First report of bivoltinism in *Isocolus* (Hymenoptera, Cynipidae): *Isolocus melikai* Pujade-Villar n. sp. from the Iberian Peninsula

Juli PUJADE-VILLAR
Universitat de Barcelona, Facultat de Biologia,
Departament de Biologia Animal, Avda. Diagonal 645,
08028 Barcelona (Spain)
jpujade@ub.edu

José TORMOS
Universidad de Salamanca, Facultad de Biología, Unidad de Zoología,
37071 Salamanca (Spain)

Manuel GONZÁLEZ-NÚÑEZ
Susana PASCUAL
Ana COBO
Laboratorio de Entomología Agroforestal,
Departamento Protección Vegetal,
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA),
Carretera de La Coruña Km 7.5, 28040 Madrid (Spain)

Pujade-Villar J., Tormos J., González-Núñez M., Pascual S. & Cobo A. 2014. — First report of bivoltinism in *Isocolus* (Hymenoptera, Cynipidae): *Isolocus melikai* Pujade-Villar n. sp. from the Iberian Peninsula. *Zoosystema* 36 (1): 41-52. http://dx.doi.org/10.5252/z2014n1a3

ABSTRACT
A new species of the genus *Isocolus* Förster, 1869 (Hymenoptera, Cynipidae: Aylacini) is described. It induces galls on the capitula of *Centaurea ornata* Willd. (Asteraceae), an endemic of the Iberian Peninsula. *Isocolus melikai* Pujade-Villar n. sp. is morphologically closely related to *I. freidbergi* Melika, 2008, forming gall on *C. verutum* L., 1755, and endemic to the North West of the Arabian Peninsula. *Urophora cuspidata* (Meigen, 1826) (Diptera, Tephritidae) has also been obtained from similar galls on *C. ornata*. *Isocolus melikai* Pujade-Villar n. sp. was found in 16 of the 326 inflorescences (capitula) sampled, while both species (*I. melikai* Pujade-Villar n. sp. and *U. cuspidata*) were found together only in three of these capitula. The galls are also described and a probable parasitoid of *Ormyrus* Westwood, 1832 (Hymenoptera, Ormyridae) is mentioned. The most important characteristics to differentiate adults and galls are illustrated. Biological data are provided showing that *I. melikai* Pujade-Villar n. sp. is bivoltine.

KEY WORDS
Cynipidae,
Aylacini,
Spain,
new species.

RÉSUMÉ
Premier cas de bivoltinisme chez Isocolus *(Hymenoptera, Cynipidae) :* Isolocus melikai *Pujade-Villar n. sp. à la Péninsule Ibérique.*
Une nouvelle espèce du genre *Isocolus* Förster, 1869 (Hymenoptera, Cynipidae: Aylacini) est décrite ; cette espèce endémique de la Péninsule Ibérique induit des galles sur les capitules de *Centaurea ornata* Willd. (Asteraceae). *Isocolus melikai* Pujade-Villar n. sp. est morphologiquement proche de *I. freidbergi* Melika, 2008, endémique du Nord Ouest de l'Arabie qui induit des galles sur *C. verutum* L., 1755. *Urophora cuspidata* (Meigen, 1826) (Diptera, Tephritidae) a également obtenu de galles similaires sur *C. ornata*. *Isocolus melikai* Pujade-Villar n. sp. a été trouvé dans 16 des 326 capitules échantillonnés tandis que les deux espèces (*I. melikai* Pujade-Villar n. sp. et *U. cuspidata*) n'ont été trouvées ensemble que dans trois des capitules échantillonnés. Les galles sont aussi décrites et un parasitoïde probable du genre *Ormyrus* Westwood, 1832 (Hymenoptera, Ormyridae) est mentionné. Les caractéristiques les plus importantes pour différencier les adultes et leurs galles sont illustrées. Le caractère bivoltin de *I. melikai* Pujade-Villar n. sp. est démontré.

MOTS CLÉS
Cynipidae,
Aylacini,
Espagne,
espèce nouvelle.

INTRODUCTION

The Cynipidae (Hymenoptera) are divided into two main trophic groups: The gall-inducers and the gall-associated inquilines are divided into eight tribes (Pujade-Villar, 2013), one of which is Aylacini Ashmead, 1931. The Aylacini are a possible paraphyletic tribe which includes the basal lineages of Cynipidae Billberg, 1820 (Liljeblad & Ronquist 1998; Ronquist 1999). It is characterized by producing galls on herbaceous plants, except *Diastrophus* Hartig, 1840 which produces them on *Rubus* spp (Rosaceae). Their life cycle is typically univoltine except in the species *Xestophanes potentillae* (Retzius *in* De Geer, 1773) and *Aulacidea pilosellae* (Kieffer, 1901) which are bivoltine in western France (Folliot 1964; Askew 1984).

Isocolus Förster, 1869 has a Palaearctic distribution. It is characterized by producing galls on Asteraceae. Most species are associated with *Centaurea* L. (Asteraceae) (Nieves-Aldrey & Parra 2003; Melika 2006). Among the 20 described species (Melika 2006), six have been cited from the Iberian Peninsula (Nieves-Aldrey 2001, 2012; Nieves-Aldrey & Parra 2003): *Isocolus fitchi* (Kieffer, 1898), *I. jaceae* (Schenck, 1863), *I. lichtensteini* (Mayr, 1882) and *I. scabiosae* (Giraud, 1859) mainly associated with *Centaurea aspera* L. and *C. scabiosa* L., *I. leuzeae* Nieves-Aldrey & Parra, 2003 associated with *Leuzea conifera* (L.) DC. and *I. serratulae* (Mayr, 1882) associated with *Serratula nudicaulis* L. (Asteraceae). Some *Urophora* Robineau-Desvoidy, 1830 species (Diptera: Tephritidae) also produce galls on *Centaurea* L. *Urophora cuspidata* (Meigen, 1826) is associated with *Centaurea* spp. of *Acrocentron* section (White & Korneyev 1989).

The new species of the *Isocolus* genus described here is morphologically similar to a species recently described from Israel, *Isocolus freidbergi* Melika, 2008 (in Karimpour *et al.* 2008). The most important characteristics to differentiate adults of the two species are given and illustrated. Description of galls and biological data of the new *Isocolus* species are also provided.

MATERIAL AND METHODS

Methodology

Capitula of *Centaurea ornata* Willd. (Asteraceae) used in this study were collected by INIA in several localities of Madrid province (Morata de Tajuña,

Valdaracete, Becerril de la Sierra and Robledo de Chavela).

Capitula from Morata de Tajuña and Valdaracete were incubated in a growth chamber at 25°C, 70 % HR and 16:8 light:dark photoperiod. Capitula from Becerril de la Sierra were incubated at room temperature. In both cases adults emerged from galls were kept in ethanol 70 % with some glycerol drops before being studied.

Capitula collected from Robledo de Chavela were immediatly dissected to investigate the presence of a second generation inside the galls.

In the material studied the galls collecting data are between parenthesis and the adult emergence data without parentheses.

Morphological structures terminology follows Liljeblad & Ronquist (1998) and Melika (2006). Venation abbreviations of the forewing are from Ronquist & Nordlander (1989). Terminology of the cuticle surface is from Harris (1979). The material has been determined using Melika (2006), Melika & Gharaei (2006) and Karimpour *et al.* (2008).

The SEM images were taken using Leica Stereoscan-360 at low voltage (700 V), without gold-coating in order to preserve the specimens.

The width of the forewing radial cell is measured from the margin of the wing to the base of Rs vein.

Abbreviations

Morphology

Cu1b	short descending branch of the cubital vein;
F1–F12	first and subsequent flagellomeres;
LOL	lateral-frontal ocellar distance, distance between lateral and frontal ocelli;
OOL	ocellar-ocular distance, distance from the outer edge of a posterior ocellus to inner margin of the compound eye;
POL	post-ocelar distance, distance between inner margins of the posterior ocelli;
R1	first branch of the radial vein;
Rs	second branch of the radial vein.

Institutions

The type material is deposited at the following institutions:

INIA	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria;
MNHN	Muséum national d'Histoire naturelle, Paris;
PDL	Pest Diagnostic Laboratory (called before Systematic Parasitoid Laboratory, SPL), Tanakajd;
UB	Universidad de Barcelona;
USNM	U.S. National Museum of Natural History, Smithsonian Institution, Washington, DC.

SYSTEMATICS

Family Cynipidae Billberg, 1820
Subfamily Cynipinae Billberg, 1820
Tribe Aylacini Ashmead, 1903

Genus *Isocolus* Förster, 1869

Type species. — *Diastrophus scabiosae* Giraud, 1859: 368, original designation.

Diagnosis. — Morphologically the *Isocolus* genus is characterized by the combination of the following features: female antennae 13-15 segmented (male 14-15 segmented), first flagellar segment (F1) shorter than second (F2), placodeal sensilla from F2 in female (F1 in male); radiating striae on face usually not reaching the base of the compound eyes; rough-coriaceous scutum (alutaceous on *I. leuzeae*) usually with more or less conspicuous transversal carinae; rough scutellum with deep and well-defined scutellar foveae; longitudinally striated mesopleuron; forewing with glabrous or slightly pubescent margin, radial cell opened on wing margin (except in *Isocolus volgensis* Dyakonchuk, 1982 which it is partially open); third metasomal tergum and the following terga (as well as the hypopygium) densely punctuated (sometimes punctures also present on T2): ventral spine very short.

Isocolus melikai Pujade-Villar n. sp. (Figs 1-3)

Holotype. — Morata de Tajuña (Madrid, Spain), (22.VI.2011) 14.VII.2011: 1 ♀, A. Cobo, rec. ex floral capitulum of *Centaurea ornata*. JP-V col. (Juli Pujade-Villar's collection deposited provisionally in UB).

Paratypes. — Same data as holotype, 5 ♂♂, 2 ♀♀; Becerril de la Sierra (Madrid, Spain), (22.VII.2012) 23.VII.2012: 3 ♂♂, 5 ♀♀, A. Cobo rec., ex floral capitula of *Centaurea ornata*; Valdaracete (Madrid, Spain), (06.VII.2011) 28.VII.2011: 3 ♂♂, 7 ♀♀ A. Cobo rec., ex floral capitula of *Centaurea ornata*. Paratypes deposited in: 4 ♂♂, 7 ♀♀ (UB); 3 ♂♂, 3 ♀♀ (INIA); 1 ♂, 1 ♀ (PDL); 1 ♂, 1 ♀ (USNM); 2 ♂, 2 ♀ (MNHN).

Type locality. — Morata de Tajuña (Madrid, Spain) situated at UTM coordinates X: 460624; Y: 4452619.

ETYMOLOGY. — This species is dedicated to our friend and colleague: George Melika (Pest Diagnostic Laboratory, Budapest, Hungary).

DIAGNOSIS. — *Isocolus melikai* Pujade-Villar n. sp. belongs to the *Isocolus* group whose females have 13-segmented antennae; the second metasomal tergum without dense white setae on the latero-basal plate, which is densely punctuated on posterior margin; mesoscutum with strong transverse carinae; mesoscutal medial line absent or slightly differentiated, sometimes forming a very short triangle. This species is similar to *I. freidbergi* but both species can be differentiated by: coloration (completely black in *I. melikai* Pujade-Villar n. sp., dark brownish with black areas in *I. freidbergi*); piliferous points above the toruli and on the laterals of the scutum (present in *I. melikai* Pujade-Villar n. sp., absent in *I. freidbergi*); distance between pronotal foveae (as long as foveae width in *I. melikai* Pujade-Villar n. sp., shorter in *I. freidbergi*); morphology of scutellar foveae (triangular in *I. melikai* Pujade-Villar n. sp., rounded in *I. freidbergi*); scutum sculpture (carinae more or less complete in *I. melikai* Pujade-Villar n. sp., interrupted carinae in *I. freidbergi*); shape of propodeal carinae (very thick in *I. melikai* Pujade-Villar n. sp., thin in *I. freidbergi*), and space between them (small in *I. melikai* Pujade-Villar n. sp., wide in *I. freidbergi*).

DESCRIPTION

Lenght

Female: 3.8-4.5 mm (n=11).
Male: 2.7-3.5 mm (n=10).

Colour

Head, mesosoma and metasoma completely black; dark antennae, scape and pedicel black, flagellomeres brown; mandibles and clypeus from brown to black; dark legs, coxae, trochanters and basal half of femora black; apical half of femora, tibiae and tarsi yellowish brown, distal tarsi darker.

Head (Fig. 1A, B)

In frontal view, around 1.4 times wider than high. In dorsal view, 2.1 times longer than wide, slightly narrower than mesosoma; gena slightly broadened behind compound eyes; POL 1.1 times longer than OOL and 2.2 than LOL; LOL 2.0 times longer than wide lateral ocellus; a small triangular concavity pointing in front of ocellus; frons, vertex, inter-ocelar area and occiput coriaceous, with some piliferous points; post-occiput and post-gena slightly coriaceous, with white setae, more abundant than in frons; centre of face more pubescent than frons and vertex; area between toruli and compound eye with fine coriaceous sculpture; transfacial distance 2.2 times as long as the compound eye height, 1.3 times longer than lower face height (from toruli to clypeus margin) and, 4.0 times longer than distance between antennal toruli and margin of eye; toruli diameter 2.75 times larger than inter-toruli distance. Lower part of head, in frontal view, strongly coriaceous, raised medially, with fine striae radiating to base of eye and toruli. Malar space coriaceous, 0.7 times longer than the height of the compound eye. Clypeus shiny, alutaceous, slightly higher than wide, slightly impressed, incised ventrally; tentorial foveae small but well-defined; epistomal sulcus well differentiated, widened; clypeo-pleurostomal line well differentiated.

Antenna

Female (Fig. 1C, D), 13-segmented, pedicel 1.6 times shorter than F1, F1 slightly shorter than F2; the smallest flagellomere is F10; F11 almost as long as F8+F9; placodeal sensilla from F2, although they are superficial and sparse. Antennal formula: 8: 4.5: 7.5: 7.5: 8: 8: 8: 7.5: 7: 6.5: 6.5: 6: 12.

Male antenna (Fig. 1E, F) similar to that of female, 14-segmented; F1 slightly curved and excavated; placodeal sensilla from F1.

Mesosoma (Figs 1B, 2A-C)

On lateral view, 1.1 times as long as high. Pronotum dorso-medial length 2.1-2.2 times shorter than its greater lateral margin width; pronotal sub-medial pits well differentiated, transversal, narrow and deep, separated by a space a slightly shorter than foveae width; pronotal plate carinae visible but incomplete; pronotum with white abundant setae along anterior edge, scarcer on lateral margin, fewer and shorter dorsal-medially; dorso-medial area coriaceous with some weak rugae close to the margin. Propleuron coriaceous, with some transversal rugae mainly at base. Mesoscutum around 1.3 times wider than long on dorsal view, with few, short and sparse setae. Notauli percurrent, uniformly impressed, not wider at base, alutaceous and dull; anterior parallel lines well differentiated, coriaceous and shiny, impressed

FIG. 1. — *Isocolus melikai* Pujade-Villar n. sp.: **A**, frontal view of female head; **B**, dorsal view of female head and pronotum; **C**, female antenna; **D**, detail of the first antennomeres of female antenna; **E**, male antenna ; **F**, detail of the first antennomeres of male antenna. Scale bars: 250 µm.

on the anterior third of mesoscutum; parapsidal lines well differentiated, convergent, extending on the posterior 2/3 of the mesoscutum; medial mesoscutal line absent; area between notauli as well as between parapsidal line and notauli strongly transversally interrupted wrinkles, especially on the posterior 2/3

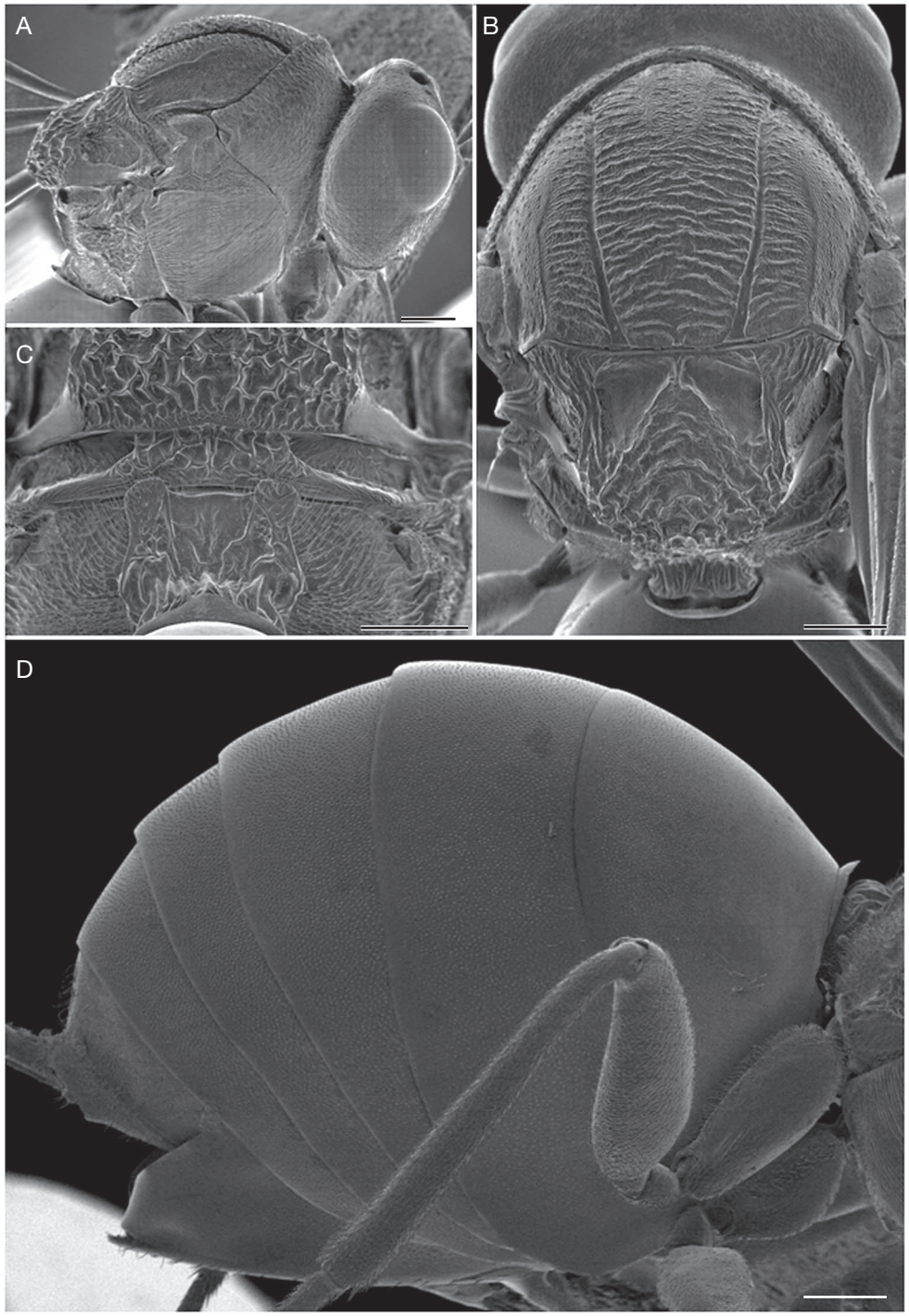

Fig. 2. — *Isocolus melikai* Pujade-Villar n. sp. female: **A**, head and mesosoma in lateral view; **B**, mesonotum in dorsal view; **C**, propodeum; **D**, metasoma in lateral view. Scale bars: 250 µm.

FIG. 3. — **A**, Floral capitulum of *Centaurea ornata* Willd.; **B**, female of *Isocolus melikai* Pujade-Villar n. sp. ovipositing on the base of the floral capitulum; **C**, floral capitulum attacked by: 1, *Urophora cuspidata* (Meigen, 1826); 2, *I. melikai* Pujade-Villar n. sp.; **D**, longitudinal section of floral capitulum attacked by *I. melikai* Pujade-Villar n. sp.: 3, gall with larvae; 4, abandoned gall; 5, adult leaving gall; **E**, galls of *I. melikai* Pujade-Villar n. sp.; **F**, forewing venation of *I. melikai* Pujade-Villar n. sp. Scale bars: A, 1 cm; B-E, 2 mm; F, 0.5 mm.

of the mesoscutum, more delicate to the 1/3 anterior, at the level of anterior parallel lines; inter-spaces weakly coriaceous and dull; distance separating to transversally wrinkles at least 2 times longer than their width; area between parapsidal lines and lateral edge of mesoscutum coriaceous, with some piliferous points in the anterior area; lateral edge of the mesoscutum emarginated. Mesoscutellum in dorsal view as long as wide, slightly emarginated posteriorly, uniformly rugose in arches more or less complete, interspaces coriaceous; scutellar foveae triangular, slightly longer than wide, alutaceous and shiny, anteriorly separated by a fine carina on the 1/3 anterior, its length is a bit less than the half length of the metascutellum. Dorso-axilar area shiny, rugose longitudinally. Mesopleuron transversally striated, striae weak ventrally, with short black setae in ventral part; specular area impressed; triangle of the mesopleuron dull, alutaceous and densely pubescent. Metapleuron furrow reaching the half length of the mesopleuron; upper area of the metapleuron (between metapleural furrow and propodeal spiracle) dull and rugous; axillula alutaceous, with abundant white short setae. Metascutellum weakly rugous; ventral area of metascutellum shiny, weakly rugous, triangular, occupying 2/3 of the dorsomedial area of the metascutellum; metanotal foveae alutaceous, dull, with scarce white setae. Propodeum laterally alutaceous, dull, with few long and white setae, not hiding propodeal sculpture, with some rugae basally; propodeal lateral carinae well differentiated, thick, slightly divergent anteriorly, slightly arched and wider basally; central area of propodeum shiny, alutaceous and with some rugae, glabrous; transversal propodeal spiracle with a strong carena on anterior margin; nucha with strong parallel longitudinal ridges.

Forewing (Fig. 3F)
Shorter than the body and ciliated on margin. Radial cell opened, 2.5 times as long as wide; vein R1 uniformly pigmented, not reaching the wing margin; vein Rs not reaching the margin; triangular areola differentiated; vein Cu1b straight, with a nebulous curvature outward wing.

Legs
Tarsal claws dark, simple, without basal lobe.

Metasoma (Fig. 2D)
Second metasomal tergum exceeding in dorsal view 1/3 of the metasoma length, with few setae in the baso-lateral area, its posterior half punctuated; the following terga and hypopygium uniformly punctuate; prominent part of the hypopygial spine very short, longer than wide, with few scattered white setae.

Host
The only known host plant is *Centaurea ornata* Willd. (Asteraceae).

Galls (Fig. 3C-E)
The galls are fused at their base (rarely scattered) and situated at the base of the floral capitula of *C. ornata*; the galls are separated laterally by abundant pubescence formed by flat white setae. Each floral capitula may contain several galls (between one and five in the studied material). The galls are formed by the modification of the ovary; first, they forming a whitish cylindrical gall, with a thick wall which at the same time as the floral capitula does, turns to yellow as it is growing. The galls are elongated and elliptical (5.0-6.0 mm × 3.4-4.1 mm), green when are young and with a very rigid and smooth yellow wall in mature galls. In three capitula, galls of *Urophora cuspidata* (Meigen, 1826) were found coexisting with *I. melikai* Pujade-Villar n. sp. galls. *U. cuspidata* galls were usually present in high number, between one and eighteen per capitulum. These galls form a callus at the base of the receptacle and are fused. They are elongated and have a pointed upper end (4.2-7.1 mm × 3.0-4.9 mm)(Fig. 4C, D).

Parasitoids
An *Ormyrus gratiosus* (Förster, 1860) female emerged from one capitulum where *I. melikai* Pujade-Villar n. sp. and *U. cuspidata* have coexisted.

Biology
Isocolus melikai Pujade-Villar n. sp. causes galls on the capitula of *C. ornata*, a perennial and rhizomatous plant, endemic from the Iberian Peninsula. Capitula formation of *C. ornata* starts at the end of May and bloom continues until August-September.

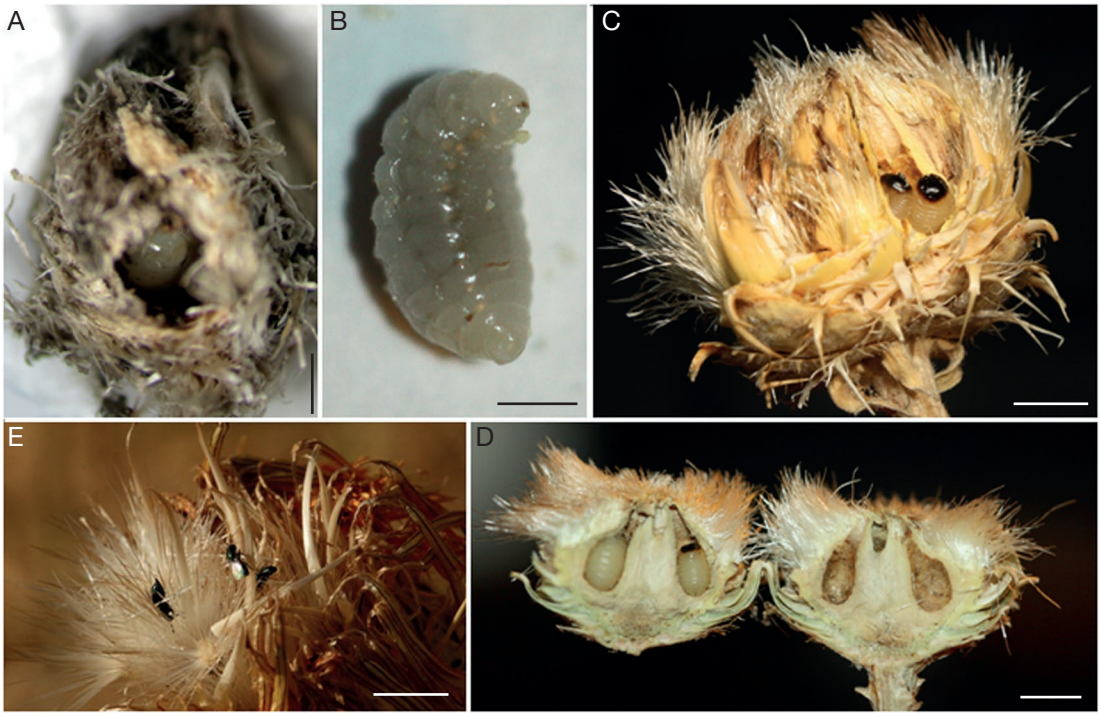

FIG. 4. — **A, B,** *Isocolus melikai* Pujade-Villar n. sp.: **A**, gall cell; **B**, larva; **C-E**, *Urophora cuspidata* (Meigen, 1826): **C**, gall cells; **D**, larvae; **E**, *Ormyrus gratiosus* (Förster, 1860) attacking capitula galls of *Centaurea ornata* Willd., 1803. Scale bars: A, B, 1 mm, C-E, 5 mm.

First generation. At the time of the first collections in Morata de Tajuña and Valdaracete (late June – early July 2011) inflorescences were beginning to open and galls of *I. melikai* Pujade-Villar n. sp. were already formed (Fig. 3A). In the laboratory adults emerged from these capitula after 22 days incubation period during the mid-end of July of the same year. Althought the collected capitula were kept incubated for a whole year, no more adults emerged, and after this period of incubation, no larvae or pupae of *I. melikai* Pujade-Villar n. sp. were found after capitula dissection.

At a later collection in Becerril de la Sierra (late July 2012), we also observed an adult emerging only after one day incubation. A female emerged from this material was observed ovipositing on the same capitulum from which it had emerged in laboratory conditions (Fig. 3B). Males comprise 42 % of the total emerged adults.

Second generation. In posterior field observations at Robledo de Chavela (late July – early August 2013) females of *I. melikai* Pujade-Villar n. sp. were also seen ovipositing in the same plant species. Galls and larvae of *I. melikai*. Pujade-Villar n. sp. were found in newly formed capitula collected at the end of August in this location (Fig. 4B).

The number of galls, larvae and adults of *I. melikai* Pujade-Villar n. sp. obtained and the number and percentage of capitula infested in every location are provided in Table 1.

Distribution

So far, *I. melikai* Pujade-Villar n. sp. has only been found in four municipalities of the Community of Madrid where samples were taken for this study. However, surveys are needed in other areas where its host is present. The host plant, *C. ornata,* is an Iberian endemic species widely distributed in the

TABLE 1. — Data on capitula of *Centaurea ornata* Willd. collected and, adults and larvae captures of *Isocolus melikai* Pujade-Villar n. sp. in four locations in the Community of Madrid. Symbol: *, 9 galls were found but only 5 of them were occupied by larvae.

Location (Madrid)	Sampling date	Number of *I. melikai* Pujade-Villar n. sp. reared	Number of capitula sampled	Number of capitula infested	Capitula infested (%)
Becerril de la Sierra	22.VII.2012	3 ♂♂, 5 ♀♀	93	5	5.3
Morata de Tajuña	22.VI.2011	5 ♂♂, 3 ♀♀	77	3	3.9
Valdaracete	06.VII.2011	3♂♂, 7 ♀♀	100	4	4.0
Robledo de Chavela	30.VIII.2013	5*	56	4	7.1

north, east and centre of the Iberian Peninsula, where it is usually found on sandy, stony and barren soils in sunny and arid areas of montane and lower altitude levels (Willkomm & Lange 1870). A distribution map of *C. ornata* is available in the Spanish plants information system "Anthos" (Anthos 2012).

DISCUSSION

Isocolus melikai Pujade-Villar n. sp. is morphologically distinct from all the species recorded in the Iberian Peninsula. It differs from *I. lichtensteini* by lacking a setose plate on the lateral margin of the second metasomal tergum; from *I. fitchi* by having the second metasomal tergum micropunctuated on distal third and a strong mesoscutum sculpture; from *I. scabiosae* by having the second metasomal tergum micropunctuated on 1/3 distal part absent in *I. scabiosae* according to Melika (2006) or with some points on distal part of second metasomal tergite according to Nieves-Aldrey (2001) and to have a strong mesoscutum sculpture; and finally, from *I. leuzeae* and *I. serratulae* (besides the hosts) by having strong transversal wrinkles on the mesoscutum. Regarding to the species which produce galls on *Centaurea*, *I. lichtensteini* induces galls on stems, *I. scabiosae* on stems and floral capitula and *I. fitchi* at the base of stems or petioles (Nieves-Aldrey 2001).

According to Melika (2006) *I. melikai* Pujade-Villar n. sp. is morphologically similar to *Isocolus cirsii* Diakontshuk, 1987 and *I. freidbergi* by the characters mentioned above to define *I. melikai* Pujade-Villar n. sp. and also by having R1 away from the wing margin and for the mesoscutum sculpture. *Isocolus freidbergi* was described by Melika in Karimpour *et al.* (2008) while cited as "*Isocolus* sp. (Israel) in Melika (2006)". *Isocolus melikai* Pujade-Villar n. sp. differs from *I. cirsii* for having alar areola (among other characters) and by the host species (*Cirsium ukrainicu*m (Diakontshuk, 1987) in *I. cirsii*); it differs from *I. freidbergi* by the characters mentioned in the diagnosis.

Isocolus melikai Pujade-Villar n. sp. and the tephritid *Urophora cuspidata* cause galls on the capitula of *Centaurea ornata*. Both species were found coexisting together in three of the samples collected, and althought their galls are similar they can be distinguished morphologically, as described in the results section. Data for four localities sampled are exposed in Table 1.

An *Ormyrus* species has been detected as parasitoid on the capitula where *I. melikai* Pujade-Villar n. sp. and *U. cuspidata* coexisted: *Ormyrus gratiosus* (Förster, 1860). This species attacks galls induced on Asteraceae capitula, among them those of *Urophora* and *Isocolus* according to Noyes (1998) and Zerova & Seryogina (2006) (Fig. 4E). For this reason, we cannot assure that *O. gratiosus* attack galls of *I. melikai* Pujade-Villar n. sp. At this moment, the only species specific of galls of *Isocolus* is *Ormyrus salmanticus* Nieves-Aldrey, 1984 (Askew *et al.* 2006).

Concerning the host plant, *Centaurea* is a phylogenetic complicated genus. The last molecular studies based on this genus release more doubts in this regard (García-Jacas *et al.* 2001). *Centaurea scabiosa* (host of *Isocolus scabiosae*) and *C. ornata* (host of *I. melikai* Pujade-Villar n. sp.) are very close phylogenetically, although in some studies they come out into different clades (Font *et al.* 2002, 2009). Both species hybridize easily result-

ing in a very heterogeneous complex which has received many names in the taxonomic literature, the most known being *Centaurea* x *polymorpha* Lag. (García-Jacas & Susanna 1993). The records of the Iberian populations of *C. scabiosa* do not correspond actually to this species but to *C. cephalariifolia* Willk., a very similar species. The two species can hardly be morphologically differentiated nevertheless they present different chromosome number (E. López *pers. com.*). These two species belong to the *Acrocentron* section which includes vivacious and rhizomatous plants, whose rhizomas remain alive when aerial part is renewed each year. On the other hand, *C. verutum* (distributed in Israel, Palestine territory, Jordan and Syria) the host of *I. freidbergi*, which is the morphologically closest species of *I. melikai* Pujade-Villar n. sp., is included in the *Mesoncentron* section , and its synonym Sect. *Solstitialia*. This section is phylogenetically distant to the *Acrocentron* section (E. López y N. García *pers. com.*), it groups annual plants as *C. solstitialis* L., 1753, *C. melitensis* L., 1753 or *C. sulphurea* Willd., 1809 between other peninsular species. Our results show for the first time that some *Isocolus* species morphologically similar can be found on galls of *Centaurea* species phylogenetically different. The morphological variability mentioned in Nieves-Aldrey (1994; 2001) for *I. scabiosae*, and its synonym *I. rogenhoferi* (Mayr, 1882), could be explained including a sibling species complex yet undefined (Nieves-Aldrey 2001) and/or by the great hybridization that the host species suffers and the diversity of the plant organs which are attacked, being the new species a case of convergent evolution. As it has been mentioned, both species have morphological differences, *I. melikai* Pujade-Villar n. sp. has a strong fairing in the mesoscutum, never present nor described in *I. scabiosae* (they are completely absent in the rest of the species present in the Peninsula Iberica), and an extended micropunctated from the second metasomal tergite. A more precise study on the relationship between *Isocolus* species and their hosts is required to know the existing morphological groups, as it has been done in other groups of Cynipidae (Melika *et al.* 2010).

Concerning biological data, a strong synchronyzation between the plant phenology and the gallwasp life cycle was observed. The fact that *Centaurea ornata* has a long period of bloom allows for the existence of two annual generations. The dates of emergence of the adults of the first generation suggest that the eggs are laid in late May – early June, concurring with the formation of the first capitula in the host plant. The posterior field observations of adults from both sexes and female ovipositions mean that there is a second oviposition period in late July – early August. This second generation probably overwinters as mature larvae inside the galls. Two types of Aylacini life cycles are known: bisexual species, where both sexes occur and they are usually univoltine except *X. potentillae* populations in western France wich are bivoltine. Unisexual species, where males are absent or very rare, typically univoltine except *A. pilosellae* populations in western France which are bivoltine (Folliot 1964; Askew 1984). According to our observations *I. melikai* Pujade-Villar n. sp. can be included in bisexual bivoltine life cycle group species of the tribe Aylacini.

Acknowledgements
We are very grateful to Ismael Sánchez Ramos and Zeus Hornos del Monte (Plant Protection, INIA) for participating in the collection of samples, to Palmira Ros-Farré (UB) for taking the SEM pictures which illustrate this study, to Dr. R. R. Askew for determining *Ormyrus* species, to George Melika (PDL) for his comments and to Valery A. Korneyev (I. I. Schmalhausen Institute of Zoology, Kiev, Ukraine) for helping with the determination of *U. cuspidata*. Also we grateful to Núria Garcia Jacas (Institut Botànic de Barcelona) and Eusebio López (Universidad de Córdoba) for comments of *Centaurea* genus included in this study. Finally, we thanks to Claire Villemant (MNHN) and Fabiana Gallardo (Museo de la Plata, Argentina) for the criticisms and comments.

REFERENCES

ANTHOS 2012. — Information System of the plants of Spain. Real Jardín Botánico, CSIC – Fundación Biodiversidad. Available at www.anthos.es. (19.XII.2012).

ASKEW R. R. 1984. — The Biology of gall wasps, *in* ANANTHAKRISHNAN T. N. (ed.), *Biology of Gall Insects*. Edward Arnold, London: 223-271.

ASKEW R. R., PLANTARD O., GÓMEZ J. F., NIEVES M. H. & NIEVES-ALDREY J. L. 2006. — Catalogue of parasitoids and inquilines in galls of Aylacini, Diplolepidini and Pediaspidini (Hymenoptera, Cynipidae) in the West Palaearctic. *Zootaxa* 1301: 1-60.

FOLLIOT R. 1964. — Contribution a l'étude de la biologie des cynipides gallicoles (Hymenopters, Cynipoidea). *Annales des Sciences Naturelles, Zoologie*,12 ser 6: 407-564.

FONT M., GARNATJE T., GARCÍA-JACAS N. & SUSANNA A. 2002. — Delineation and phylogeny of *Centaurea* sect. *Acrocentron* based on DNA sequences: a restoration of the genus *Crocodylium* and indirect evidence of introgression. *Plant Systematics and Evolution* 234: 15-26.

FONT M., GARCÍA-JACAS N., VILATERSANA R., ROQUET C. & SUSANNA A. 2009. — Evolution and biogeography of *Centaurea* section *Acrocentron* inferred from nuclear and plastid DNA sequence analyses. *Annals of Botany* 103: 985-997.

GARCÍA-JACAS N. & SUSANNA A. 1993. — *Centaurea x polymorpha* Lagasca: los problemas de un híbrido. *Fontqueria* 36: 65-66

GARCÍA-JACAS N., SUSANNA A., GARNATJE T. & VILATERSANA R. 2001. — Generic Delimitation and Phylogeny of the Subtribe Centaurinae (Asteraceae): A combined Nuclear and Chloroplast DNA Analysis. *Annals of Botany* 87: 503-515

HARRIS R. 1979. — A glossary of surface sculpturing. State of California, Department of Food and Agriculture, *Occasional Papers in Entomology* 28: 1-31.

KARIMPOUR Y., TAVAKOLI M. & MELIKA G. 2008. — New species of herb gallwasps from the Middle East (Hymenoptera, Cynipidae, Aylacini). *Zootaxa* 1854: 16-32.

LILJEBLAD J. & RONQUIST F. 1998.- A phylogenetic analysis of higher-level gall wasp relationships (Hymenoptera: Cynipidae). *Systematic Entomology* 23: 229-252.

MELIKA G. 2006. — Gall Wasps of Ukraine. Cynipidae. *Vestnik Zoologii* Supplement 21(1-2): 1-300, 301-644.

MELIKA G. & GHARAEI B. 2006. — New species of herb galling-cynipids (Hymenoptera: Cynipidae: Aylacini) from Iran. *Acta Zoologica Academiae Scientiarum Hungaricae* 52(4): 385-399.

MELIKA G., PUJADE-VILLAR J., ABE Y., TANG C.-T., NICHOLLS J., WACHI N., IDE T., YANG M.-M., PÉNZES Z., CSÓKA, G. & STONE, G. N. 2010. — Palaearctic oak gallwasps galling oaks (*Quercus*) in the section Cerris: re-appraisal of generic limits, with descriptions of new genera and species (Hymenoptera: Cynipidae: Cynipini). *Zootaxa* 2470: 1-79.

NIEVES-ALDREY J. L. 1994. — Revision if West-European genera of tribe Aylacini Ashmead (Hymenoptera, Cynipidae). *Journal of Hymenoptera Research*, 3: 175-206.

NIEVES-ALDREY J. L. 2001. — Hymenoptera, Cynipidae, *in* RAMOS M. A., ALBA TERCEDOR J., BELLÉS I ROS X., GOSÁLBEZ I NOGUERA J., GUERRA SIERRA A., MACPHERSON MAYOL E., MARTÍN PIERA F., SERRANO MARINO J., TEMPLADO GONZÁLEZ J. (eds), *Fauna Ibérica* Vol. 16. Museo Nacional de Ciencias Naturales, CSIC, Madrid: 636.

NIEVES-ALDREY J. L. 2012. — Two new herb gall wasps from Spain, including the description of a new species of *Aulacidea* Ashmead, 1897 (Hymenoptera, Cynipidae, « Aylacyni ») inducing galls on *Serratula nudicaulis* L. DC (Asperaceae). *Graellsia* 68(2): 325-339.

NIEVES-ALDREY J. L. & PARRA L. A. 2003. — A new species of *Isocolus* (Hymenoptera, Cynipidae) from Spain, inducing galls in flower heads of *Leuzea conifera* (Asteraceae). *Annales de la Société entomologique de France* 39(1): 49-54.

NOYES J. S. 1998. — Catalogue of the Chalcidoidea of the World. Biodiversity Catalogue. CD-ROM.

PUJADE-VILLAR J. 2013. — Las agallas de los encinos: un ecosistema en miniatura que hace posible estudios multidisciplinares. *Entomología Mexicana* 12(1): 2-22.

RONQUIST F. 1999. — Phylogeny, classification and evolution of the Cynipoidea. *Zoological Scripta* 28: 139-164.

RONQUIST F. & NORDLANDER G. 1989. — Skeletal morphology of an archaic cynipoid, *Ibalia rufipes* (Hymenoptera, Ibaliidae). *Entomologica Scandicavica* Suppl 33: 1-60.

WHITE I. M. & KOMEYEV V. A. 1989. — A revision of the western Palaearctic species of Urophora Robineau-Desvoidy (Diptera: Tephritidae). *Systematic Entomology* 14: 327-374.

WILLKOMM M. & LANGE J. 1870. — *Prodromus florae hispanicae. Vol. II. E. Schweizerbart'sche Verlagsbuchhandllung Stuttgart: 680 p.* Available at http://archive.org/details/prodromusfloraeh02will. (19.XII.2012).

ZEROVA M. D. & SERYOGINA L. Y. 2006. — Review of Palearctic Ormyridae (Hymenoptera, Chalcidoidea), with description of two new species. *Vestnik Zoologii* 40 (1): 27-40.

Submitted on 5 April 2013;
accepted on 15 October 2013;
published on 28 March 2014.