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Notas / *Notes*

First record in Iberia of *Oobius rudnevi* (Nowicki, 1928) (Hymenoptera: Encyrtidae), a poorly-known egg parasitoid of *Cerambyx* species (Coleoptera: Cerambycidae)

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ABSTRACT

Oobius rudnevi (Nowicki, 1928) (Hymenoptera: Encyrtidae) is first reported from Iberia and southern Europe using sentinel eggs. The parasitoid was detected in July 2016 in three oak open woodlands in Extremadura (SW Spain), located in Almendral (La Jara) and Mérida (La Serrana and Cornalvo Natural Park). *O. rudnevi* parasitized both the eggs of *Cerambyx cerdo* Linnaeus, 1758 (its only known host to date) and *C. welensii* (Küster, 1845), which constitutes a new host-parasitoid association (*C. welensii*-*O. rudnevi*). Parasitism rate was quite variable among sites and dates (range 0–93%), with a higher prevalence in *C. cerdo* than in *C. welensii*. New studies are conducted in the field and laboratory to explore the ecology, behaviour and parasitic potential of *O. rudnevi* in Mediterranean oak open woodlands.

Keywords: parasitoid wasp; sentinel eggs; *Cerambyx cerdo*; *Cerambyx welensii*; Iberian Peninsula; Extremadura.

RESUMEN

Primer registro en la Península Ibérica de *Oobius rudnevi* (Nowicki, 1928) (Hymenoptera: Encyrtidae), un parasitoide oófago poco conocido de *Cerambyx* sp. (Coleoptera: Cerambycidae)

Se cita por primera vez a *Oobius rudnevi* (Nowicki, 1928) (Hymenoptera: Encyrtidae) de la Península Ibérica y el sur de Europa usando huevos centinela. El parasitoide se detectó en julio de 2016 en tres dehesas en Extremadura (SO de España), localizadas en Almendral (La Jara) y Mérida (La Serrana y Parque Natural de Cornalvo). *O. rudnevi* parasitó tanto los huevos de *Cerambyx cerdo* Linnaeus, 1758 (su único hospedador conocido hasta la fecha) como los de *C. welensii* (Küster, 1845), lo que supone una nueva asociación hospedador-parasitoide (*C. welensii*-*O. rudnevi*). El porcentaje de parasitismo se mostró bastante variable entre sitios y fechas (rango 0–93%), con mayor prevalencia en *C. cerdo* que en *C. welensii*. Nuevos estudios se desarrollan en campo y laboratorio para explorar la ecología, comportamiento y potencial parasitario de *O. rudnevi* en las dehesas mediterráneas de quercíneas.

Palabras clave: avispa parasitoide; huevos centinela; *Cerambyx cerdo*; *Cerambyx welensii*; Península Ibérica; Extremadura.

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The genus *Oobius* Trjapitzin, 1963, belongs to the subfamily Encyrtinae, tribe Oobiini, but before Noyes (2010) it was classified in Discodini (former Microteryini). After the description in recent years of several new species, and Noyes' (2010) review in which the genera *Avetianella* Trjapitzin, 1968, *Szelenyiola* Trjapitzin, 1977, and *Oophagus* Liao, 1987, were synonymised under *Oobius*, the genus currently includes almost 50 species distributed worldwide (Noyes, 2019). The genus *Oobius* includes some important species for the biological control of alien insect pests in forest ecosystems, such as *O. longoi* (Siscaro, 1992) and *O. agrili* Zhang & Huang, 2005, which parasitize *Phoracantha semipunctata* (Fabricius, 1775) (Coleoptera: Cerambycidae) and *Agrilus planipennis* Fairmaire, 1888 (Coleoptera: Buprestidae), respectively.

The species *Tyndarichus rudnevi* Nowicki, 1928, is the type species of the genus *Oobius* by original designation (Trjapitzin, 1963). However, species of *Tyndarichus* Howard, 1910 are considered to be hyperparasites, while *Oobius* species are primary egg parasitoids mainly of wood-boring Coleoptera in the families Cerambycidae and Buprestidae. This biological inconsistency led Trjapitzin (1963) to include *T. rudnevi* in his newly-described genus *Oobius*, establishing thus the currently accepted combination *Oobius rudnevi* (Nowicki, 1928). Note that the form *rudnewi*, used occasionally, is merely a misspelling of *rudnevi*. For more details on the interesting taxonomic history of *O. rudnevi* see Trjapitzin & Volkovitsh (2011).

O. rudnevi (Fig. 1) is known from Ukraine (Transcarpathian, Zhitomir, Cherkassy, and Kirovograd provinces) (Nowicki, 1928; Trjapitzin, 1978; Trjapitzin & Volkovitsh, 2011), Georgia (incl. Abkhazia) (Trjapitzin, 1978), Russia (Thompson, 1954) and Morocco (La Maâmora cork oak forest) (El Antry, 1999; Kissayi & Benhalima, 2016). An imprecise record from "western Europe" and another from Poland are apparently erroneous, and a third record from Croatia requires confirmation (Trjapitzin & Volkovitsh, 2011). For an updated distribution of the species see also Noyes (2019). To date, *O. rudnevi* has been only recorded as a gregarious egg parasitoid of the great capricorn beetle *Cerambyx cerdo* Linnaeus, 1758, a well-know oak-living saproxylic beetle. *C. cerdo* is a univoltine species and in the studied area adults usually fly from middle-late May to early August.

It follows that the known distribution of *O. rudnevi* is rather modest and strikingly scattered at the synoptic scale, contrasting with the wide distribution of *C. cerdo* across the western Palaearctic. This discrepancy suggests a quite fragmentary knowledge of the real biogeography of the small wasp *O. rudnevi*, almost a century after the species was described. In this scenario, the main goals of this study were: 1) to detect if *O. rudnevi* occurs in the region of Extremadura (SW Spain) parasitizing *C. cerdo* eggs, and 2) to explore if *O. rudnevi* could also parasitize the eggs of *Cerambyx welensii* (Küster, 1845), an emerging oak pest in Iberia (Torres-Vila *et al.*, 2016).

To detect the presence of *O. rudnevi* adults in the field, we used sentinel eggs of *C. cerdo* and *C. welensii* obtained in the laboratory from field-derived adults caught with baited traps in oak open woodlands at Mérida (Badajoz). The methodology used to obtain the eggs follows previous studies with both *Cerambyx* species (Torres-Vila *et al.*, 2016; Torres-Vila, 2017). We used standardized 16 l cardboard cages with a transparent cover as mating and oviposition chambers. Males shared cages with females during mating and were kept separately during female egg-laying in well-aerated 240 ml clear plastic containers. Caged adults were regularly sprayed with water and fed ad libitum on a saturated sugar-water paste simulating host tree exudates.

Freshly cut cork oak branches were sliced with a circular saw to produce wood disks (7–8 cm in diameter, 2 cm thick) that were used as egg-laying substrate for females and then as support for sentinel eggs in the field (Fig. 2). If necessary, wood disks were frozen until use to prevent excessive drying. The cork layer was detached from the cambium in one piece with a penknife, returned to its place and affixed with a rubber band. The narrow space between the cambium and cork layer was found to be extremely attractive for ovipositing females and most eggs were found in this place (>95%). Decorticating the disks also greatly facilitated inspections and removal of the eggs after their field exposition (see below). One wood disk labelled with the *Cerambyx* species was used per cage to obtain eggs during a single dusk/scotophase period. We usually introduced 2–4 females per cage (depending on their size and age) trying to obtain 10–30 eggs per disk. Special care was taken not to contaminate the eggs of a host species with semiochemicals of the other. In practice, we used different material (cages, wood disks, rubber bands and other elements) for each species and two controlled environmental chambers, one for *C. cerdo* and another for *C. welensii*. Chambers were adjusted at 25 ± 1°C, 60 ± 10% relative humidity and a L16:D8 photoperiod, simulating typical summer conditions in the studied area (July). Under these conditions, adults fed, mated and laid eggs normally.

Wood disks housing newly-laid eggs (<24 h old) were taken to the field the next day in the morning and fixed with double-threaded (wood thread/machine thread) metal bolts (11.5 cm x 8 mm in diameter) to the tree trunk. The wood thread end was screwed to the trunk and in the machine thread the disk was fastened with two nuts (Fig. 2). A small drop of entomological glue was also applied at the bolt base to prevent the predation of eggs by ants. Wood disks were placed in three selected dehesas (forming a triangle with sides of 38, 46 and 70 km long) in which populations of both *Cerambyx* species were known to occur. They were located at Almendral (La Jara) and Mérida (La Serrana and Cornalvo Natural Park) (see Table 1

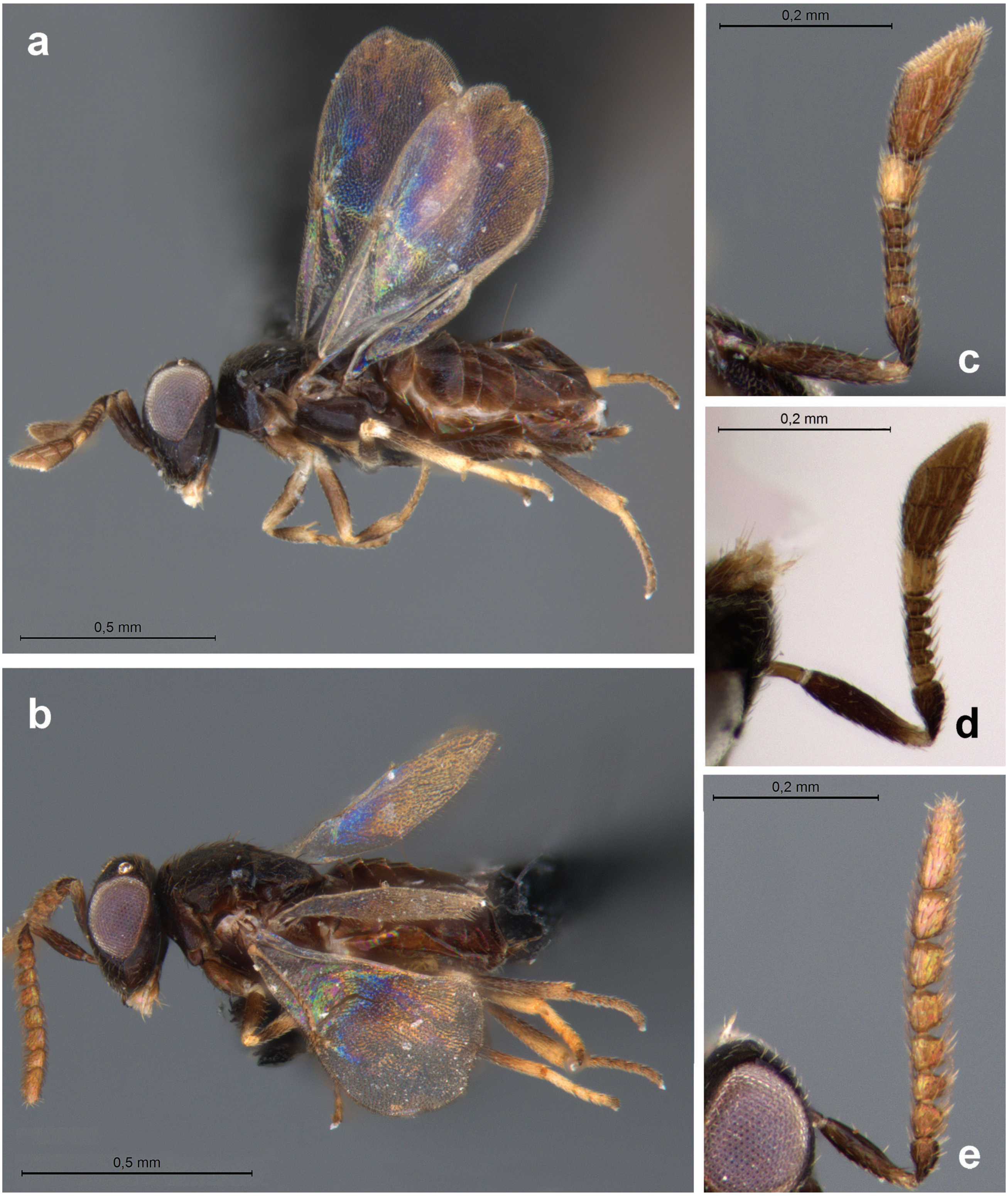

Fig. 1.— *Oobius rudnevi* (Nowicki, 1928) female 2019-01 (a), male 2019-02 (b), female antenna in specimen 2019-01 (c) and 2019-03 (d), and male antenna in specimen 2019-02 (e). (Photo: L. Fusu).

Fig. 1.— *Oobius rudnevi* (Nowicki, 1928) hembra 2019-01 (a), macho 2019-02 (b), antena de la hembra 2019-01 (c) y 2019-03 (d), y antena del macho 2019-02 (e). (Foto: L. Fusu).

for more details), and at each site, 2–3 trees at least 50 m apart were chosen. Disks were placed at a height of 1.40–1.50 m oriented northwards to avoid excessive insolation and to prevent sentinel egg dying from overheating. Two disks 10–15 cm apart were usually arranged in each tree, housing either eggs of *C. cerdo* or *C. welensii*. In total, 37 disks were set in July 2016 coinciding with the flight period of the host species.

Fig. 2.— A cork oak wood disk housing sentinel eggs of *Cerambyx welensii* (Cw) arranged in the field for the detection of *Oobius rudnevi*, in (a) frontal and (b) lateral view (Photo: L.M. Torres-Vila).

Fig. 2.— Disco de madera de alcornoque portando huevos centinela de *Cerambyx welensii* (Cw) dispuesto en el campo para la detección de *Oobius rudnevi*, en vista frontal (a) y lateral (b) (Foto: L.M. Torres-Vila).

Disks were kept 7–9 days in the field and then taken to the laboratory for inspection. After removing the rubber band and cork layer, all eggs from each disk were carefully detached, counted and individually placed in glass tubes (7 cm x 9 mm in diameter) stoppered with cardboard plugs to check for the presence of *O. rudnevi* and eventually assess the parasitism rate. We estimated two parasitism rates (considering or not unviable eggs), as explained in Table 1.

The emerged parasitoids were preserved in ethanol and some were chemically dried using hexamethyldisilazane (HMDS) (Heraty & Hawks, 1998) to prevent

Table 1.— Parasitism rates by *Oobius rudnevi* on sentinel eggs of *Cerambyx cerdo* and *Cerambyx welensii* at three sites in Extremadura (SW Spain) during July 2016.

Tabla 1.— Porcentajes de parasitismo por *Oobius rudnevi* sobre huevos centinela de *Cerambyx cerdo* y *Cerambyx welensii* en tres sitios de Extremadura durante julio de 2016.

						Egg number per disk[5]				Parasitism rates[6,7]	
Wood disk	**Municipality**	**Site[1]**	**Host egg[2]**	**Tree[3]**	**Date[4]**	**P (n)**	**H (n)**	**U (n)**	**Total (n)**	**P_U (%)**	**P_N (%)**
1	Almendral	La Jara	Cc	ho	5-jul	28	2	3	33	85	93
2	Almendral	La Jara	Cw	ho	5-jul	5	1	0	6	83	83
3	Almendral	La Jara	Cc	ho	5-jul	10	2	1	13	77	83
4	Almendral	La Jara	Cw	ho	5-jul	3	6	4	13	23	33
5	Mérida	La Serrana	Cc	ho	5-jul	12	1	7	20	60	92
6	Mérida	La Serrana	Cw	ho	5-jul	11	7	1	19	58	61
7	Mérida	La Serrana	Cc	ho	5-jul	0	28	10	38	0	0
8	Mérida	La Serrana	Cw	ho	5-jul	0	15	7	22	0	0
9	Almendral	La Jara	Cc	ho	12-jul	21	7	7	35	60	75
10	Almendral	La Jara	Cw	ho	12-jul	2	18	1	21	10	10
11	Almendral	La Jara	Cc	ho	12-jul	3	19	3	25	12	14
12	Almendral	La Jara	Cc	ho	12-jul	9	9	2	20	45	50
13	Almendral	La Jara	Cw	ho	12-jul	0	2	1	3	0	0
14	Mérida	La Serrana	Cc	ho	12-jul	2	4	1	7	29	33
15	Mérida	La Serrana	Cw	ho	12-jul	0	12	3	15	0	0
16	Mérida	La Serrana	Cc	ho	12-jul	0	8	2	10	0	0
17	Mérida	La Serrana	Cw	ho	12-jul	0	18	2	20	0	0
18	Mérida	Cornalvo	Cc	co	19-jul	3	11	9	23	13	21
19	Mérida	Cornalvo	Cw	co	19-jul	0	20	10	30	0	0
20	Mérida	Cornalvo	Cc	co	19-jul	2	7	3	12	17	22
21	Mérida	Cornalvo	Cw	co	19-jul	1	22	5	28	4	4
22	Mérida	Cornalvo	Cc	co	19-jul	0	23	7	30	0	0
23	Mérida	Cornalvo	Cw	co	19-jul	0	9	2	11	0	0
24	Mérida	Cornalvo	Cc	co	19-jul	0	19	0	19	0	0
25	Mérida	Cornalvo	Cw	co	19-jul	0	11	2	13	0	0
26	Mérida	Cornalvo	Cc	co	19-jul	1	6	5	12	8	14
27	Mérida	Cornalvo	Cc	co	19-jul	0	10	1	11	0	0
28	Mérida	Cornalvo	Cc	co	19-jul	0	6	0	6	0	0
29	Mérida	Cornalvo	Cc	co	19-jul	0	3	2	5	0	0
30	Mérida	Cornalvo	Cc	co	19-jul	0	20	1	21	0	0
31	Mérida	Cornalvo	Cw	co	19-jul	0	33	11	44	0	0
32	Mérida	Cornalvo	Cc	co	19-jul	0	14	0	14	0	0
33	Mérida	Cornalvo	Cw	co	19-jul	0	18	3	21	0	0
34	Mérida	Cornalvo	Cc	co	19-jul	0	23	8	31	0	0
35	Mérida	Cornalvo	Cw	co	19-jul	0	13	4	17	0	0
36	Mérida	Cornalvo	Cc	co	19-jul	0	2	1	3	0	0
37	Mérida	Cornalvo	Cw	co	19-jul	1	22	6	29	3	4
Total Cc						91	224	73	388	23.5	28.9
Total Cw						23	227	62	312	7.4	9.2
Total Cc + Cw						114	451	135	700	16.3	20.2

[1] Site coordinates (decimal WGS84): La Jara (38.64178, -6.85777), La Serrana (39.0154, -6.63251), Cornalvo Natural Park (38.99887, -6.20154)
[2] Cc: *Cerambyx cerdo*, Cw: *Cerambyx welensii*
[3] ho: holm oak (*Quercus ilex*), co: cork oak (*Quercus suber*)
[4] Wood disks with sentinel eggs were removed 7-9 days after the installation date indicated
[5] P, H and U are the number of parasitized, hatched and unviable eggs per disk, respectively
[6] P_U: uncorrected parasitism rate calculated as $P_U = 100P / (P + H + U)$
[7] P_N: net parasitism rate calculated as $P_N = 100P / (P + H)$

them from collapsing and mounted on black triangular points (black points are better for imaging as they are much less reflective compared to the traditional white ones). Images were taken at the CERNESIM facility of the "Al. I. Cuza" University in Iaşi with a Leica DFC450C camera fitted on a Leica M205-A motorized stereomicroscope, and illuminated with a Leica LED5000 HDI light source. Individual images were then combined using Zerene Stacker (Zerene Systems LLC, http://www.zerenesystems.com/) and retouched using Adobe Photoshop. Imaged specimens were labelled with a unique identification number in the form 2019-NN to ensure their future recognition.

Sentinel eggs parasitized by *O. rudnevi* occurred in all three sampled sites, parasitism being detected both in eggs of *C. cerdo* and *C. welensii* (Table 1). Eggs parasitized by *O. rudnevi* are easily recognisable because, after a few days, they acquire a characteristic light green colour, a result of the combination of the pale yellow colour of the egg chorion and the bright brown colour of the numerous chambers of parasitoids formed inside the host egg. A single parasitized egg produced on average 10.8 ± 0.72 adult wasps (mean ± SE) (range 1–29), often both female and male parasitoids emerging from the same host egg, but with a clearly female-biased sex ratio (as female frequency) of 0.69 ± 0.03 (mean ± SE) (range 0–1). The net parasitism rate (P_N) was more than triple in *C. cerdo* (28.9%) than in *C. welensii* (9.2%), the difference being highly significant (G test, $G_1 = 35.92$, $p < 0.001$) (Table 1). Quite similar results were obtained when uncorrected parasitism rate (P_U) was considered (*C. cerdo*: 23.5%, *C. welensii*: 7.4%, $G_1 = 35.24$, $p < 0.001$) (Table 1). Most parasitoids overwintered inside the host eggs and emerged in the spring of the following year (May 2017), coinciding with the appearance of *Cerambyx* adults in the field. However, in a small fraction of parasitized eggs (6 of 114, 5.26%, all *C. cerdo* eggs) the adults of *O. rudnevi* emerged the same summer (August 2016).

The Spanish specimens of *O. rudnevi* compare well with those from Ukraine (Nowicki, 1928), except that we found the colour of the first funicular segment of female to be variable. It can be similarly light, brownish-yellow as the sixth funicular segment (Fig. 1d), or only slightly lighter than funicular segments two to five (Fig. 1c). Consequently, some *O. rudnevi* specimens might not be correctly identified when using the key provided by Trjapitzin & Volkovitsh (2011).

Natural enemies recorded on *C. cerdo* are rather scarce, and the reported species (including *O. rudnevi*) do not exceed ten (Duffy, 1953; Kenis & Hilszczanski, 2007). The known natural enemies of *C. welensii* are far less numerous: only the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin, 1912 (Morales-Rodríguez *et al.*, 2015) and the tachinid fly *Billaea adelpha* (Loew, 1873) (Torres-Vila & Tschorsnig, 2019) were recently reported.

Our results provide the first records of *O. rudnevi* in Iberia (and southern Europe) and suggest that the species is widespread and relatively frequent in the region of Extremadura (SW Spain), as we found the parasitoid in all three sampled sites. We also show a new host-parasitoid association as *O. rudnevi* was able to successfully parasitize *C. welensii* eggs in the wild. This finding indicates that *O. rudnevi* is not a specific parasitoid of C. *cerdo* as previously thought, albeit parasitic prevalence was significantly higher in *C. cerdo* than in *C. welensii*. Parasitism rates averaged about 10–30% (depending on host) and sometimes even reached very high levels (>90%). Rudnev (1936) reported that parasitism rates above 35–50% are not frequent in Ukraine. We also found that *O. rudnevi* populations occurred in both holm oak (*Quercus ilex* L.) and cork oak (*Q. suber* L.) forests. The emergence pattern of the adult parasitoids indicates that *O. rudnevi* undergoes obligate diapause and thereby has a univoltine life cycle, even if a small fraction of the population exhibited facultative diapause, playing the strategy of being bivoltine. Such a strategy seems at first sight risky when hosts are also univoltine, especially in often unpredictable habitats such as those in the Mediterranean region: if bivoltine individuals develop slowly and second-flight appears too late in the season, they could die without producing offspring as there would be no *Cerambyx* eggs available to be parasitized. In any case, the mere presence of some bivoltine individuals in the wild indicates by itself that this strategy fits. The genetic and/or environmental factors that determine the diapause in *O. rudnevi* are unknown. Our results permit to hypothesize that additional studies will show that *O. rudnevi* is widespread throughout the western Palaearctic, adjusting its distribution range to the presence of its hosts.

Finally, *O. rudnevi* represents the third species of the genus *Oobius* recorded in Iberia after *O. pinicola* (García-Mercet, 1921) and *O. longoi*. The first, *O. pinicola*, is a species native to Spain, originally described as *Coccidencyrtus pinicola* García-Mercet, 1921, which has been recently transferred to the genus *Oobius* (Trjapitzin & Volkovitsh, 2011). Just one female is known, collected in Madrid on pine trees (*Pinus halepensis* Mill.), its host and biology being unknown (García-Mercet, 1921). The second, *O. longoi*, is a foreign species native to Australia that was introduced in Spain (possibly unintentionally from Portugal) for the biological control of *P. semipunctata* (Mansilla-Vázquez *et al.,* 1999). New studies are currently conducted in the field and in the laboratory to explore the biology, ecology, behaviour and parasitic potential of *O. rudnevi* in Mediterranean oak open woodlands.

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