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Integrative taxonomy confirms two new West-Palaeartic species allied with *Chrysotoxum vernale* Loew, 1841 (Diptera: Syrphidae)

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Abstract

The taxonomy of the syrphid genus *Chrysotoxum* Meigen, 1803 (Diptera: Syrphidae), is complex and currently under scrutiny. Two new species allied with *Chrysotoxum vernale*, one from the Western Mediterranean, *Chrysotoxum hispanicum* sp. n. and the other from the Eastern Mediterranean, *Chrysotoxum anatolicum* sp. n., are described and illustrated. *Chrysotoxum hispanicum* sp. n. is distinguished from the similar *C. vernale* Loew by the size of the yellow abdominal fasciae and shape of surstyli. *Chrysotoxum anatolicum* sp. n., known only from females, possesses an almost entirely yellow-pigmented wing, unusual amongst the other studied species of the *C. vernale* group. Additionally, *C. hispanicum* sp. n. and *C. anatolicum* sp. n. are separated from each other, as well as from other species of the *C. vernale* group by COI and ITS2 gene markers. An identification key to the West Palaeartic species of the *C. vernale* group is provided.

Keywords Adult morphology · COI · ITS2 · *Chrysotoxum hispanicum* sp. n. · *Chrysotoxum anatolicum* sp. n. · Identification key

Introduction

Within the family Syrphidae (Diptera), the subfamily Syrphinae (syrphines) consists of four tribes and 57 genera, 42 of which belong to the widespread tribe Syrphini (Mengual et al. 2008). *Chrysotoxum* Meigen, 1803, is one

of the most distinctive Syrphini genera, with adults appearing as Batesian mimics of social wasps due to their long antennae, black and yellow bodies and oval to elongate, convex abdomens (Van Veen 2004). Larval biology is poorly known, but some species have been recorded in association with ant-attended root aphids (Aphididae)

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(Rotheray 1993). According to molecular phylogenetic work, *Chrysotoxum* is near *Epistrophe* Walker, 1852; *Epistrophella* Dušek et Láska, 1967; and *Xanthogramma* Schiner, 1861 (Mengual et al. 2008).

Chrysotoxum is widespread over all biogeographic regions except for Australasia and Antarctica, but is most speciose in the Palaearctic region with 85 described species (Thompson and Rotheray 1998; Mutin and Barkalov 1999; Huo and Zheng 2004; Huo and Ren 2007; Huo et al. 2006, 2007; Nedeljković et al. 2013, 2015, 2018a; Vujić et al. 2017). In the Western Palaearctic, i.e. excluding China and the Russian Far East (Lattin 1967; Van Steenis and Lucas 2011), there are 78 species, 13 of which are present in the Iberian Peninsula (Van Eck 2011; Ricarte and Marcos-García 2017) and 18 in Turkey, including the Anatolian Peninsula (Sarıbiyık 2014; Vujić et al. 2017; Nedeljković et al. 2018a). Iberian species of *Chrysotoxum* have not been revised yet, while those of the Balkan and Anatolian peninsulas have experienced scrutiny in recent years, with seven new species erected (Nedeljković et al. 2013, 2015, 2018a; Vujić et al. 2017).

Although identification of *Chrysotoxum* is usually straightforward at the genus level, separation of some species has proven difficult and often dependent on subtle morphological characters and molecular, morphometric or even ecological analyses (e.g. Nedeljković et al. 2013, 2015). In fact, some individual species have been split into more than one taxon (a species complex) as a product of an integrative study of their taxonomy. This is the case, for example, of *Chrysotoxum vernale* Loew, 1841, and the similar *Chrysotoxum montanum* Nedeljković et Vujić in Nedeljković et al. (2015) described from the Balkans.

The species *C. vernale* was regarded as part of the species group of *Chrysotoxum festivum* (Linnaeus, 1758) by Masetti et al. (2006). However, Nedeljković et al. (2015) and Vujić et al. (2017) started developing an implicit morphological concept of the *C. vernale* group for the species sharing a basoflagellomere shorter than scape and pedicel combined, pro- and mesofemora black basally and abdominal terga with yellow fasciae not reaching the lateral margins. Nonetheless, the bases of the pro- and mesofemora are sometimes dark yellow or with brown markings in *C. orthostylum* Vujić in Nedeljković et al. (2015).

Using both morphological and molecular characters, we examined Spanish and Turkish material of the *C. vernale* species group to determine its taxonomic status. Descriptions are provided for two new species, as well as a key to the Western Palaearctic species allied with *C. vernale*. The aim of this paper is to provide further steps in resolving the systematics of the genus *Chrysotoxum* by diagnosing and differentiating new taxa of the *C. vernale* group from the Western Palaearctic.

Material and methods

Examined material

A total of 52 specimens belonging to two potentially-new species were examined. These putative new species were compared with other species similar to *C. vernale* (Nedeljković et al. 2015; Vujić et al. 2017). The examined material is deposited in the following collections: Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad (FSUNS); CIBIO Institute, University of Alicante, Spain (CEUA); Dieter Doczkal's private collection, Germany (DD). Acronyms of the repository collections are presented between square brackets (‘[]’) after each series of specimens. Square brackets are also used when some additional information is provided, apart from that shown in the printed label. Abbreviations of the Spanish provinces, given between brackets in the examined material list, are as follows: CC, Cáceres; CR, Ciudad Real; GR, Granada; LE, León; O, Asturias; S, Santander; SA, Salamanca; V, Valencia. For location of the Spanish provinces, see map in Ricarte and Marcos-García (2017). In Cabañeros national park (Spain), specimens were collected with permits under the agreement to undertake research in the 040/2002 project (‘Ministerio de Medio Ambiente’, Spain). In Sierra Nevada national and natural parks collecting permits were issued by the ‘Junta de Andalucía, Consejería de Medio Ambiente y Ordenación del Territorio’ [Ref. number: ENSN/FJSG/IMJ (232)]. The fieldwork did not involve red-listed species, neither species protected in legislation.

Morphological study

To describe and diagnose species, characters were examined using a Nikon SMZ 745T and a Leica MZ12 binocular microscopes. Colour characters always refer to dry specimens. Body size was measured as the length (‘L’) from the tip of the frontal prominence (excluding antennae) to the tip of the abdomen. The proportional length of the antennal segments is given as a ratio (‘r’) of x/y/z (‘x’, scape; ‘y’, pedicel; ‘z’, basoflagellomere). The length of each antennal segment was measured along its dorsal edge.

Male genitalia were removed from relaxed specimens and stored in microvials containing glycerol, after clearing in warm 10% KOH for 3–5 min and then washing in acetic acid, followed by ethanol, to neutralize KOH. Species were illustrated with photos produced as stacks of individual images made with a camera (Leica DFC 450) attached to a binocular stereomicroscope (Leica M205 C). Stacks and measurements were made with Leica Application Suite X (LAS X)®, v. 3.0.4.16529. Morphological terms follow Thompson (1999), except for the term ‘proepimeron’, which follows Speight and Sarthou (2017). ‘Tergum’ is abbreviated as ‘T’ and ‘sternum’ as ‘S’ in the species descriptions and diagnoses.

New species were described based on the holotypes to warrant the stability of our species concepts by providing an unequivocal correlation between the descriptions and the name-bearing types (holotypes). The intraspecific variability found within the type series of each new species is recorded and included in different ways in the diagnoses, identification key and discussion.

Molecular study

In addition to the morphological character description, we performed molecular analyses using sequences from the 3'COI region and the ITS2 region. We used the same matrix of concatenated COI and ITS2 sequences as in Nedeljković et al. (2018a) upgraded with the additional sequences of *C. vernale* group specimens. For detailed information on analysed specimens, see Table 1.

Protocols for DNA extraction, PCR amplification and sequencing of 3'COI and ITS2 regions are described in Nedeljković et al. (2018b). The newly-produced DNA sequences were edited for base-calling errors using BioEdit version 7.2.5. (Hall 1999). The rest of the sequences were downloaded from GenBank. COI sequences were aligned manually, whereas ITS2 sequences were aligned using the E-INS-I strategy as implemented in MAFFT v. 7 (Kato and Standley 2013). Maximum-parsimony (MP) and maximum-likelihood (ML) phylogenetic trees were constructed for COI sequence matrix and for combined matrix of COI and ITS2 sequences. Parsimony analysis was performed in NONA (Goloboff 1999), spawned with the aid of ASADO (Nixon 2008) using the heuristic search algorithm with 1000 random addition replicates (mult_1000), holding 100 trees per round (hold/100), max trees set to 100,000 and applying tree-bisection–reconnection branch swapping. The ML tree was constructed using RAxML 8.2.8 (Stamatakis 2014) using the CIPRES Science Gateway web portal (Miller et al. 2010) under the general time-reversible (GTR) evolutionary model with a gamma distribution (GTRGAMMA) (Rodríguez et al. 1990). The bootstrap support values for clades were calculated with 1000 replicates for both MP and ML trees. The uncorrected pairwise distance (p) matrix for COI gene sequences and a number of variable positions were estimated using MEGA 7 software (Kumar et al. 2016). The trees were rooted on *Melanostoma mellinum* (Linnaeus, 1758) (GenBank accession numbers: KJ848101 for COI, KJ848059 for ITS2).

Results

New species of the *Chrysotoxum vernale* group

The two new species described here belong to the *C. vernale* species group as defined in the introduction.

Chrysotoxum anatolicum Nedeljković et Vujčić, sp. n. (Figs. 1a, 2, 4a, 5a)

Examined material. Holotype: 1♀, Turkey, İzmir, Bozdağ, Ski Centre 1, [38.332626 N 28.108250 E], 24 June 2016, leg. Vujčić, Ačanski & Uzal [FSUNS].

Paratype: 1♀, Turkey, İzmir, Bozdağ, Ski Center 1, [38.332626 N 28.108250 E], 25 June 2016, leg. Vujčić, Ačanski & Uzal [FSUNS].

Additional material of other species. Specimens of *C. vernale* and *C. montanum*, including types, published in Nedeljković et al. (2015).

Type locality. İzmir, Bozdağ (Turkey).

Diagnosis. $L=14-15$ mm ($n = 2$). Gena dark brown to black; $r = 1:0.66:1.42$ ($n = 2$); basoflagellomere 3.28 times longer than wide ($n = 2$); pro- and mesofemora black in their basal thirds, metafemur yellow; wing extensively but unevenly pigmented, darker towards the anterior margin and apex: cells R_1 and R_{2+3} dark yellow pigmented basally and dark brown to black pigmented apically, cells R_{4+5} and DM dark brown to black pigmented, cell CuA_1 dark brown pigmented, cells R and BM dark yellow pigmented, cell CuP yellow pigmented (Fig. 4a).

Remarks. The other species of the *C. vernale* group, including *C. hispanicum* sp. n., have at least posterior half of wing without pigmentation. *Chrysotoxum anatolicum* sp. n. can also be separated from the similar *C. vernale* by the shape of the frontal pollinose maculae, bar-shaped in *C. anatolicum* sp. n. (Fig. 1a) but triangular in *C. vernale* [Figure 5D in Vujčić et al. (2017)], and colour of the posterior margin of T3, wholly black in *C. anatolicum* sp. n. but at least with a small central yellow macula in *C. vernale*.

Description. Female. $L = 14$ mm.

Head (Figs. 1a, 2). Eye with sparse, straight, yellow pile; frons shiny black, with short, black pile and with two separate, bar-shaped, white pollinose maculae (Fig. 1a); ocellar triangle equilateral, shiny black with short black pile; occiput shiny black behind the ocellar triangle and white pollinose in other parts, narrowly yellow ventrally, with yellow pile intermixed with black pile dorsally; antenna black; scape and pedicel with black pile; $r = 1:0.62:1.45$; arista bare, dark brown; face yellow, with yellow pile; face with a medial black vitta extending from antennal base to mouth edge; facial vitta as wide as a third of the facial width; face black postero-ventrally; gena yellow pilose, entirely shiny black (Fig. 2).

Thorax. Scutum black, with two medial, silvery, pollinose vittae extending for a third of the scutum length from its anterior margin, with short black pile; pleuron shiny black, except yellow maculae on posterior anepisternum and katatergum; katapisternum wholly black; scutellum yellow, except black on lateral corners and a medial macula; scutellum with short black pile; legs yellow except black on basal thirds of pro- and mesofemora; metafemur yellow; wing (Fig. 4a)

Table 1 *Chrysotoxum* specimens genetically analysed, all deposited at FSUNS

DNA ID	3'COI GenBank accession number	ITS2 GenBank accession number	Species	Sex	Locality	Date	Leg.
AU1254	MH290053	MH277392	<i>C. bozdagensis</i>	♂	Turkey, Bozdağ, Ski Centre 1	25/06/2016	Vujić A., Ačanski J. & Uzal A.
AU1255	MH290054	MH277393	<i>C. bozdagensis</i>	♀	Turkey, Bozdağ, Ski Centre 1	24/06/2017	Vujić A., Ačanski J. & Uzal A.
C27	MH290041	KF408327	<i>C. festivum</i>	♂	Serbia, Fruška Gora, above 300 m	10/05/2011	Vujić A.
C19	MH290040	KF408304	<i>C. festivum</i>	♀	Serbia, Stara planina, Babin zub	09/07/2011	Vujić A.
C29	MH290042	KF408307	<i>C. festivum</i>	♂	Serbia, Fruška Gora, above 300 m	10/05/2011	Vujić A.
C35	MH290043	KF408308	<i>C. festivum</i>	♀	Serbia, Fruška Gora, above 300 m	10/05/2011	Vujić A.
C40	MH290044	KF408311	<i>C. elegans</i>	♂	Serbia, Dubašnica, Demizlok	08/06/2011	Vujić A.
C43	MH290045	KF408313	<i>C. elegans</i>	♂	Serbia, Dubašnica, Demizlok	08/06/2011	Vujić A.
C44	MH290046	KF408314	<i>C. elegans</i>	♀	Montenegro, Prokletije, Ropojanska dolina	Jul-2011	Vujić A.
C47	MH290047	KF408317	<i>C. elegans</i>	♀	Serbia, Kopaonik, Samokovska reka	02/08/2010	Vujić A.
C51	MH290048	KF408320	<i>C. elegans</i>	♀	Serbia, Đerdap	01/09/2011	Vujić A.
C118	MH290051	KF408339	<i>C. tomentosum</i>	♂	Serbia, Kopaonik, Kukavica	14/07/2012	Vujić A. & Radenković S.
C119	MH290052	KF408340	<i>C. tomentosum</i>	♂	Serbia, Kopaonik, Marine vode	16/07/2012	Vujić A. & Radenković S.
C22	MH290049	KF408306	<i>C. tomentosum</i>	♂	Serbia, Kopaonik, Metode	03/08/2010	Vujić A.
C26	MH290050	KF408326	<i>C. tomentosum</i>	♀	Montenegro, Prokletije, Ropojanska dolina	July-2011	Vujić A.
AU1252	MW159863	MW161162	<i>C. orthostylum</i>	♂	Turkey, Isparta	July-2015	Vujić A.
C104	KR019026	MH277388	<i>C. montanum</i>	♂	Serbia, Kopaonik, Klisura Samokovske reke	16/06/2012	Vujić A.
C105	KR019027	MH277389	<i>C. montanum</i>	♂	Serbia, Kopaonik, Klisura Samokovske reke	16/06/2012	Vujić A.
C13	KR019022	MH277386	<i>C. vernale</i>	♂	Greece, Olimp, Litochoras, Prionia	18/05/2011	Šimić S.
C3	KR019014	MH277387	<i>C. vernale</i>	♀	Serbia, Malinik, Manastirište	30/04/2011	Vujić A.
C12	KR019021	MH277384	<i>C. vernale</i>	♀	Greece, Olimp, Litochoras, Prionia	18/05/2011	Vujić A.
C130	KR019037	MH277385	<i>C. vernale</i>	♂	Serbia, Đerdap, Ciganski potok	22/04/2012	Vujić A.
MS155	MW052731	MW051571	<i>C. vernale</i>	♂	Greece, Chelmos, Kalavryta ski center	06/06/2017	Vujić A.
MS156	MW052732	MW051572	<i>C. vernale</i>	♂	Greece, Chelmos, Kalavryta ski center	06/06/2017	Vujić A.
MS157	MW052733	MW051573	<i>C. vernale</i>	♂	Greece, Chelmos, Kalavryta ski center	08/06/2017	Vujić A.
MS168	MW052734	MW051574	<i>C. vernale</i>	♀	Serbia, Vlasina, Delnice	12/07/2017	Tot T. & Vujić M.
MS169	MW052735	MW051575	<i>C. vernale</i>	♂	Serbia, Zlot prema Maliniku	24/07/2017	Miličić M. & Ivošević B.
MS170	MW052726	MW051566	<i>C. hispanicum</i> sp. n.	♂	Spain, Sierra Nevada, first valley	17/06/2014	Vujić A.
MS171	MW052727	MW051567	<i>C. hispanicum</i> sp. n.	♀	Spain, Ski Centar Sierra Nevada	16/06/2014	Vujić A.
MS172	MW052728	MW051568	<i>C. hispanicum</i> sp. n.	♀	Spain, Ski Centar Sierra Nevada	16/06/2014	Vujić A.
AU1256	MW052729	MW051569	<i>C. anatolicum</i> sp. n.	♀	Turkey, Bozdağ, Ski Center 1	24/06/2016	Vujić A., Ačanski J. & Uzal A.
AU1257	MW052733	MW051570	<i>C. anatolicum</i> sp. n.	♀	Turkey, Bozdağ, Ski Center 1	24/06/2016	Vujić A., Ačanski J. & Uzal A.

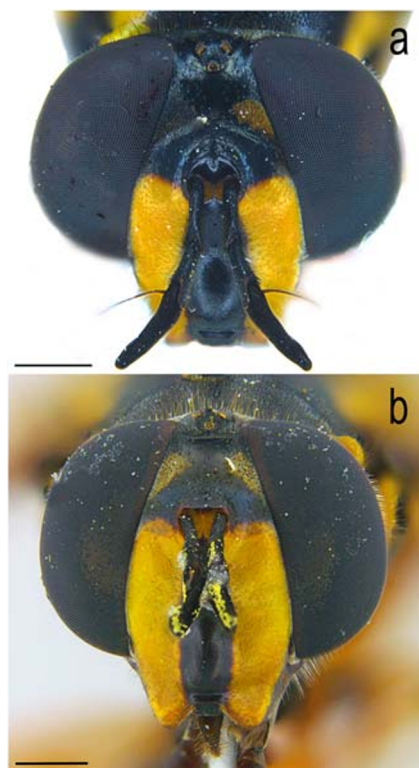


Fig. 1 Female heads of *Chrysotoxum* spp., frontal view. **a** *C. anatolicum* sp. n., İzmir, Turkey. **b** *C. hispanicum* sp. n., Salamanca, Spain. Scale lines: 0.75 mm

membrane extensively microtrichose; wing cells R_1 and R_{2+3} dark yellow pigmented basally and dark brown to black pigmented apically; cells R_{4+5} and DM dark brown to black pigmented; cell CuA_1 dark brown pigmented; cells R and BM dark yellow pigmented; CuP yellow pigmented; calypter yellow with yellow pile at edge; halter dark brown basally, the remainder yellow, with lighter capitulum.

Abdomen (Fig. 5a). Shiny black, with short black pile, except for T1 and anterior part of T2 having long yellow and black pile intermixed; all terga with two separate, elongate, yellow maculae not reaching the lateral margins; yellow



Fig. 2 Head of *C. anatolicum* sp. n., female, latero-ventral view. Scale line: 0.75 mm.

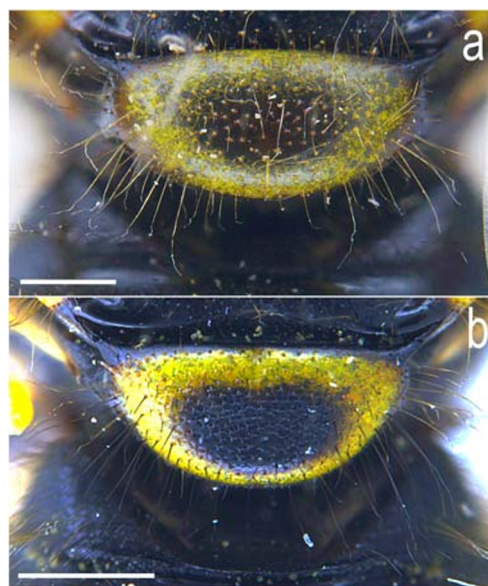


Fig. 3 Scutellums of *Chrysotoxum* spp., dorsal view. **a** *C. hispanicum* sp. n., holotype, male, Granada, Spain. **b** *C. vernale*, male, Dubašnica, Serbia. Scale lines: 0.5 mm (a), 0.75 mm (b).

maculae bearing yellow pile; all sterna shiny black, with short yellow pile anteriorly and black pile posteriorly; S3 with two interconnected yellow maculae, not reaching the lateral margins; S4 with two yellow maculae not reaching the lateral margins; all sternal markings yellow pilose.

Male. Unknown.

Etymology. The specific epithet ‘anatolicum’ refers to the geographic origin of the specimens of the type series, a locality in the Anatolian Peninsula.

Habitats. Specimens were collected in a Mediterranean mountain steppe with *Astragalus tmoleus* var. *tmoleus* and *Genista lydia* var. *lydia*, between 1800 and 1900 m asl. This montane habitat is found at altitudes above forests of *Pinus brutia* and *P. nigra pallasiana* and below subalpine-vegetation habitats (i.e. communities of *Sideritis taurica*, *Euphorbia anacamperos* var. *tmolea*, *Minuartia juressi*, *Campanula teucrioides*).



Fig. 4 Wings of *Chrysotoxum* spp. **a** *C. anatolicum* sp. n., female, İzmir, Turkey. **b** *C. vernale*, male (similar wing pigmentation as that found usually in *C. hispanicum* sp. n.), Bezdán, Serbia. Scale line: 1 mm.

Fig. 5 Abdomens of *Chrysotoxum* spp., dorsal view. **a** *C. anatolicum* sp. n., female, İzmir, Turkey. **b** *C. hispanicum* sp. n., holotype, male, Granada, Spain. **c** *C. hispanicum* sp. n., paratype, female, Salamanca, Spain. **d** *C. vernale*, male, Dubašnica, Serbia. Scale line: 1mm.



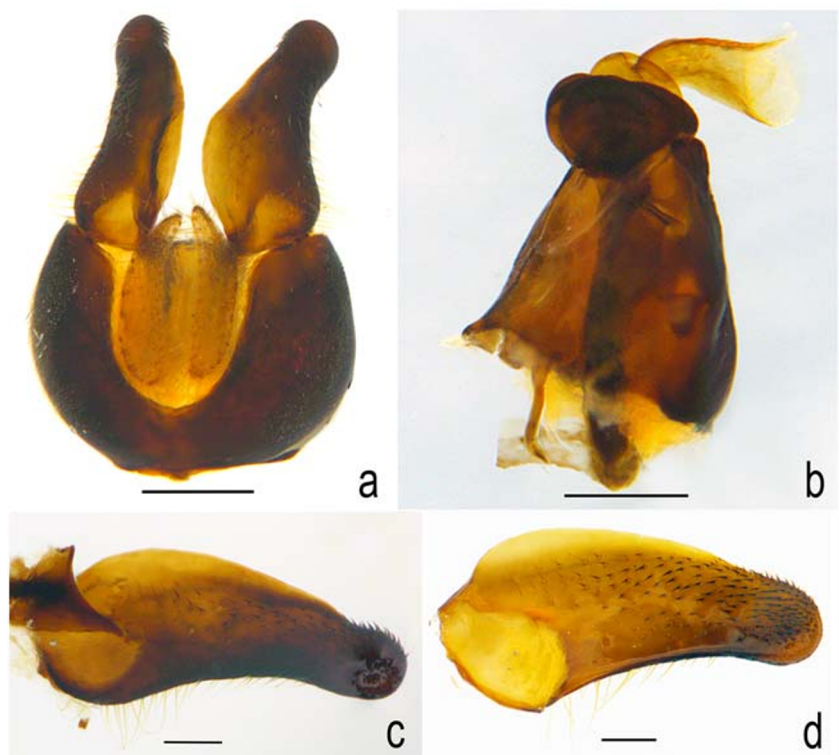
***Chrysotoxum hispanicum* Nedeljković, Ricarte et Marcos-García, sp. n. (Figs. 1b, 3a, 5b-c, 6a-c)**

Examined material. Holotype: 1♂, Spain, Sierra Nevada (GR), first valley [‘Barranco de las Víboras’, above ‘Cabaña Sierra Nevada’], 17 June 2014 [37°07'40"N 3°26'44"W, 1626m] leg. Vujić [FSUNS].

Paratypes: 3♂♂, Andalucía, Sierra de Gador, Barjalí (AL), [2060 m], 10 June 2003 [36°53'51"N 2°48'04"W], leg. D. Doczkal [S11374, S11375, S11376]; 4♀♀, Andalucía, Sierra de Gador (AL), Barjalí, 2060m, 10 June 2003 [36°53'51"N 2°48'04"W], leg. D. Doczkal [S11377, S11378, S11379, S11380] [DD]; 1♀, Sierra Nevada (GR), first valley [‘Barranco de las Víboras’, above ‘Cabaña Sierra Nevada’], 17 June 2014, 37°07'40"N 3°26'44"W, 1626 m,

leg. Vujić (07329); 2♀♀, Ski Centre, Sierra Nevada (GR), 16 June 2014 [37°06'45"N 3°25'10"W], 2191 m, leg. Vujić [MS171, MS172] [FSUNS]; 6♂♂, 2♀♀, Chelva (V), 9-24 June 1994 (5♂♂, 1♀), 22-27 Mar. 1994 (1♂), 25 April-9 May 1994 (1♀), leg. Pérez Bañón; 1♀, Utiel (V), 9 May 1994, leg. Pérez Bañón; 1♂, Requena (V), 7 Sept. 1994, leg. Pérez Bañón; 1♀, Valverde del Fresno (CC), 950 m, 19 June 1981, leg. M.A. Marcos García; 1♂, Puerto El Torno (CC), 950m, 4 May 1982, leg. M.A. Marcos García; 1♀, Hoyos (CC), 19 June 1981, leg. M.A. Marcos García; 1♀, La Garganta (CC), 1000m, 27 June 1980, leg. M.A. Marcos García; 1♀, P.N. de Cabañeros (CR), Pal [Valle de Santiago], 14 May 2004, leg. A. Ricarte; 1♀, P.N. de Cabañeros (CR), maJ1 [Melojar de Viñuelas], 14 April-8 May 2004, leg. A. Ricarte; 1♀, La Alberca (SA), 17

Fig. 6 Male genitalia of *Chrysotoxum* spp., specimens from Spain. **a** *C. hispanicum* sp. n., epandrium, dorsal view (shape of each surstylus differs according to a slight view inclination). **b** *C. hispanicum* sp. n., hypandrium, lateral view. **c** *C. hispanicum* sp. n., right surstylus mounted in a slide. **d** *C. vernale*, right surstylus mounted in a slide. Scale lines: 250 µm (a and b), 100 µm (c and d).



June 1980, leg. M.A. Marcos García; 1♀, Ledesma (SA), 26 June 1977, leg. M.A. Marcos García; 2♀♀, Peñacaballera (SA), 18 May 1980, leg. M.A. Marcos García; 1♀, Los Llanos, Bejar (SA), 30 May 1980, leg. M.A. Marcos García; 1♀, Monterrubio de la Armuña (SA), 11 June 1978, leg. M.A. Marcos García; 1♂, Salamanca, 22 June 1984, leg. P. Calzada; 1♂, Navasfrías, Monte Mezas (SA), 19 June 1980, leg. M.A. Marcos García; 1♀, Negrilla de la Palencia (SA), 11 June 1978, leg. M.A. Marcos García; 2♂♂, Puerto Perales, El Payo (SA), 860 m, 24 May 1980, leg. M.A. Marcos García; 1♀, Escorial de la Sierra (SA), 15 May 1980, leg. M.A. Marcos García; 1♂, Salamanca (SA), 7 May 1978, leg. M.A. Marcos García; 1♀, Béjar (SA), 5 June 1977, leg. M.A. Marcos García; Puerto de Vallejera (SA), 26 June 1980, leg. M.A. Marcos García [CEUA]; 1♀, Rinconada de la Sierra (SA), 17 June 1980, leg. M.A. Marcos García; 2♀♀, El Cabaco (SA), 29 June 1980, leg. M.A. Marcos García; 1♀, Pino de Tormes (SA), 19 June 1979, leg. M.A. Marcos García; 1♀, Puerto Magdalena (LE), 1434 m, 13 June 1986, leg. M.A. Marcos García; 1♀, Pereda de Ancares (LE), 12 June 1986, leg. M.A. Marcos García; 1♀, Geras (LE), 3 June 1987, leg. M.A. Marcos García; 1♀, Murias de Paredes (LE), 13 June 1986, leg. M.A. Marcos García; 1♀, Valdeteja (LE), 17 July 1977, leg. M.A. Marcos García.

Additional examined material of other species. Specimens of *C. vernale* and *C. montanum*, including types, published in Nedeljković et al. (2015). Spanish specimens morphologically fitting to the concept of *C. vernale* sensu Nedeljković et al. (2015): 1♀, Plan d'Están, Valle Benasque (HU), 1850m, 26 July 1983, leg. M.A. Marcos García; 1♂, 1♀, Plan de Sarrá, Valle de Benasque (HU), 1750m, 26 July 1983, leg. M.A. Marcos García; 1♂, 1♀, Sotres (S), 5 June 1988, leg. M.A. Marcos García; 1♂, Villar de Santiago (LE), 1 June 1988, leg. M.A. Marcos García; 1♀, Tielve (O), 5 June 1988, leg. M.A. Marcos García; 1♀, Hervás (CC), 17 May 1980, leg. M.A. Marcos García [CEUA].

Type locality. Sierra Nevada, Granada (Spain).

Diagnosis. L = 9–12 mm ($n = 6$). T2 yellow fascia about a third as wide as the width of the tergum; T3–T5 yellow fasciae about as half as wide as the width of the tergum (Figs. 5b–c); the long scutellum pile all yellow (Fig. 3a); gena at least partly yellow; $r = 1:0.85:1.68$ ($n = 12$); basoflagellomere 3.16 times longer than wide ($n = 12$).

Remarks. Abdomen with yellow maculae on terga much wider than those in *C. montanum* and *C. vernale* (Fig. 5d); the longest scutellum pile all yellow (sometimes just 1–2 long black pile present) (Fig. 3a), unlike *C. vernale* (Fig. 3b) and *C. montanum* which have all or most of the longest scutellum pile black; male genitalia in dorsal view, with surstylus apex more slender (Fig. 6c) than that in *C. vernale* (Fig. 6d).

Description. Male. L = 9 mm.

Head. Eye pile straight and yellow; vertical triangle black, with long, yellow pile (sometimes black pile also present anteriorly), white pollinose only in the posterior part of the triangle; frontal triangle about twice as long as region of eye contiguity, shiny black, with eye margins narrowly white pollinose from eye contiguity to antennae; frontal triangle with black pile anteriorly and mostly yellow pile posteriorly; antenna black, scape and pedicel with black pile, basoflagellomere 3.8 times longer than wide elongate and with rounded apex; arista bare and dark brown; $r = 1:0.66:1.43$; face yellow pilose, yellow with a medial black vitta extending from the antennal bases to the mouth edge; facial black vitta as wide as a fourth of the facial width; mouth edge wholly but narrowly black; gena yellow, also with yellow pile; occiput white pollinose, with long yellow and black pile intermixed dorsally.

Thorax. Scutum black with two medial, silvery pollinose vittae extending from anterior margin for two thirds of the scutum length; scutum with intermixed long and short, yellow pile anteriorly, and with long, yellow and short, black pile posteriorly; pleuron shiny black except with yellow maculae on posterior anepisternum and katatergum; scutellum yellow, except for black lateral corners and a small medial macula, with long yellow pile (Fig. 3a); legs mainly yellow except for the black basal thirds of pro- and mesofemora; metafemora entirely yellow; wing membrane extensively microtrichose; wing cell R_1 brown pigmented apically; cell R_{2+3} brown pigmented anteriorly; calypter yellow, with yellow pile at edge; halter yellow, with pedicel darker than capitulum.

Abdomen (Fig. 5b). Shiny black, with short black pile, except for the yellow pile on T1, anterior part of T2 and lateral parts of T3–T5; all terga with a yellow fascia interrupted in the middle; T2 yellow fascia as wide as a third of the tergum width; each T3–T5 yellow fasciae nearly as wide as half of the tergum width; yellow fasciae on terga with yellow pile; posterior margin of T2 black; posterior margin of T3 with a medial, narrow, yellow macula; posterior margin of T4 with a narrow, yellow fascia; T5 with a semicircular macula on the posterior margin; all sterna shiny black, S1 and anterior part of S2 with long yellow pile; posterior part of S2 and S3–S5 with adpressed black pile; S3 with two interconnected, yellow maculae not reaching the lateral margins; S4 with two yellow maculae not reaching the lateral margins. Genitalia as in Figs. 6a–c.

Female (Fig. 5c). Same as the male except for the frons having two separate triangular pollinose maculae on eye margins (Fig. 1b) and the scutum bearing short black pile.

Etymology. The specific epithet 'hispanicum' derives from Hispania, the Latin name for Spain, referring to the type locality of this species. Hispania was a Roman Empire province that also included the current Portugal.

Distribution. Only confirmed from Spain, where it is apparently widespread (provinces: AL, CC, CR, GR, LE, SA, V). It is found mainly in highlands, at altitudes from 500 to 2190 m asl.

Habitats. This species is found in a broad range of habitats, including mixed woodlands of *Quercus faginea*, *Q. pyrenaica* and *F. angustifolia*, and woodlands of *Q. pyrenaica*, both with temporary brooks and small grassy clearings (Cabañeros national park); ‘dehesas’ of *Q. rotundifolia*; croplands next to oak forests (*Q. rotundifolia*). The holotype was collected in a meadow with a stream in the forests of ‘Barranco de las Viboras’, Sierra Nevada.

Molecular analysis of the species allied with *Chrysotoxum vernale*

We analysed 33 COI gene sequences (including the outgroup) of 662 bp in length. The ingroup sequences have 40 variable and 27 parsimony informative positions. The p-distances ratios range from 0.9 to 3%, when comparing COI sequences between different species (Table S1). The topologies of COI-based ML and MP trees do not support the division between

the *C. festivum* and the *C. vernale* species groups (*C. festivum* group understood as a set of species with basoflagellomere shorter than scape and pedicel combined and pro- and mesofemora wholly yellow; *C. vernale* group sensu Introduction). Additionally, *C. tomentosum* Giglio-Tos, 1890, *C. festivum* and *C. elegans* Loew, 1841 are not resolved as separate. *Chrysotoxum hispanicum* sp. n. forms a nested clade with high bootstrap support (99/98) within the *C. vernale* clade, while the position of *C. anatolicum* sp. n. is questionable (Fig. 7a, b).

For the analyses on combined COI+ITS2 sequences, we produced a matrix of 1071 bp. The ingroup sequences have 73 variable positions from which 60 are parsimony informative. ML analysis based on COI+ITS2 resolved the *C. vernale* species group (*C. vernale*, *C. montanum*, *C. orthostylum*, *C. hispanicum* sp. n. and *C. anatolicum* sp. n.) as monophyletic with moderate bootstrap support (82) (Fig. 8a). The new species within *C. vernale* group, *C. anatolicum* sp. n. and *C. hispanicum* sp. n., are also resolved as monophyletic with medium to high clade bootstrap support (87 and 100). *Chrysotoxum hispanicum* sp. n. clade is nested within *C. vernale* clade but yet clearly divergent from it.

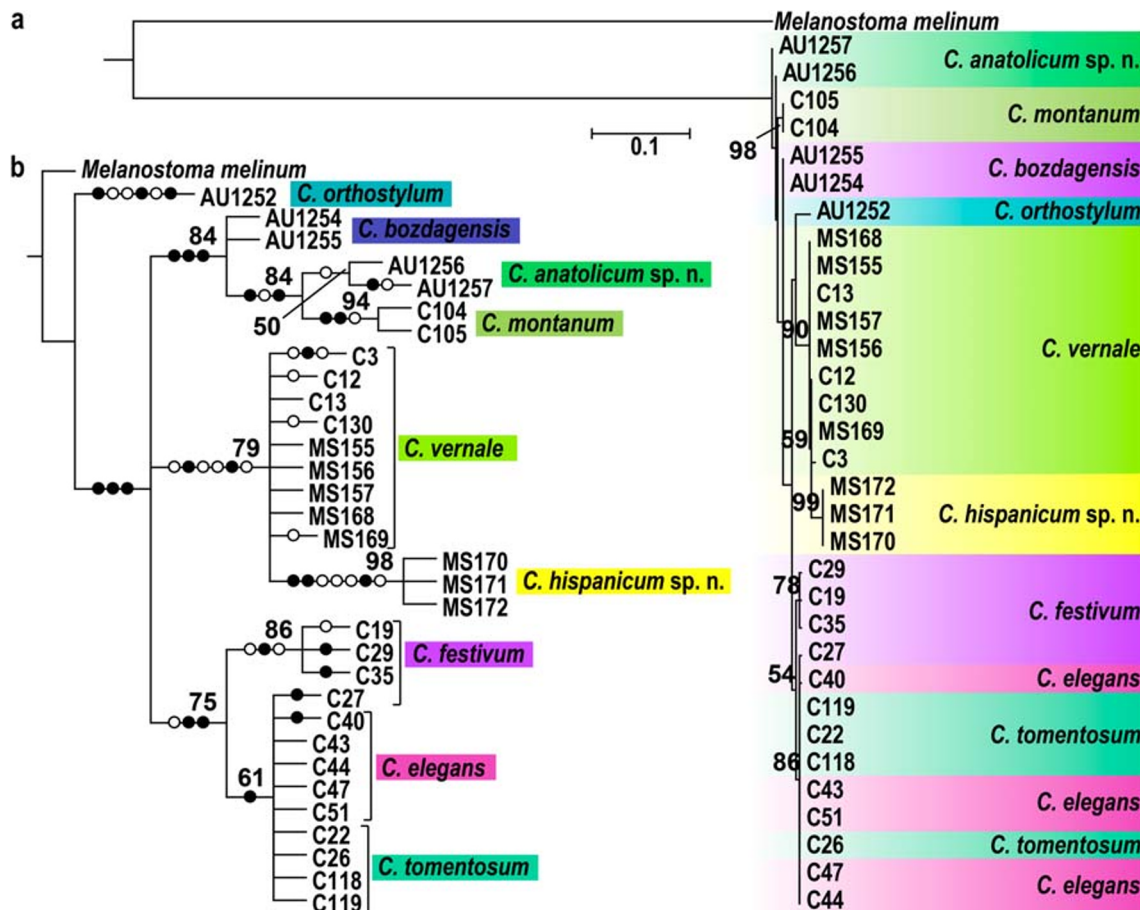


Fig. 7 COI gene trees of *Chrysotoxum* spp.. **a** Maximum likelihood tree. **b** Strict consensus of eight equally parsimonious trees.; filled circles denote non-homoplasious changes and open circles homoplasious

changes. Length = 292 steps; consistency index = 85; retention index = 90. Bootstrap values higher than 50 are indicated near nodes.

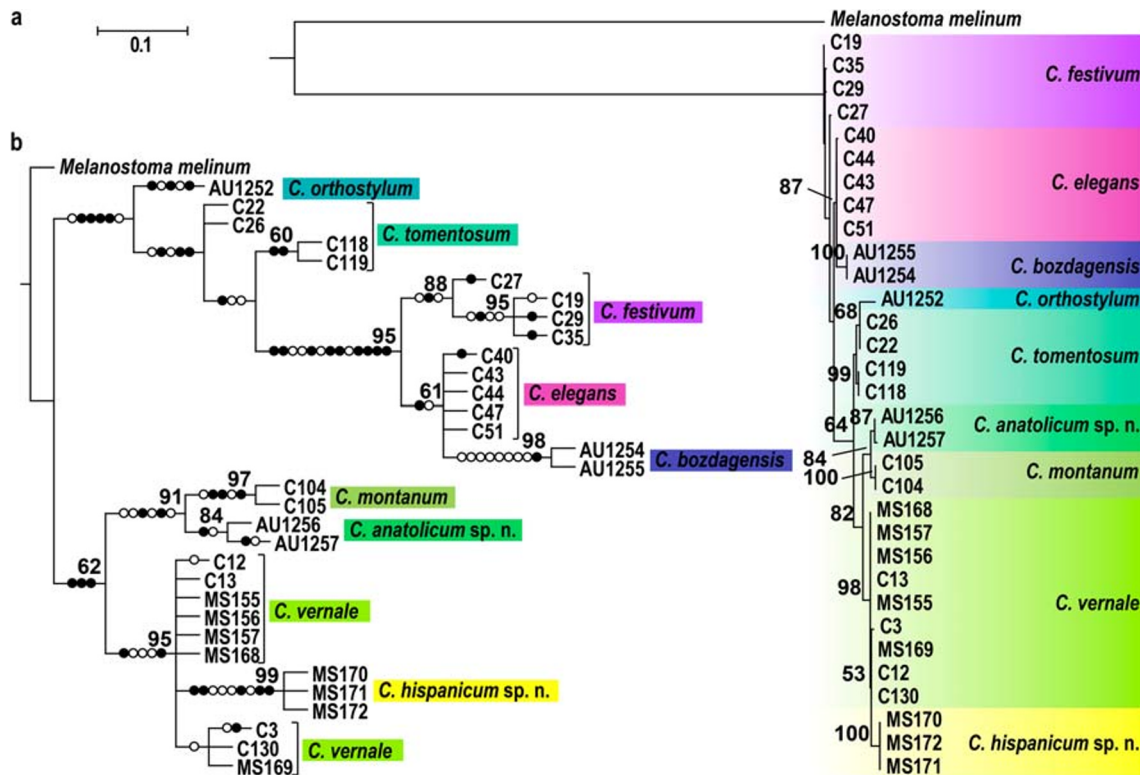


Fig. 8 Combined COI and ITS2 trees of *Chrysotoxum* spp.. **a** Maximum likelihood tree. **b** Strict consensus of four equally parsimonious trees; filled circles denote non-homoplasious changes and open circles homoplasious changes. Bootstrap values higher than 50 are indicated near nodes.

Maximum parsimony analysis based on COI+ITS2 produced four equally parsimonious trees of 279 steps in length (consistency index = 86, retention index = 91). The strict consensus tree shows two main clades (Fig. 8b). The first main clade comprises species of the *C. festivum* group [*C. bozdagensis* Nedeljković, Vujić et Hayat in Nedeljković et al. (2018a), *C. tomentosum*, *C. festivum* and *C. elegans*], but the bootstrap support for this clade is below 50. The second main clade comprises the *C. vernale* group and has low bootstrap support (62). *Chrysotoxum anatolicum* sp. n. and *C. hispanicum* sp. n. are resolved as monophyletic with medium to high clade bootstrap support (84 and 99) as on ML tree, and *C. hispanicum* sp. n. clade is also nested within *C. vernale* clade.

Key to the Western Palaeartic species of the *Chrysotoxum vernale* group

The *C. vernale* group, understood as a set of species sharing a basoflagellomere shorter than scape and pedicel combined, pro- and mesofemora black basally and terga 2–4 with yellow fasciae not reaching the lateral margins, consists now of the following eight species in the Western-Palaeartic: *C. anatolicum* sp. n., *C. antennalis* Vujić, Nedeljković et

Hayat in Vujić et al. (2017), *C. clausseni* Vujić, Nedeljković et Hayat in Vujić et al. (2017), *C. hispanicum* sp. n., *C. lineare* (Zetterstedt, 1819), *C. montanum*, *C. orthostylum* and *C. vernale*. All species of the *C. festivum* group (*C. bozdagensis*, *C. elegans*, *C. festivum* and *C. tomentosum*) (Figs. 7, 8), as well as light-legged specimens of *C. orthostylum* have pro- and mesofemora uniformly yellow or with their bases dark yellow. Light-legged specimens of *C. orthostylum* would key out with Nedeljković et al. (2015).

- 1a. Scape longer than pedicel; abdomen elongate 2
- 1b. Scape shorter than pedicel; abdomen oval 3
- 2a. Scutellum yellow, with a black central macula; mesofemora black in their basal thirds *C. antennalis*
- 2b. Black scutellum, with the posterior margin narrowly yellow; mesofemora black in their basal halves ... *C. lineare*
- 3a. Widest sections of the T3–4 yellow maculae extend for a third or more of each tergum length (Figs. 5b-c) *C. hispanicum* sp. n.
- 3b. Widest sections of the T3–4 yellow maculae extend clearly less than a third of each tergum length (Figs. 5a, d) ... 4
- 4a. Posterior half of wing pigmented (Fig. 4a), including parts of cells CuP, CuA₁ and DM *C. anatolicum* sp. n.
- 4b. Posterior half of wing without pigmentation (Fig. 4b), at most pigmented in reduced areas of cells CuP, CuA₁ or DM ... 5



5a. Anterior margin of scutum with two medial, silvery pollinose vittae extending for less than half of the scutum length; scutellum with a medial, transparent, dark-yellow macula *C. claussemi*

5b. Anterior margin of scutum with two medial, silvery pollinose vittae extending for at least half of the scutum length, usually more; scutellum with a medial, opaque, black macula (Fig. 3b) 6

6a. Abdomen slender, T5 medial length more than half of the tergum maximum width; male surstylus gradually tapering towards the apex, more than 3 times longer than its maximum width *C. orthostylum*

6b. Abdomen stocky, T5 medial length half or less of the tergum maximum width (Fig. 5d); male surstylus expanded basally (Fig. 6d), less than 2.5 times longer than its maximum width 7

7a. Medial vittae of scutum evenly pollinose in their entire length; in male, long pile of scutum yellow; male surstylus less than 2.5 times longer than its maximum width (Fig. 6d); female with frontal pollinose maculae bar-shaped *C. vernale*

7b. Medial vittae of scutum unevenly pollinose, less conspicuous in their posterior halves; in male, long pile of scutum black; male surstylus 3 times longer than its maximum width; female with frontal pollinose maculae triangular-shaped *C. montanum*

Discussion

This study integrates information from both adult morphology and gene sequences to establish species limits within the Western Palearctic species of the *Chrysotoxum vernale* group. The results confirmed the presence of two separate *Chrysotoxum* species at opposite sides of the Mediterranean Basin: *C. hispanicum* sp. n., from the Iberian Peninsula, and *C. anatolicum* sp. n., from the Anatolian Peninsula. *Chrysotoxum hispanicum* sp. n. is distinguished from the similar *C. vernale* by the relative width of the yellow abdominal maculae, which are wider in relation to each tergum length in *C. hispanicum* sp. n. (Figs. 5b–c) than in *C. vernale* (Fig. 5d). The relative width of the yellow abdominal maculae proved to be useful to separate other *Chrysotoxum* species, for instance, *C. festivum*/*C. tomentosum* (Nedeljković et al. 2011, 2013) and *C. bozdagensis*/*C. octomaculatum*/*C. elegans* (Nedeljković et al. 2018a). The relative width of the abdominal maculae is also used in other syrphine genera such as *Dasysyrphus* Enderlein, 1938 and *Epistrophe* Walker, 1852 to separate species (Van Veen 2004; Doczkal and Schmid, 1994). Another useful character to separate *C. hispanicum* sp. n./*C. vernale* is the colour of the long scutellum pile, most of which are yellow in *C. hispanicum* sp. n. (Fig. 3a) and black in *C. vernale* (Fig. 3b).

Studied specimens of *C. anatolicum* sp. n. had a remarkable wing pigmentation pattern which covered most of the wing membrane (Fig. 4a), including cells BM, CuP and CuA₁. The other species of the *C. vernale* group usually lack pigmentation on the posterior part of wing (Fig. 4b): only a female of *C. hispanicum* sp. n. was found to have pigmentation in the posterior part of wing but just in the membranous areas next to veins. Similar differences in the wing pigmentation are found in species of other syrphine genera such as *Xanthogramma* (Nedeljković et al. 2018b) and *Leucozonia* Schiner, 1860 (Doczkal 1998, 2000). Another remarkable character in *C. anatolicum* sp. n. is the gena colour, which is wholly black, while in other species of the *C. vernale* group is at least partly pale. Nonetheless, some specimens of *C. claussemi* and *C. montanum* have a black gena.

Recent *Chrysotoxum* studies such as Nedeljković et al. (2018a) describing *C. bozdagensis* already showed the utility of a combined COI+ITS2 analysis for species delimitation in this genus. These two markers were also used independently, and with success, for resolving species within the *C. festivum* and *C. vernale* groups separately (Masetti et al. 2006; Nedeljković et al. 2013, 2015). However, in this study we showed a limited ability of COI gene sequences in resolving taxonomy of *Chrysotoxum* species when both *C. vernale* and *C. festivum* groups are analysed together. For example, *C. festivum* and *C. tomentosum*, both part of the *C. festivum* group, cannot be delimited using COI, but are resolved as separate using ITS2 sequences (Nedeljković et al. 2013). Thus, only analyses of combined COI and ITS2 sequences can provide successful delimitation of *Chrysotoxum* species. Unlike COI trees, *C. vernale* species group is resolved as monophyletic on combined COI+ITS2 trees, although with low to moderate bootstrap support (ML = 82, MP = 62). Additionally, *C. hispanicum* sp. n. and *C. anatolicum* sp. n. are resolved as genetically divergent from the other species of *C. vernale* group (Fig. 8). *Chrysotoxum orthostylum* does not belong to the *C. vernale* clade, neither to the *C. festivum* in the COI-based maximum parsimony tree (Fig. 7b), while in the tree based on COI+ITS2 groups together with species allied with *C. festivum* (Fig. 8b). In addition, the remarkable shape of the *C. orthostylum* male genitalia together with the reported variability in colour of pro- and mesofemora (Nedeljković et al. 2015) make the placement of this species difficult in any of the above mentioned species groups. Thus, we decide to leave *C. orthostylum* as an outlier within the genus *Chrysotoxum* until further systematic studies are undertaken.

Many syrphid species are rare and/or endemic to the Mediterranean Basin hotspot (e.g. Doczkal 1996; Vujić et al. 1999, 2001, 2007, 2011; Marcos-García et al. 2007, 2011; Ricarte et al. 2012; Grković et al. 2016), and the two new species described here further contribute to increase the biodiversity of this hotspot. Within the Mediterranean Basin, Turkey and mainland Spain have now a combined total of

24 *Chrysotoxum* species, 19 in Turkey (Saribiyik 2014; Vujić et al. 2017; Nedeljković et al. 2018a; present study) and 14 in mainland Spain (Ricarte and Marcos-García, 2017; van Steenis et al. 2020; present study). The Spanish provinces of León and Salamanca have the highest numbers of *Chrysotoxum* species recorded in Spain (8 spp. each) (Ricarte and Marcos-García, 2017; present study), probably due to higher sampling efforts in these provinces. Italy and France equal mainland Spain in the number of *Chrysotoxum* species (14 spp.), but in countries further north, the numbers reduce to, for example, 11 in Germany, 8 in the UK and 7 in Norway, all three with well-studied hoverfly faunas (Speight et al. 2016). Turkey and mainland Spain share about 35% (8 spp.) of their *Chrysotoxum* faunas, which is slightly higher than the percentage found in other syrphid genera such as *Merodon* Meigen (22%) (Vujić et al. 2011). The number of *Chrysotoxum* species in the Iberian Peninsula will certainly increase after the *C. vernale* and other species groups such as that of *C. intermedium* Meigen, 1822 are revised (Nedeljković et al. in prep).

In Turkey, *C. anatolicum* sp. n. was found in Bozdağ, which extends as a mountain range (with the peak at 2157 m asl) for about 170 km in an east-west direction between the provinces of İzmir and Manisa (Toksöz and Ustaoglu 2005). This locality includes forests with ancient chestnut (*Castanea sativa*), pines (*Pinus brutia* and *Pinus nigra*) and oaks (*Quercus ithaburensis* subsp. *macrolepis* and *Quercus infectoria*), Mediterranean scrublands ('macchia') and mountain steppes (Kurtaslan and Demirel 2011; Anlaş et al. 2010), all of them providing habitats and resources for a high diversity of invertebrates such as Coleoptera (Tenebrionidae) (Merican et al. 2004), Dermaptera (Anlaş et al. 2010), Diptera (Chironomidae and Chaoboridae) and Oligochaeta (Toksöz and Ustaoglu 2005). At the moment, Bozdağ is the only known locality where both *C. bozdagensis* and *C. anatolicum* sp. n. occur (Nedeljković et al. 2018a).

Chrysotoxum hispanicum sp. n. is widespread in the Iberian Peninsula and present in a broad variety of habitats. The records of *C. vernale*, confirmed here to occur in mainland Spain based on morphological evidence, originate from the Cantabrian Mountains (Provinces of Asturias, León and Santander) [Marcos-García (1990) in part], the 'Sistema Central' (Cáceres) [Marcos-García (1986) in part] and the Pyrenees (Huesca) (Marcos-García 1985), all high altitude mountain ranges. In Turkey, *C. vernale* is also found at high altitudes (2000–3000 m asl.), in the Erzurum Province (Saribiyik 2014; Vujić et al. 2017), while *C. anatolicum* sp. n. is found in highlands but at a lower altitude (1567m). Nonetheless, new studies may contribute to better define the altitudinal range where *C. anatolicum* sp. n. occurs.

The detailed and often integrative study of the taxonomy of the *C. vernale* group in other parts of the Western Palaeartic (e.g. Balkan Peninsula) has also resulted in the definition and

redefinition of several species concepts, some of them corresponding to new taxa (Nedeljković et al. 2015, Vujić et al. 2017). The present paper represents the first attempt to resolve the taxonomy of the *C. vernale* group in the Iberian Peninsula. As a result, *C. hispanicum* sp. n. has been discovered among material that was previously thought to belong to the species *C. vernale* (Marcos-García 1981, 1986, 1990; Ricarte and Marcos-García 2008). A higher level of taxonomic scrutiny of adults—i.e. further DNA, geometric morphometric, and ecological analyses in a more extensive study area—of *Chrysotoxum* populations, as well as a more complete knowledge of their early stages and biology are expected to increase the alpha diversity of this genus in the Iberian Peninsula and other parts of Western Europe (Speight 2018).

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Data availability statement The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval All applicable international, national and international guidelines for the care and use of animals were followed.

This article does not contain any studies with human participants performed by any of the authors.

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