberiae* (De Lotto, 1961), originally from South Africa, is an alien species that has also been recorded in Eastern Spain since 2009 causing significant damage to citrus and persimmon crops (Beltrà *et al*. 2012; 2013; 2015; Martínez-Blay *et al.* 2017).

There are 23 species of *Anagyrus* recorded in Europe, and eleven of them were listed from Spain by Fusu (2013): *Anagyrus aranzadii* (Mercet, 1921), *Anagyrus belibus* (Walker, 1837), *Anagyrus bohemanni* (Westwood, 1837), *Anagyrus longicornis* Mercet, 1923, *Anagyrus matritensis* (Mercet, 1921), *Anagyrus minium* (Mercet, 1921), *Anagyrus novickyi* Hoffer, 1953, *Anagyrus orbitalis* (Ruschka, 1923, not Timberlake), *Anagyrus pseudococci* (Girault, 1915), *Anagyrus punctulatus* Agarwal & Alam, 1959 and *Anagyrus subflaviceps* (Girault, 1915). However, *Anagyrus orbitalis* (Ruschka) (not Timberlake) is a junior synonym of *Anagyrus matritensis* (Koponen & Askew 2002), and *Anagyrus punctulatus* is a junior synonym of *Anagyrus aligarhensis* Agarwal & Alam, 1959 (Noyes & Hayat 1994, Noyes 2000). *Anagyrus diversicornis* Mercet, 1921, recorded from Spanish mainland (García-Mercet 1921; Askew *et al*. 2001) and Canary Islands (Koponen & Askew 2002) is also a junior synonym of *Anagyrus aligarhensis* (Noyes 2000). Finally, the *Anagyrus* sp. near *pseudococci* often recorded from Spain has been formally described as *Anagyrus vladimiri* Triapitsyn, 2019 (Andreason *et al.* 2019). Therefore, the Spanish fauna of *Anagyrus* is represented by 11 species. Four of these eleven species are associated to pseudococcid hosts belonging to genera *Planococcus* Ferris and *Pseudococcus* Westwood found on citrus, persimmon and ornamentals trees (Noyes 2019): *A. aligarhensis, A. matritensis, A. pseudococci* and *A. vladimiri*.

This study reviews the species of *Anagyrus* collected as primary parasitoids of the obscure mealybug *Pseudococcus viburni* in Eastern Spain, including the description of a new species.

Material and Methods

The specimens of the study were collected from 2015 to 2017 in several towns in Eastern Spain as listed in the results section (the coordinates are given in decimal degrees Grid: Lat/Lon hddd.dddd°; datum: WGS84). Although

they can be observed year-round, they are more abundant in Spring and Summer. The specimens were collected using 10x20-cm adhesive-covered chromatic traps (Econex®) and "Econex Polillero" (Econex®) water traps, both baited with *Pseudococcus viburni* pheromones (Pherocon® CAP and Storgard® CAP, from TRÉCÉ® Incorporated). The traps were checked weekly and the pheromones replaced every 45 days. The insects were extracted from the adhesive surface using the histological solvent Histo-Clear II, made by National Diagnostics. Initially, the insects were preserved in 70% ethanol. In their subsequent preparation, they were air-dried to remove the ethanol, followed by the hexamethyldisilazene (HMDS) technique described by Noyes (2019). Nesbitt's fluid (chloral hydrate, 40 g, concentrated hydrochloric acid, 2,5 mL, distilled water, 25 mL) was used for the maceration of internal tissues, especially muscles, in order to soften the cuticle for dissection and increase transparency for the preparation and microscopic observation of the genitalia of males and females and the scale-like sensilla on the antenna of males. Heinze PVA (HPVA) fluid (polyvinyl alcohol, 10 g, chloral hydrate, 100 g, glycerol, 10 g, distilled water, 60 mL, 85%-92% lactic acid, 35 mL) was used to mount the permanent slide preparations of the genitalia of males and females and antennas of males.

The hymenopterans emerged from scale insects parasitized naturally in the field were reared at the insectarium of the Plant Health Service of Generalitat Valenciana located in Almassora (province of Castelló). The conditions of the insectarium were kept constantly at 26–27°C, relative humidity of 40–55% and 24 hour photoperiod. The insects were identified using a Nikon SMZ 1500 stereomicroscope with NIS-BR Basic Research imaging software (Nikon Instruments Inc., USA) at the Instituto Agroforestal Mediterráneo of the Universitat Politècnica de València.

The anatomical characteristics follow Gibson's terminology (1997). All measurements are given in micrometers (µm), as length or as length: width. The following abbreviations are used in the text:

F1-6 funicle segments 1–6
POL minimum distance between posterior ocelli
OOL minimum distance between a posterior ocellus and eye margin
AOL minimum distance between a posterior ocellus and anterior ocellus

The following acronyms are used for the depositories:

ENV Laboratory of Entomology and Pest Control, Institute Cavanilles of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain
NHM Natural History Museum, London, United Kingdom

Identifications are based in the descriptions and taxonomic keys by several authors: Çalişkan et al. (2016) and Japoshvili (2012) from Tukey, De Santis (1963) from Argentina, Hayat (2003) from India, Noyes & Hayat (1994) from Oriental region, Timberlake (1919) from Pacific region, Viggiani and Battaglia (1983) from Italy, Zu & Li (2015) from China, Andreason et al. (2019) and Triapitsyn *et al.* (2007).

The studied specimens are deposited in the entomological collection of the University of Valencia (collection ENV); at the NHM, and in the private collection of one of the authors (J.Mª. Soler), as indicated under material examined. All the slides of the microscopic preparations of this paper are kept at the Entomology Department of the Mediterranean Agroforestry Institute of the Universitat Politècnica de València.

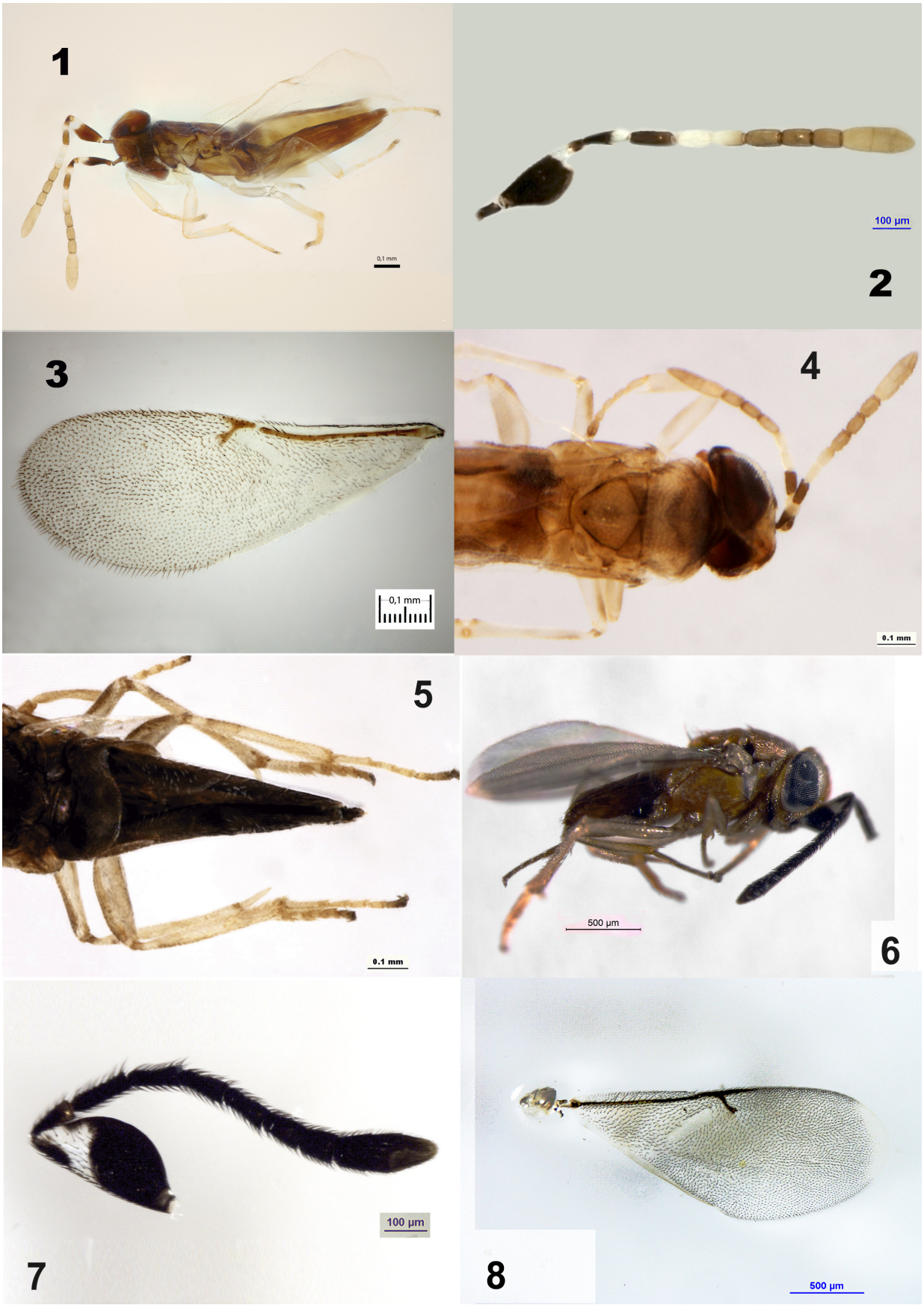
Results

Species associated to *Pseudococcus viburni* (Signoret)

Anagyrus aligarhensis Agarwal and Alam

Figs. 1–5.

Anagyrus aligarhensis (Man Mohan) Agarwal & Alam, 1959: 392, Holotype female, India, Aligarh. Synonym of *A. diversicornis* Mercet. Next available name for *A. diversicornis* Mercet.
Anagyrus diversicornis Mercet, 1921: 134-135, Lectotype female, Spain. Preoccupied by *A. diversicornis* (Howard).



FIGURES 1–5. *Anagyrus aligarhensis*, female: **1**, *habitus* in dorsolateral view; **2,** antenna; **3**, fore wing; **4**, mesosoma; **5,** metasoma. **FIGURES 6–8.** *Anagyrus fusciventris*, female: **6**, *habitus* in side face view; **7,** antenna; **8**, fore wing.

Philoponectroma opacum Mercet, 1921: 105, Lectotype male, Spain.
Anagyrus punctulatus (Man Mohan) Agarwal & Alam, 1959: 392, Holotype female, India.
For further synonyms, see Noyes (2019).
For a detailed description, see Noyes & Hayat (1994: 109 as *A. diversicornis* Mercet) and Çalişkan *et al*. (2016: 157).

Diagnosis. *Female*. Body length: 1110-1420. Body color non-metallic (Fig. 1), varying from orange to dark brown, except as follows: occiput behind eyes and frontovertex along eye margins orange yellow; malar space with a yellow streak from below eye margin; mouth margin below each torulus marked dark brown; collar of pronotum white, interrupted by a dark brown band in lateral fourth and sides anteriorly brownish yellow (in fresh specimens, collar of pronotum clearly black); mesoscutum and scutellum (Fig. 4) with rugose-reticulate sculpture; tegula orange, apically dark brown (Fig. 4); mesopleuron orange, but anteriorly, dorsally and posteriorly yellow. Antenna (Fig. 2) with scape except a small basal spot and a subapical curved band, pedicel in about distal third, F2 and basal half of F3, white; radicle, rest of scape, basal 2/3 of pedicel and F1 dark brown; distal half of F3 and F4–F6 pale brown; clava pale brownish yellow. Wings hyaline (Fig. 3). Postmarginal vein shorter than stigmal vein. Legs white to pale yellow, with femora and tibiae dorsally brown. Gaster (Fig. 5) varying from orange-brown to dark brown. Forewing 2.6x as long as wide. Female antenna length/width proportions: scape: 2.4x, pedicel: 3.0x, F1: 2.9x, F2: 2.3x, F3: 2,0x, F4: 1.8x, F5: 1.7x, F6: 1.5x, clava: 2.9x. F1-F6 decreasing in length and increasing in width. Antenna male F6 and clava basally with scale-like sensilla.

Material examined. Spain, País Valenciano, province Castelló, town Borriol, parcel Benadressa, lat. 40°2'7.09" N & long. 0°7'51.05" W, leg.: J.Mª. Soler, vii.2015 [2 female NHM], ex *Pseudococcus viburni*, on 'Clemenules' clementine mandarin (*Citrus reticulata*), det.: J.Mª Soler. Spain, País Valenciano, province Castelló, town Castelló, parcel Sanatori, lat. 40°0'36.12" N & long. 0°4'36.11" W, leg.: J.Mª. Soler, vii.2016 [2 female NHM], ex *Pseudococcus viburni*, on Navel Lane Late orange (*Citrus sinensis*), det.: J.Mª Soler. Spain, País Valenciano, province Valencia, town Godelleta, lat. 39°25'50.91" N & long. 0°42'41.82" W, leg.: B. Herrero and J.Mª. Soler, 19.viii.2016, ex *Pseudococcus viburni* collected 5.viii.2016 [1 female ENV] on persimmon (*Diospyros kaki*), det.: J.Mª Soler. Spain, País Valenciano, province Valencia, town Picassent, lat. 39°22'33.35" N & long. 0°33'33.10" W, leg.: J.Mª. Soler, vii-viii.2017 [3 female NHM], ex *Pseudococcus viburni*, on Navel Lane Late orange (*Citrus sinensis*), det.: J. Mª Soler.

Host. This is the first known record of *A. aligarhensis* parasitizing the obscure mealybug *Pseudococcus viburni*. The relationship has been found on *Citrus reticulata* Blanco, *Citrus sinensis* (L.) Osbeck and *Diospyros kaki* L.f.

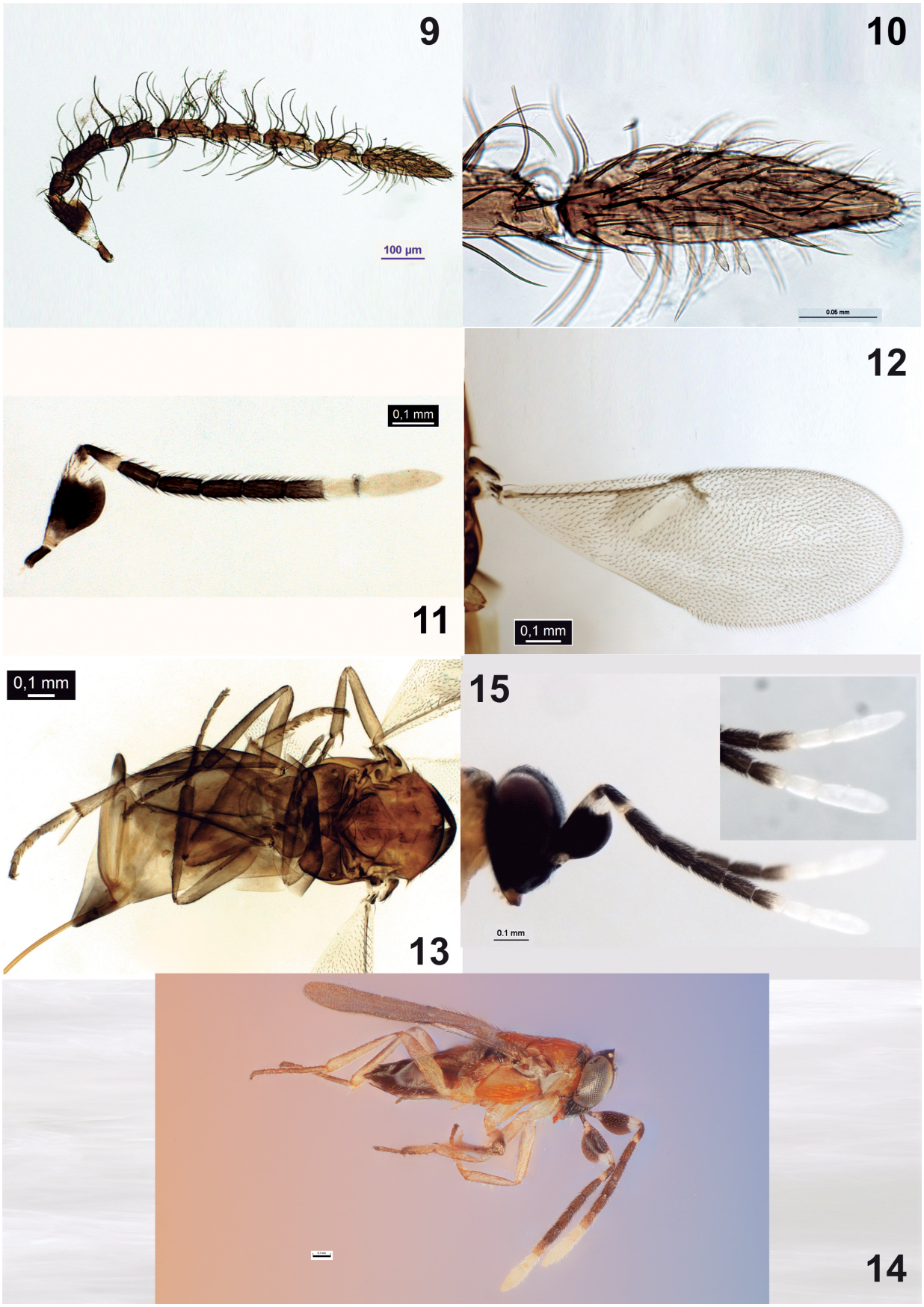
Comments. It seems to be a widely distributed species in the Old World and introduced in USA (Noyes & Hayat 1994; Çalişkan *et al.* 2016). This species has been long treated in Spain as *Anagyrus diversicornis* Mercet, with *A. aligarhensis* as synonym established by Noyes and Hayat (1994); but being a preoccupied name by *A. diversicornis* (Howard), the next available name is *A. aligarhensis* (Noyes 2000). The specimens were identified by comparison with literature descriptions.

Anagyrus fusciventris (Girault)

Figs. 6–8.

Epidinocarsis fusciventris Girault, 1915: 144. Holotype female, Australia.
Anagyrus nigricornis Timberlake, 1919: 199. Holotype female, Hawaii.
For a detailed description, see Timberlake (1919: 197, as *A. nigricornis*).

Diagnosis. *Female*. Body length: 1200-1800. Body (Fig. 6) color non-metallic, generally orange, except as follows: dark brown spot from postgena until mouth margin; frontovertex yellowish, lighter than rest of the thorax; eyes distinctly black, densely setose; scape with characteristic white spot, rest of antenna dark brown (Fig. 7); collar of pronotum white, with a row of long black setae, sometimes interrupted by white setae; metasoma brown; tegula white. Forewing hyaline but apical area with distinctly paler setae (Fig. 8); postmarginal vein a little longer than stigmal and 2.5x as long as wide; linea calva interrupted by four or five setae lines dorsally and four ventrally (Fig. 8). Legs yellowish white, except coxae, apex of tibiae and tarsi yellow. Female antenna length/width proportions: scape: 2.2x, pedicel: 3.6x, F1: 3.5x, F2: 2.8x, F3: 2.3x, F4: 2.0x, F5: 1.4x, F6: 1.2x, clava: 2.8x. F1–F6 decreasing in length and increasing in width. Ovipositor not exserted. Male antenna (Fig. 9) with a line of 5–6 (usually 5) scale-like sensilla present only on base of the clava (Fig. 10).



FIGURES 9–10. *Anagyrus fusciventris*, male: **9**, antenna; **10,** scale-like sensilla on clava (only). **FIGURES 11–13.** *Anagyrus jucundus*, holotype female: **11**, antenna; **12,** fore wing; **13**, body; **FIGURES 14-15**. *Anagyrus borrianensis* **sp. nov.**, holotype female: **14**, *habitus* in dorsal view; **15**, head and antenna.

Material examined. Spain, Comunidad Valenciana, province Valencia, town Guadassuar, lat. 39°10'44.92" N & long. 0°28'22.39" W, leg.: B. Herrero and J.Mª. Soler, 9.viii.2016, [1 female ENV] collected 5.viii.2016; 19.viii.2016 [2 female ENV] collected 5.viii.2016; 26.viii.2016 [1 female ENV], collected 1.viii.2016, 29.ix.2016 [1 female, 1 male ENV] collected 16.ix.2016; 11.xi.2016 [2 male, 1 female ENV], collected 30.ix.2016, ex *Pseudococcus viburni* collected 16.ix.2016 and x.2016, on persimmon (*Diospyros kaki*) [13 male ENV], det.: J.Mª Soler. Spain, País Valenciano, province Valencia, town Godelleta lat. 39°25'50.91" N & long. 0°42'41.82" W, leg.: B. Herrero and J.Mª. Soler, 26.viii.2016, [1 female ENV] collected 1.viii.2016; ex *Pseudococcus viburni*, on persimmon (*Diospyros kaki*), det.: J.Mª Soler. Spain, País Valenciano, province Castelló, town Burriana, lat. 39°52'32.10" N & long. 0°3'27.87" W, leg.: J.Mª. Soler, 3.x.2017, [6 female, 4 male ENV] ex *Pseudococcus viburni* and *Pseudococcus longispinus*, on Cycas (*Cycas revoluta*), det.: J. Mª Soler.

Host. *Anagyrus fusciventris* is a parasitoid of pseudococcids and, among them, it is associated to the obscure mealybug *Pseudococcus viburni* although this relationship seems to be established in laboratory rearing experiments (Blumberg & Driesche 2001; Noyes 2018). We have been able to observe *A. fusciventris* emerging from young *P. viburni* females collected on persimmon crops (*Diospyros kaki* L.f.) and kept in controlled insectariums, and also as a parasitoid of *P. longispinus* on persimmon and Japanese sago palm (*Cycas revoluta* Thunb.). We considered that a confirmation of the association of this parasitoid with *P. viburni* under natural field conditions in Spain.

Comments. *Anagyrus fusciventris* is a species originally found in Australia but widely distributed around the world; in Europe is only cited from Italy, where it is attacking *Pseudococcus calceolariae* (Maskell) on citrus crops (Viggiani & Battaglia 1983). This is a new record from Spain. The specimens were identified by comparison with literature descriptions.

Non-European species

Anagyrus jucundus De Santis
Figs. 11–13

Anagyrus jucundus De Santis, 1963: 59. Holotype female. Argentina. Examined.

Diagnosis: Body length: 1490. Body color non-metallic, yellowish orange (Fig. 13), except head, scape except base and apical area, base of pedicel and first five funicle segments black; pronotum, distal half of tegulae, lateral and posterior margin of axillae, lateral margin of scutellum, metanotum, propodeum and almost all dorsum of metasoma blackish. F6 and clava white. Legs yellow, except hind tibiae and tarsi blackish. Wings sub-hyaline, venation brown and infuscated under apical third of submarginal, marginal and stigmal veins (Fig. 12). Forewing 2.69x as long as wide. Female antenna length/width proportions: scape 2.27x, pedicel: 2.4x, F1: 2.6x, F2: 2.4x, F3: 2.3x, F4: 2.3x, F5: 2.2x, F6: 1.8x, clava: 3.8x. F1–F6 decreasing in length and increasing in width (Fig. 11).

Material examined. Female (slide mounted), Holotype: Argentina, Tucumán, San Javier, 13.iii.1960, Exp. Museo La Plata, leg.; MLP Type number 1933/1.

Host. Unknown.

Comments. *A. jucundus* was described from a single female collected by sweeping on vegetation (De Santis 1963). It is a Neotropical species, known only from Argentina, and shows similarities with the next described new species. This type specimen was compared with the literature description and key in De Santis (1963). Although this species appears in the literature indicated as described in 1964 (Loiácono *et al.* 2001, Noyes 2018), the date on the cover of the publication is clearly the year 1963 (De Santis 1963, CICBA 2015).

New species

Anagyrus borrianensis Soler, Falcó & Aquino, sp. nov.
Figs. 14–23

Type material. HOLOTYPE [female ENV]. Spain, Comunidad Valenciana, province Castelló, town Borriol, parcel

Benadressa, lat. 40°2'7.09" N & long. 0°7'51.05" W, leg.: J.Mª. Soler, viii.2017 ex *Pseudococcus viburni*, on 'Clemenules' clementine mandarin (*Citrus reticulata*). PARATYPES: Spain, País Valenciano, province Castelló, town Burriana, parcel Vinarragell, lat. 39°55'21.72" N & long. 0° 2' 44.20" W, leg.: J.Mª. Soler, vii.2015 [12 females ENV] and viii.2015 [7 females ENV], ex *Pseudococcus viburni*, on 'Nadorcott' clementine mandarin (tangor, or mandarin–sweet orange hybrid); Spain, País Valenciano, province Castelló, town Borriol, parcel Benadressa, lat. 40°2'7.09" N & long. 0°7'51.05" W, leg.: J.Mª. Soler, viii.2015 [5 female NHM] and [6 female NHM] xii.2015-vi.2016, ex *Pseudococcus viburni*, on 'Clemenules' clementine mandarin (*Citrus reticulata*). Spain, País Valenciano, province Castelló, town Borriol, parcel Benadressa, lat. 40°2'7.09" N & long. 0°7'51.05" W, leg.: J.Mª. Soler, vii-viii.2017 [10 females, 2 males, ENV], ex *Pseudococcus viburni*, on 'Clemenules' clementine mandarin (*Citrus reticulata*). Spain, País Valenciano, province Valencia, town Guadassuar, lat. 39°10' 44.92" N & long. 0°28'22.39" W, leg.: B. Herrero and J.Mª. Soler, 29.ix.2016, ex *Pseudococcus viburni* collected 16.ix.2016, [8 females, 3 males, ENV], on persimmon (*Diospyros kaki*). Spain, País Valenciano, province Valencia, town Godelleta, lat. 39°25'50.91" N & long. 0°42'41.82" W, leg.: B. Herrero and J.Mª. Soler, 29.ix.2016, [12 females, 2 males, ENV], ex *Pseudococcus viburni* collected 16.ix.2016, on persimmon (*Diospyros kaki*).

Diagnosis and Description.

FEMALE (Holotype) (Figs. 14–18).

Color. Non-metallic (Fig. 14) Head (Fig. 15) completely black, the eye darkly reddish, the mesosoma yellow-orange overall, including the mesoscutum, scutellum, axillae and mesopleuron, except for the pronotum, which is completely black (Fig. 17), and part of the propodeum, which is brown; the gaster is brownish (Fig. 17). Antenna (Fig. 15): radicle black, scape broad foliaceous with base and apical area white, rest black; pedicel completely black except apex orange-white; F1 to F4 black, F5 basal 2/3 (90 μm) black and apically white (50 μm), F6 and clava completely white. Legs, including coxae, entirely white-yellow except for dark tarsus.

Sculpture. Head, especially frontovertex, mesoscutum, scutellum axilla, mesopleuron, propodeum, and gaster with fine but conspicuous rugose reticulate sculpture.

Pubescence. Eyes pilose, frontovertex and mesosoma with short and inconspicuous setae, except for a few long black setae in the scutellum, gradually longer towards the apex.

Head as wide as high, with middle part of frontovertex wider than the eyes. Large, oval and pilose eyes. Ocelli arranged in an isosceles triangle, POL approximately 2.4x OOL. Toruli around the lower margin of the eye and outline of the mouth. Antenna 0.97x as long as the body, scape flat, foliaceous, 2.0x as long as wide; pedicel thin and short and 2.2x long as wide, shorter than flagellomere F1; funicle 6 segmented, all longer than wide; decreasing in length progressively to clava; clava 3-segmented, 4.0x long as wide and approximately as long as F5+F6 (Fig. 15). Mandible bidentate.

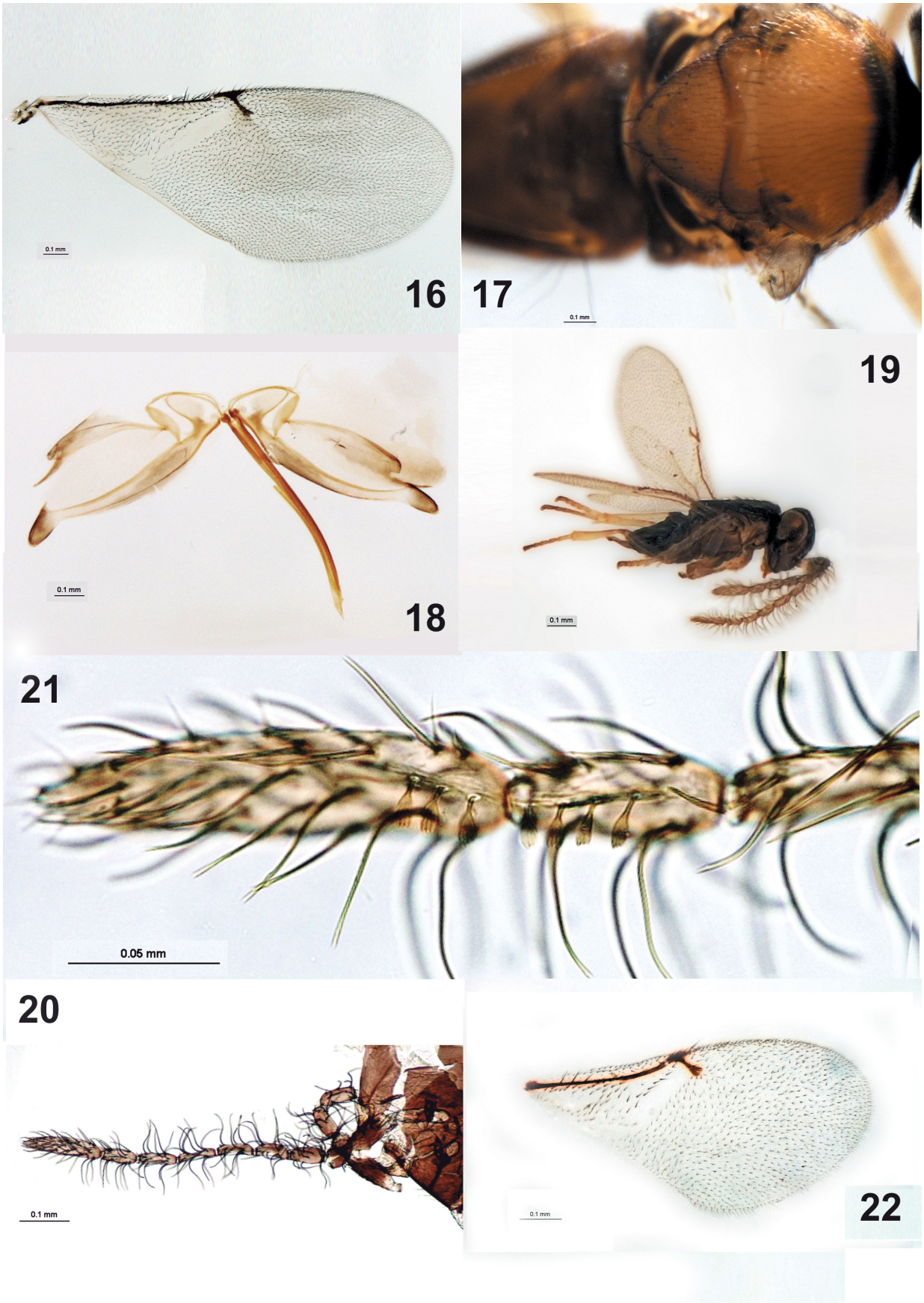
Mesosoma (Fig. 17). Mesoscutum 1.65x wider than long; scutellum in flat right-angled triangle, acute margins, including scutellar apex.

Wings (Fig. 16). Completely hyaline, large and developed, extending beyond gastral apex. Fore wing 2.40x as long as wide, hind wing 4.0x as long as wide. Marginal vein short, longer than wide and slightly longer than postmarginal vein. Stigmal vein long, a little shorter than marginal and postmarginal veins combined. Speculum interrupted by 3–4 rows of setae.

Legs. Mesotibial spur significantly shorter than mesobasitarsus.

Metasoma. Gaster 1.2x longer than mesosoma, apex acute. Very long ovipositor (Fig. 18), 1.08x mid tibia length, occupying slightly more 80% of the length of the gaster, slightly exserted. The exserted part of ovipositor 0.55x as long as mid tibial spur in microscopic preparation, but about 0.2x in dry mounted specimens. Hypopygium reaches the gaster apex.

Measurements (μm), female. Body length 1840. Head length:width:height 192:610:600; eye 380:240, POL 120, AOL 80, OOL 50, anterior ocellus to eye margin 80. Head width to minimum width of the frontovertex 610:230, i.e. 2.65x. *Antenna*: toruli separated 110; radicle 90; scape 370:180; pedicel 130:60; flagellum 1210; funicle 930; F1 180, F2 170, F3 160, F4 150, F5 140, F6 130; clava 280:70, a little longer than combined length of F5-F6. *Mesosoma* 748, pronotum height 75, mesoscutum 290:480, scutellum base 350, height 330, longest scutellum seta 120. Fore wing 1660:690; 2.4x as long as wide; submarginal vein 680, 5.2x longer than marginal+postmarginal; marginal



FIGURES 16–18. *Anagyrus borrianensis* **sp. nov**., female: **14**, habitus in dorsal view; holotype female: **15**, head and antenna; **16,** fore wing; **17**, mesosoma; **18** genitalia. **FIGURES 19–22.** *Anagyrus borrianensis* **sp. nov**., paratype male: **19**, habitus in dorsolateral view; **20**, antenna; **21,** scale-like sensilla on F6 and clava; **22**, fore wing.

vein 80; postmarginal vein 50; marginal+postmarginal combined 1.1x as long as marginal, stigmal vein 120; longest marginal seta 30; longest seta of the submnarginal vein 70; wing disc seta 40. Hind wing 1130:280; 4.0x as long as wide, longest marginal seta 40. Legs with mesotibia 730, mesofemur 690, mesotibial spur 200, and mesobasitarsus 510. *Metasoma* 900:480. Ovipositor 790.

MALE (Paratype) (Figs. 19–23).

Color. Non-metallic (Fig. 19). Less color variability than in females. Head dark, eye darkly reddish, same dark brown color in pronotum, mesoscutum, scutellum, axillae and gaster; mesopleurum orange-tinged. Antenna radicle and scape whitish gray-transparent except the base whitish; pedicel dark, flagellum gray transparent (Fig. 20). Legs yellowish-white except dark tarsus.

Sculpture. Fine but conspicuous rugose reticulate sculpture.

Pubescence. Eyes pilose, frontovertex and mesosoma with short and inconspicuous setae, except for a few long black setae in the scutellum, gradually longer towards the apex.

Head. Large, oval and pilose eyes. Mandible bidentate. Toruli around the lower margin of the eye and outline of the mouth. Antenna long (Fig. 20), not very flat scape 2.6x as long as wide; pedicel shorter, as long as wide and shorter than F1; all 6 funicular segments longer than wide, equal in length except F1 slightly shorter; clava entire, 4.6x as long as wide and approximately as long as F5–F6; all with numerous long setae. Scale-like sensilla in the form of a spatula with three lobes at the apex disposed along a line of 4–5 (usually 4) on F6 and along a line of 2–3 (usually 3) on the base of clava (Fig. 21).

Mesosoma. Mesoscutum 1.75x wider than long; scutellum in flat nearly right-angled triangle, acute margins, including scutellar apex.

Wings (Fig. 22). Hyaline, extending beyond the gastral apex. Fore wing 2.0x as long as wide; hind wing 3.50x. Marginal vein short, longer than wide and slightly shorter than postmarginal vein; stigmal vein long, longer than marginal and postmarginal veins combined. Speculum interrupted by 3–4 rows of setae.

Legs. Mesotibial spur slightly shorter than mesobasitarsus.

Metasoma. Gaster short, shorter than mesosoma, in right-angled triangle. The genitalia (Fig. 23) occupies slightly more than two thirds length of the gaster, slightly exerted, exceeding its total length by slightly over 20%.

Measurements (µm), male. Observed dry, air-dried and through hexamethyldisilazene (HMDS) technique. *Body* 750; head length 290, height 250; eye 170:120; *Antenna*: toruli separated 80; radicle 30; scape 130:50; pedicel 40:40; flagellum 580; funicle 440; F1 60, F2 80, F3 70, F4 80, F5 80, F6 70; setae flagellar segment length 50; clava 140:30. *Mesosoma* 370, pronotum height 40, mesoscutum height 170 and width 300, scutellum base 160, height 180. Fore wing 810:400; submarginal vein 360; marginal vein 20:10; postmarginal vein 30; stigmal vein 60; longest marginal seta 20. Hind wing 530:150. Legs with mesotibial length 350, mesofemur 310, tibial spur 100, tarsus 250, mesobasitarsus 120. *Metasoma* 360:350; genitalia (aedeagus) length 230, exceeds gaster by 50.

Variation. Female paratypes: body size very variable, length between 1070 and 1910 µm (n=10) (using hexamethyldisilazene, HMDS- dry-mounted specimens).

Host. Species reared from the obscure mealybug *Pseudococcus viburni.*

Etymology. Specimens of *Anagyrus borrianensis* were collected for the first time at a citrus area named "Vinarragell" in the municipal territory of Borriana (province of Castellón; Valencian Country; Spain). Vinarragell was an important Iberian settlement around the 8th century BC. In addition, populations of *A. borrianensis* are much more abundant at this site from Borriana than in other localities where the new species has been collected. Lastly, a significant part of the economy of the municipality of Borriana revolves around the citrus crop.

Discussion

Since spring 2015 we have observed a significant abundance of populations of this new species of *Anagyrus* on citrus, persimmon and ornamental crops and we have confirmed their relationship as primary parasitoid of *Pseudococcus viburni*. We consider it to be a key biological control agent for *P. viburni,* for which there have been few biological control agents available and that have shown limited efficacy for the control of *P. viburni*.

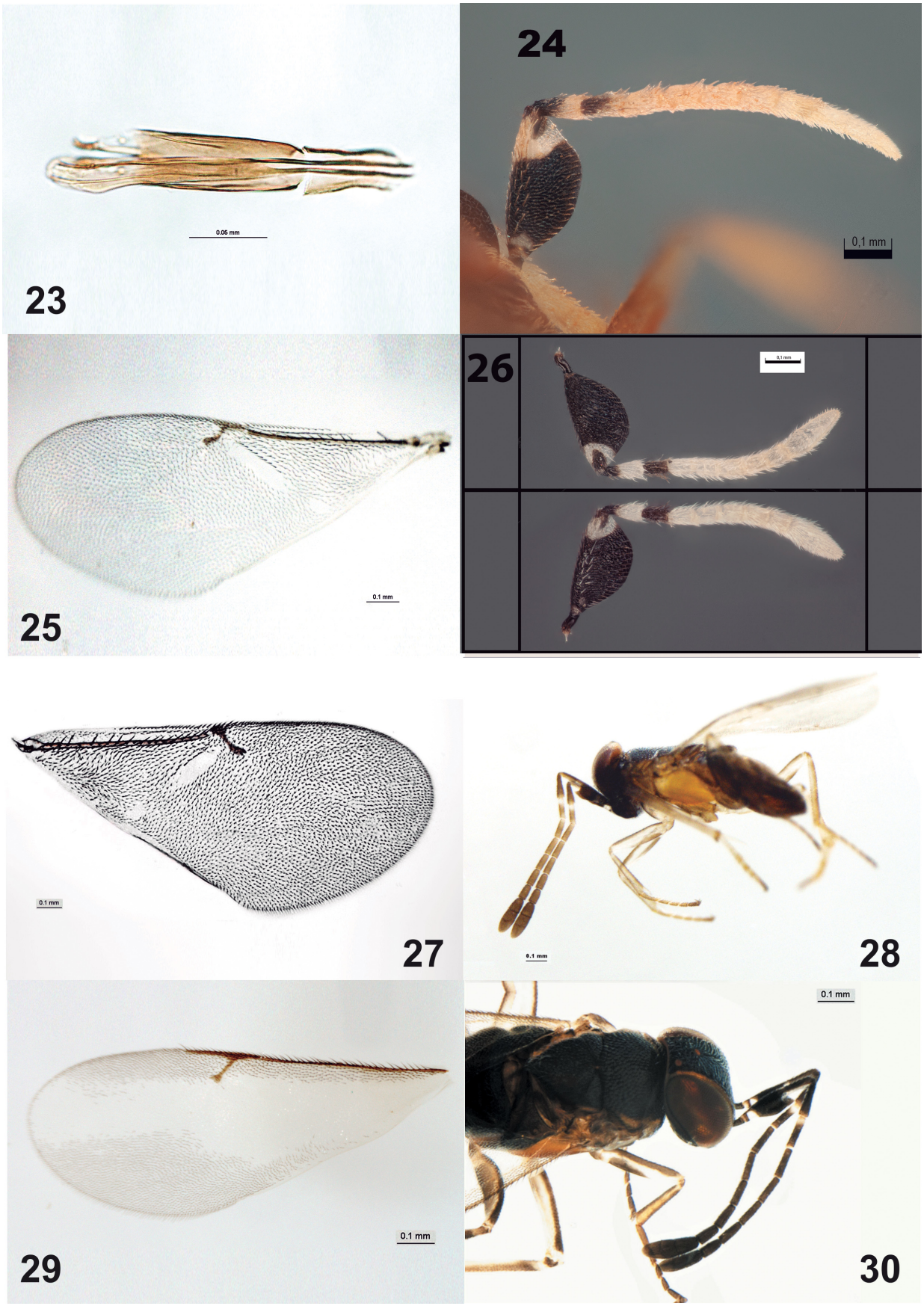


FIGURE 23. *Anagyrus borrianensis* **sp. nov**., paratype male: genitalia. **FIGURES 24–25.** *Anagyrus pseudococci*, female: **24**, antenna; **25**, fore wing. **FIGURES 26–27.** *Anagyrus vladimiri*, female: **26**, head and antenna; **27**, fore wing. **FIGURES 28–30.** *Anagyrus matritensis*, female: **28**, *habitus* in dorsolateral view; **29**, fore wing; **30**, head.

Anagyrus borrianensis shows clear anatomical differences from other European species of the genus, particularly in relation to the antenna structure and darkened areas on the surface of the wing disc.

However, this species shows similarities to some neotropical *Anagyrus* and it is particularly similar to *Anagyrus jucundus* De Santis, 1963. *A. juncundus* is known exclusively based on a female specimen collected with an entomological net on undefined vegetation in the town of San Javier, Tucumán province, Argentina (De Santis 1963); the female holotype (leg. M.L.P. Type no. ZA-158, Museo de La Plata; La Plata, Buenos Aires) has been checked. These species can be separated based on the following anatomical characteristics:

1.a.- Ocelli arranged in an equilateral triangle. Antenna with short and thin flagellum; flagellomere F5 completely black (Fig. 11). Sub-hyaline fore wing, infuscated zone below the apical third of the submarginal vein and the marginal and stigmal veins (Fig. 12). Linea calva interrupted by five rows of setae (Fig. 12). Hind tibia and tarsus blackish . . . . . ***A. jucundus***

1.b.- Ocelli arranged in an isosceles triangle. Antenna with funicle and club much longer and thicker, flagellum 1.56x longer, flagellomere F5 65% black basally and 35% white apically (Fig. 15). Fore wing completely hyaline (Fig. 16). Linea calva interrupted by three-four rows of setae, but few setae in each row (Fig. 16). Hind tibia and tarsus yellowish, slightly darkened . . . . . ***A. borrianensis* sp. nov.**

Previous studies of *Anagyrus* populations with inconspicuous anatomical differences, such as variations in antennal flagellomere coloration, have revealed sufficient reproductive incompatibility and genetic differences to consider these populations as separate species (Triapitsyn *et al.* 2007). Therefore, the anatomical characteristics as well as the biological relationships with the pseudococcid host and its nutrient plant are justified reasons to establish the validity of *A. borrianenis* as a new species and clearly different from the comparatively similar *A. jucundus*.

In addition to the similarities of *A. borrianensis* and *A. jucundus*, it becomes necessary to comment on the relationships of the new species *A. borrianensis* with the species of the genus *Anagyrus* distributed in Eastern Spain and potentially recorded as parasitoids of the pseudococcids belonging to the genera *Planococcus* and *Pseudococcus* on fruit trees, ornamental plants and citrus crops. These species are compiled in the key below.

All these *Anagyrus* species are recognized by the following generic characteristics in accordance to Noyes & Hayat (1994): *Female*, scape of female broadened and flattened, foliaceous, in male slightly broadened; pedicel longer than wide, funicle 6-segmented, funicle segments longer than broad; clava 3-segmented; eyes weakly to strongly hairy, mandibles bidentate; head with frontovertex and dorsum of thorax normally matt with finely punctuated sculpture with a silky appearance; legs slender, mesoscutum without notauli; wings hyaline, marginal long vein, postmarginal vein shorter than stigma, although it is sometimes longer, linea calva interrupted and normally closed posteriorly; gaster long, acute apex, longer than the thorax. Ovipositor hidden to strongly exserted. *Male*: scape slightly broadened and flattened, scale-like sensilla normally on both sixth funicle segment and clava, wings hyaline.

Key to *Anagyrus* parasitoids of *Planococcus* and *Pseudococcus* mealybugs in Eastern Spain (females)

1 Clava dark, testaceous, brown or black (Figs. 2, 7, 28) 2

\- Clava white (Figs. 15, 24, 26) 4

2(1) F1 brown, very dark, nearly black, F2 and basal half of F3, white, F4 to F6 pale brown; clava pale brownish yellow (Fig. 2). Forewing with the postmarginal vein clearly shorter than stigmal vein (Fig. 3) . . . . . ***Anagyrus aligarhensis***

\- F1 variable, F2-F6 and clava uniformly dark (Figs. 7, 28). Postmarginal vein of fore wing similar or longer than stigmal vein (Figs. 8, 29) 3

3(2) Antenna with all segments of flagellum completely black (Fig. 7). Head generally orange (fig. 6). Fore wing with a zone of white and pale setae only located at the apical extreme (Fig. 8) . . . . . ***Anagyrus fusciventris***

\- F1 black and basally white (Fig. 28). Head testaceous-brown, inner orbits of eyes yellowish (Fig. 30). Forewing with a pattern of white and pale setae forming a broad longitudinal medial hyaline band from the basal extreme to the apical one (Fig. 29). . . . . ***Anagyrus matritensis***

4(1) Head black (Figs. 14, 15). F1 to F4 completely black, F5 partially black (65% black basally and 35% white apically), F6 and clava completely white (Fig. 15) . . . . . ***Anagyrus borrianensis* sp. nov**.

\- Head orange to yellowish (Figs. 24, 26). F2-F6 white (Figs. 24, 26) 5

5(4) First funicle segment contrastingly partially black (basal half or so) and white (distal half or so), rest of flagellum completely white (Fig. 24) . . . . . ***Anagyrus pseudococci***

\- First funicle segment completely black and rest of flagellum white (Fig. 28) . . . . . ***Anagyrus vladimiri***

Anagyrus aligarhensis Agarwal and Alam has as main hosts the mealybugs of the genera *Phenacoccus* and *Pseudococcus* (Noyes 2019) although it had not yet been recorded until now as parasitoid of the obscure mealybug *Pseudococcus viburni*.

Anagyrus fusciventris (Girault) is a parasitoid mainly of *Pseudococcus* spp. (Noyes 2019), including the obscure mealybug *Pseudococcus viburni,* but these relationships were established in laboratory rearing conditions (Blumberg & Driesche 2001). We have been able to verify the presence of *A. fusciventris* for the first time in Eastern Spain, and its emergence from young *P. viburni* females and also as a *P. longispinus* parasitoid.

Anagyrus matritensis (Mercet) is a parasitoid of coccids and pseudococcids but has not been specifically recorded attacking species of *Planococcus* and *Pseudococcus* (Noyes 2019). Although its associated plant species are others than fruit trees (Noyes 2019), we have continuously collected for a few years on citrus crops, so it is interesting to differentiate it from *A. borrianensis*.

Anagyrus pseudococci (Girault) is mainly recorded from *Phenacoccus* spp., *Planococcus* spp. and *Pseudococcus* sp. (Noyes 2019). Daane *et al.* (2008) recorded this parasitoid attacking *Pseudococcus viburni* on vineyards from California. No other species of the *Anagyrus* genus has been associated with *P. viburni* in field conditions.

Anagyrus vladimiri Triapitsyn, described as new species in 2019, is the anteriorly named *Anagyrus* sp. near *pseudococci* in the sense of Triapitsyn *et al.* (2007), but definitively has been recognized as a separate species from *A. pseudococci* (s. str.) (Andreason *et al*. 2019). *A. vladimiri* is certainly recorded from Israel, Italy (Sicily), Russia, Turkmenistan, Tunisia and USA (Triapitsyn *et al.* 2007, Mansour 2017). It is also present in Spain (provinces of Alicante, Cádiz and Murcia) (Andreason *et al*. 2019). This species is a well-known parasitoid of *Planococcus* spp. and *Pseudococcus* spp. on citrus, figs, grapevines and persimmons, mainly the vine mealybug *Planococcus ficus* and the citrus mealybug *Planococcus citri* (Triapitsyn *et al*. 2007; Andreason *et al.* 2019). It has been associated with *Pseudococcus viburni* but the relationship has been only established in laboratory studies about defense responses of some mealybugs against the parasitoid (Bugila *et al.* 2014).

We have had the opportunity to collect adults of both *A. pseudococci* and *A. vladimiri* on citrus crops in Eastern Spain, in several sites from the provinces of València and Castelló, such that we confirmed the joint presence of both species in Spain as parasitoids of pseudococcids, and, in accordance with bibliography, with the potential ability to parasitize the obscure mealybug *P. viburni*.

Conclusions

Pseudococcus viburni (Signoret) (Hemiptera: Pseudoccidae) is considered a pest of high economic importance because of its presence on various crops, especially citrus, persimmon, apple, grapevine and ornamental plants, and because of its continuous expansion across large agricultural areas of Southern and Eastern Spain (Andalusia, Valencia, Aragon and Catalonia).

We have confirmed that three species of the genus *Anagyrus* (Hymenoptera: Encyrtidae) are attacking the obscure mealybug in eastern Spain: *Anagyrus aligarhensis* Agarwal & Alam, the first record as parasitoid on this mealybug, *Anagyrus fusciventris* (Girault), new record from Spain, and the new species *Anagyrus borrianensis* Soler, Falcó & Aquino. The latter is a particularly abundant and active parasitoid in citrus and persimmon plots, and therefore we consider it a remarkable biological control agent of *P. viburni*, and its role in future conservative biological control strategies against the obscure mealybug will be very useful, so we consider that *A. borrianensis* will be the species that will exercise the most effective biological control over *P. viburni*.

Having confirmed the identification, presence and abundance of these three parasitoid species of genus *Anagyrus* in the eastern part of the Peninsula on *P. viburni*, and other three parasitoids (*A. matritensis*, *A. pseudococci* and *A. vladimiri*) on pseudococcids, it is of the utmost importance to properly manage biological control of the Pseudococcidae family and to define and implement the most appropriate strategies to optimize conservation biological control.

New contributions on the biology of this new species will be highly significant for improving biological control strategies for *Pseudococcus viburni* and optimizing the natural control through parasitoid conservation.

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