JHR 93: 139–149 (2022)
doi: 10.3897/jhr.93.93865
https://jhr.pensoft.net

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

Description of the nest of the pollen wasp *Celonites jousseaumei* Du Buysson, 1906 (Hymenoptera, Vespidae, Masarinae) with a new host association of the cuckoo wasp *Spintharina innesi* (Du Buysson, 1894) (Hymenoptera, Chrysididae)

Volker Mauss[1], Christophe Praz[2], Andreas Müller[3], Rainer Prosi[4], Paolo Rosa[5]

1 *Staatliches Museum für Naturkunde, Abt. Entomologie, Rosenstein 1, D-70191 Stuttgart, Germany* **2** *University of Neuchâtel, Institute of Biology, Emile-Argand 11, 2000 Neuchâtel, Switzerland* **3** *ETH Zürich, Institute of Agricultural Sciences, Biocommunication and Entomology, Schmelzbergstraße 9/LFO, CH-8092 Zürich, Switzerland* **4** *Lerchenstraße 81, D-74564 Crailsheim, Germany* **5** *Laboratory of Zoology, Institute of Biosciences, University of Mons, Place du Parc, 20, 7000 Mons, Belgium*

Corresponding author: Volker Mauss (volker.mauss@gmx.de)

Academic editor: Michael Ohl | Received 23 August 2022 | Accepted 19 October 2022 | Published 31 October 2022

https://zoobank.org/DC5B62DF-3F6C-4D86-9E9F-018369FD6E7F

Citation: Mauss V, Praz C, Müller A, Prosi R, Rosa P (2022) Description of the nest of the pollen wasp *Celonites jousseaumei* Du Buysson, 1906 (Hymenoptera, Vespidae, Masarinae) with a new host association of the cuckoo wasp *Spintharina innesi* (Du Buysson, 1894) (Hymenoptera, Chrysididae). Journal of Hymenoptera Research 93: 139–149. https://doi.org/10.3897/jhr.93.93865

Abstract

Two nests of *Celonites jousseaumei* are described in detail from the Antiatlas in Morocco. The nests consisted of two or three linearly arranged earthen brood cells that were attached to the almost vertical surface of medium sized stones. The brood cell provisions consisted exclusively of *Heliotropium* pollen (Boraginaceae). Species affiliation of developmental stages by DNA barcoding revealed that one of the brood cells contained a pupa of *Spintharina innesi* in a cocoon.

Keywords

brood parasitism, COI-5P, nest construction, Palaearctic

Introduction

The knowledge about nest architecture of the more than 40 Palaearctic species of the pollen wasp genus *Celonites* is fragmentary. Detailed nest descriptions are only available for *Celonites abbreviatus* (Villers, 1789) (Ferton 1901, 1910; Bellmann 1984, 1995), *Celonites tauricus* Kostylev, 1935 (Mauss et al. 2016) and *Celonites fischeri* Spinola, 1838 (Mauss and Müller 2014), which are all members of the *C. abbreviatus*-group of the subgenus *Celonites* s.str. In contrast, information about nesting in the subgenus *Eucelonites* Richards, 1962 is not available despite a short note by Richards (1962: 224) concerning two specimens of *Celonites* (*Eucelonites*) *jousseaumei senegalensis* Richards, 1962 from Bambey, Senegal that "had pinned with them some mud cells which from their shape must have been attached end to end, longitudinally, on a plant-stem. The cells were approximately 9.0 mm long and 3.5 mm in diameter". During a field trip to Morocco in 2019 two nests of *Celonites* (*Eucelonites*) *jousseaumei* were discovered that are described in the present paper.

Materials and methods

The nests were found and dissected on the 19th of April 2019. Outer cell dimensions were measured using a calliper rule (accuracy 0.1 mm), inner cell dimensions were reconstructed from macro photos. All brood cells were dissected with fine tweezers (Dumont INOX No. 5 Biologie, No. 7 and spring steel tweezers) using a combination of two reading glasses that provided a sufficient magnification. Photos were taken with a Canon EOS 70D or 80D camera with a 50 mm or 100 mm macro lens (scale up to 1:1, resolution 20 or 24 mega pixel) and macro flash-lights. Dry specimens of all *Celonites* species were labelled with an individual, serial database number (dbM = database Mauss) printed on the determination label and placed in the collection of Volker Mauss (*C. jousseaumei* 1♂ dbM 5402 1♀ dbM 5386; *C. pictus* 5♀ dbM 5385, 5398–5401). Specimens of all plant species that were visited by pollen wasps were collected and preserved dried. The material was placed in the herbarium of the State Museum of Natural History in Stuttgart (Herbarium STU).

Pollen samples from brood cell provisions were prepared using the method outlined by Westrich and Schmidt (1986). The content of each provision was distributed over two or three slide preparations. The different pollen types were ascertained under a light microscope at magnifications of 400× and determined to generic level with the aid of a reference collection. For each slide all pollen grains were determined along three randomly chosen lines transversal to the cover glass.

1♀ *Celonites jousseaumei* (dbM 5421 [BOLD process ID: CECYP002-20]) and 1♀ *Celonites pictus* (dbM 5422 [CECYP004-20]) from the nest locality, as well as the pupa from cell B2 [CECYP001-20] and the larva from cell N1 [CECYP003-20] from the brood cells were preserved in 96% pure ethanol for DNA barcoding. For

further reference, the barcoding fragment of the mitochondrial gene was sequenced from 1♂ (dbM 4320 [AIMEJ011-20]), 4♀ (dbM 4319 [AIMEJ010-20], dbM 5590 [CECYP035-22], dbM 5592 [CECYP036-22], dbM 5594 [CECYP037-22]) of *C. jousseaumei*, and 1♂ (dbM 5117 [AIMEJ013-20]), 1♀ (dbM 4322 [AIMEJ012-20]) of *Celonites afer* from different localities in Morocco. To facilitate identification of possible cuckoo wasp nest parasites, we barcoded dry specimens of six chrysidid wasps morphologically close to *Spintharina* that had been collected at four localities in Morocco between 2011 and 2019 identified as *Spintharina procuprata* (Linsenmaier, 1959) [CECYP022-22], *Spintharina innesi* (du Buysson, 1894) [CECYP023-22, CECYP024-22, CECYP025-22, CECYP026-22] and *Chrysis patruela* Linsenmaier, 1999 [CECYP027-22]. Species identification was based on morphological characters of the imagines. DNA barcoding followed standard methods of DNA extraction from a single leg of dry specimens or specimens collected and stored in 96% pure ethanol. The barcoding fragment of the gene Cytochrome Oxidase subunit 1 (COI-5P) was amplified in PCR using the universal primers LepF and LepR (Hebert et al. 2004). Sequencing was performed bi-directionally using the same primers and the resulting chromatograms were edited in Geneious 6.0.6 (Kearse et al. 2012). DNA barcoding of the additional reference specimens that had not been collected at the nest site was accomplished by AIM Advanced Identification Methods GmbH Leipzig. All nucleotide sequences were uploaded and analysed using the BOLD database (https://www.boldsystems.org). Another four public COI-5P sequences of two taxa of *Spintharina* were added from the BOLD database. Genetic distances were computed using Kimura 2-parameter (K2P) distance model in a test version of Paup 4.0 (Swofford 2002) kindly provided by D. Swofford. Finally, a species ID tree was computed using the following parameters: distance model Kimura 2 Parameter; pairwise deletion of positions containing gaps and missing data; minimum complete overlap 0 bp; alignment with BOLD Aligner (Amino Acid based HMM); individual nucleotide sequence length varied from 252 to 675 bp.

Results and discussion

Locality

Two nests of *Celonites jousseaumei* were found at Ait Daoud (WGS 84: 29°36.977'N, 08°59.009'W), 15 km south of Tafraout in the Antiatlas in Morocco, situated at a height of 1140 m a.s.l. The climate of the area is arid with a mean annual precipitation of 235 mm and a mean annual temperature of 16.6 °C (data from Tafraout, AM ONLINE Project). The habitat consisted of a richly flowering roadside (Fig. 1b) with adjacent former terraces of almond orchards that were left fallow since approximately ten years because of increasing aridity (Fig. 1a). The stony area was heavily grazed and somewhat polluted with rubbish, i.e. along little dry drainage channels.

Figure 1. a, b habitat of *Celonites jousseaumei* at Ait Daoud, 15 km south of Tafraout, Morocco **a** nest site **b** foraging area **c** exterior view of nest B **d, e** stones used as base for nests of *C. jousseaumei* **d** nest N **e** nest B.

In the area *Celonites jousseaumei* and *C. pictus* were recorded. Imagines of both species were exclusively observed to visit flowers of *Heliotropium crispum* Desf. (number of sightings at *H. crispum* flowers: *C. jousseaumei* 1♂ 3♀, *C. pictus* 66♀), although other plants were flowering at the site, for example *Cladanthus arabicus* (L.) Cass., *Centaurea calcitrapa* L., *Pallenis spinosa* (L.) Cass., *Senecio glaucus* subsp. *coronopifolius* (Maire) C. Alexander (all Asteraceae), *Echium horridum* Batt. (Boraginaceae), *Lotus* sp. (Fabaceae) and *Convolvulus trabutianus* Schweinf. & Muschl. (Convolvulaceae).

Nest site

Both nests were situated at the steep edge of a 0.5 m high terrace with an exposure of 70° to the north (ENE) (Fig. 1a). The site was approximately 10 m away from large patches of *Heliotropium crispum* flowering on the embankment of the road (Fig. 1b). Both nests were attached to almost vertical surfaces of medium sized stones (Table 1, Fig. 1d, e), 6 cm and 29 cm respectively above the foot of the terrace. The distance between the nests was 75 cm.

Nest structure

The nests were made of fine clayey soil with a small proportion of tiny stones and consisted of 2 or 3 cells (Table 1). The cells were arranged in a longitudinal row in which each subsequent cell abutted with its basal end onto the apical end of the completed preceding cell. The resulting almost vertical or diagonal linear construction was 15.5 mm or 20.9 mm long, with the apical ends of the cells oriented downward (Figs 1c, 2a). An additional nest covering was not present. This exclusively linear arrangement of the brood cells is in congruence with the fragmentary nest description of *Celonites jousseaumei senegalensis* by Richards (1962). Linearly arranged brood cells were also observed in a few nests of *C. abbreviatus* (Lichtenstein 1869) and *C. fischeri* (Mauss & Müller, 2014), but in these nests at least a single cell was also attached longitudinally to the others in addition. Moreover, in most nests of *C. abbreviatus* (Bellmann 1984) and *C. fischeri* (Mauss & Müller, 2014) the cells are only attached longitudinally to each other, which is also the case in all of the few known nests of other members of the *C. abbreviatus*-group, in particular *C. tauricus* (Mauss et al. 2016) and *C. mayeti* Richards, 1962 (Lichtenstein 1875). Therefore, the observed exclusively linear arrangement of the brood cells of *C. jousseaumei* is of note.

Table 1. Parameters of two nests of *Celonites jousseaumei* recorded at Ait Daoud, Morocco.

Nest	Condition	Height above ground (cm) [1]	Orientation to the North (°)	Nest substrate	Σ cells	Contact between adjacent cells	Nest covering
B	sealed, current season	29	70	stone (base 18×23 cm, height 10 cm)	2	linear	absent
N	sealed, current season	6	0	stone (base 17×7 cm, height 10 cm)	3	linear	absent

[1]measured from the lowest part of the nest to the foot of the terrace.

The brood cells were cylindrical with almost parallel sides, rounded at the basal and truncate at the apical end (Fig. 2b, c). The median dimensions of the cells were: outer length 7.3 mm (n = 5), outer diameter 3.6 mm (n = 5), inner length 6.3 mm (n = 4), inner diameter at the cell opening 3.4 mm (n = 4) (Table 2). The outer cell surface showed a distinct "fish scale" pattern (Figs 1c, 2a) while the inner surface was smooth (Fig. 2c). Towards the stone it was attached to, the cell wall was not completely constructed resulting in a few spots in the median axis where the surface of the stone formed the boundary of the cell (Fig. 2c). The outer cell wall continued between adjacent cells laterally covering the small hollow space between them that resulted from the existence of two separate cell walls that is the transversal apical wall of the basal cell and the rounded basal wall of the apical cell (Fig. 2b, c). At the apical end of the nest the outer wall of the last cell was produced into a small rim protruding the apical transversal cell wall by 2.1 mm (Fig. 2b). The wall of the rim had one or two deep notches and was slightly bent outwards (Fig. 1c). Within this short tube-like entrance, the nest was sealed by a circular, curved, circa 0.2–0.3 mm thick mud plug (Fig. 2b). This nest seal was positioned about 0.2 mm above the apical wall of the last cell and 1 mm inwards from the edge of the nest opening resembling the bottom of a new cell in form, position and structure.

Brood cell content

The content of the brood cells is summarized in Table 2. The provision consisted of a yellowish-white, viscous pollen mass with shining surface forming a pollen loaf. The surface of the loaf was characteristically papillated (Fig. 2b) so that it barely touched the cell walls. Between the apical end of the pollen loaf and the apical wall of the cell remained a hollow space measuring approximately 0.8–0.9 mm (Fig. 2b). Inwards the pollen mass became more sticky and rather liquid, so that it could not be removed as a whole with a pair of tweezers. The provision in the cells of both nests consisted exclusively of *Heliotropium* pollen indicating narrow oligolecty (sensu Müller and Kuhlmann 2008) of *C. jousseaumei* at this locality. Both eggs of *Celonites jousseaumei* were whitish and curved. Each egg was situated on top of the provision close to the basal end of the cell indicating that it was laid by the female prior to brood cell provisioning (Fig. 2b). Small remnants of membranous material attached to one pole of each egg suggested that the eggs had initially been attached to the wall. The small larva from cell N1 was also situated basally on top of the pollen mass, where it fed on the provision (Fig. 2b).

Species identification

The COI-5P gene sequence of the larva from brood cell N1 was 100% identical (distance of 0) to the sequence of a *Celonites jousseaumei* female [CECYP037-22] collected northeast of Sidi Ifni at a distance of approximately 100 km from the nest site. Four additional specimens of *C. jousseaumei* from Morocco were sequenced; the average within-species genetic distance among these specimens was 0.51% (minimum 0, maximum 1.19). The

Figure 2. Structure of *Celonites jousseaumei* nest N on 19th of April: **a** exterior view **b** brood cells opened in longitudinal direction **c** cell content removed. (N1–N3 = brood cell numbers; aw = apical cell wall; bw = basal cell wall; e = egg; hs = hollow space below pollen loaf; l = larva; nr = nest rim; ns = nest seal; p = pollen loaf; pa = papilla on surface of pollen loaf; s = bare stone surface; measurements and cell content summarized in Table 2).

sequence of the larva from brood cell N1 to *C. pictus* [CECYP004-20] was 24.25%. In the distance-based tree (Fig. 3), the larva N1 clusters together with the imagines of *C. jousseaumei*. Therefore, it can be concluded that the recorded nests belong to *C. jousseaumei*. This is in congruence with the cell dimensions that are too small for *C. pictus* but match the size of *C. jousseaumei* (median total length of females 7.46 mm (n = 8) in *C. pictus* versus 5.75 mm (n = 7) in *C. jousseaumei*).

Table 2. Measurements and condition of brood cells from two nests of *Celonites jousseaumei* recorded at Ait Daoud, Morocco.

Nest	Cell No.	Condition	Outer cell length (mm)	Outer cell width (mm)	Inner cell length (mm)	Inner cell width (mm)	Content	Species affiliation	Pollen type composition of provision
B	B1	sealed	7.3	3.5	5.8	2.8	yellowish-white pollen loaf, dead dry larva		exclusively *Heliotropium*[1]
	B2	sealed	8.2	3.6			pupa in light yellowish cocoon	*Spintharina innesi*	
N	N1	sealed	7.1	3.6	6.4	3.3	yellowish-white pollen loaf, small larva feeding on basal end of provision	*Celonites jousseaumei*	exclusively *Heliotropium*[1]
	N2	sealed	7.0	4.0	6.2	3.4	yellowish-white pollen loaf, curved egg basally on top of provision		exclusively *Heliotropium*[1]
	N3	sealed	7.9	3.7	7.3	3.4	yellowish-white pollen loaf, curved egg basally on top of provision		exclusively *Heliotropium*[1]

[1]at most very rarely with single pollen grains of other plant taxa.

The pupa from brood cell B2 turned out to belong to a species of Chrysididae, as the obtained COI-5P gene sequence of the pupa closely matched the public sequences of *Spintharina versicolor* Spinola, 1808 within BOLD database. However, the identity of the sequences was only 92%, indicating that the pupa belongs to a different but related species. After additional sequencing of the reference chrysidid specimens from Morocco, a match was found between the pupa and a specimen of *Spintharina innesi* collected in Tizourgane [CECYP026-22] (Fig. 3). Three additional specimens of *Spintharina innesi* were sequenced; the average within-species genetic distance was 0.71% (minimum 0.33, maximum 1.31). The genetic distances between the pupa from brood cell B2 and *Spintharina procuprata* or *Chrysis patruela* were 17.20% and 12.13%, respectively. Therefore, it can be concluded that *Celonites jousseaumei* is a host of *Spintharina innesi*. Host-parasite associations of *Spintharina* with *Celonites* seem to be common. In Europe *Spintharina versicolor* has been recorded as a brood parasite of *Celonites abbreviatus* (Blüthgen 1961; Erlandsson 1972), while in the Afrotropical Region *Spintharina arnoldi* (Brauns, 1928) and *Spintharina bispinosa* (Mocsáry, 1902) were reared from brood cells of two different *Celonites* species (Brauns 1913; Gess 1996). Moreover, Pauli et al. (2019) found a close phylogenetic relationship between *Spintharina* and the Nearctic *Chrysurissa*, which also appear to parasitize exclusively pollen wasps. This phylogenetic placement indicates that both genera are descendants of a last common ancestor which may already have exploited pollen wasps as hosts, suggesting an old and exclusive association with pollen wasps in this clade. However, the host specificity of numerous *Spintharina* species is poorly known and it remains to be shown whether *Spintharina innesi* is restricted to *C. jousseaumei* or if it may also parasitize brood cells of other species of *Celonites* or even of other pollen wasp genera. Conversely, there are at least three other species of *Spintharina* recorded from Morocco that are all suitable in size to fit the brood cells of *C. jousseaumei* (cf. Linsenmaier 1999, classified as species of the *Chrysis versicolor*-group), so that this pollen wasp may be parasitized by more than one *Spintharina* species.

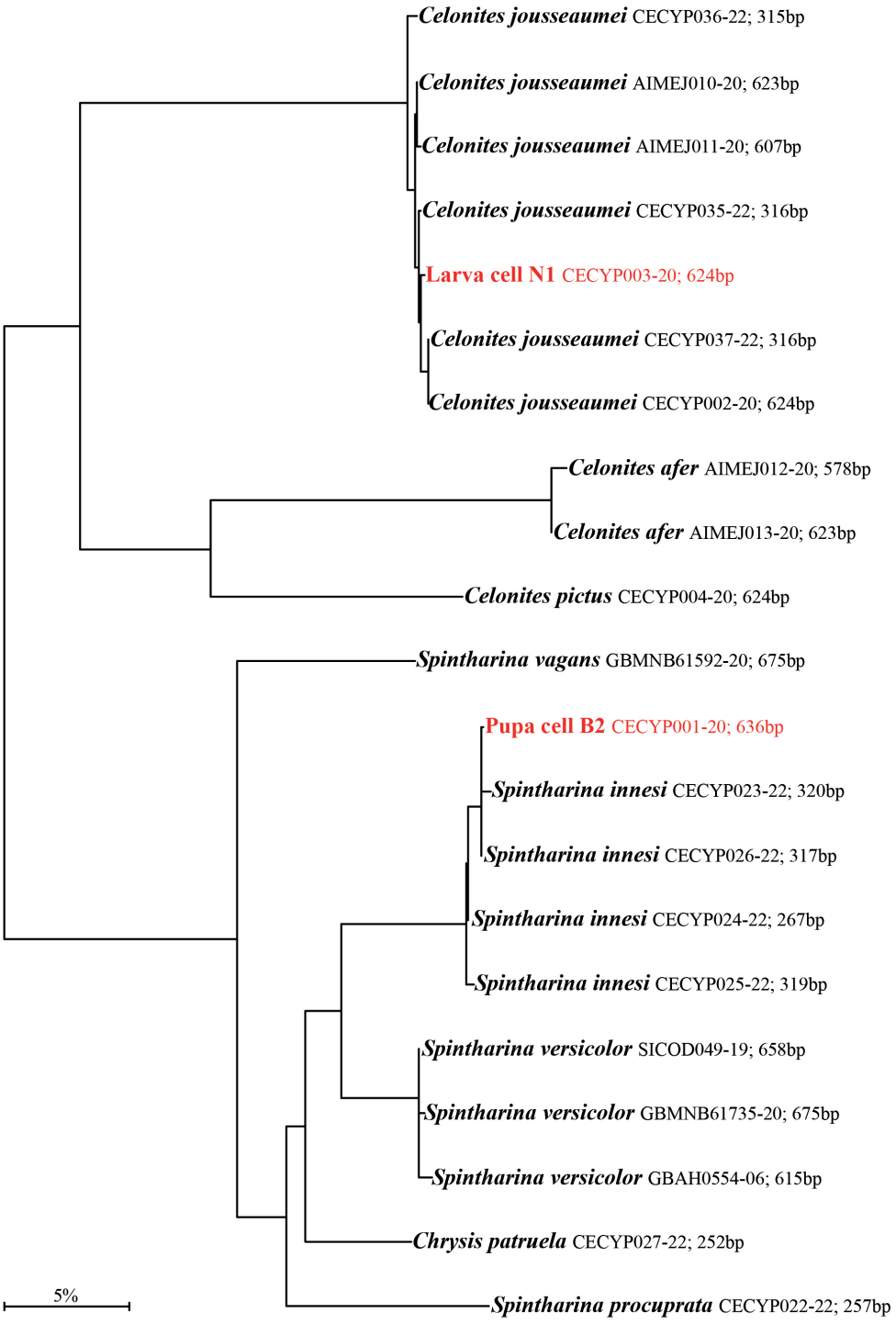

Figure 3. Neighbour joining tree of COI-5P sequences of 9 imagines of *Celonites* (Masarinae) from Morocco, 10 imagines of *Spintharina* (Chrysididae) and 2 immature stages collected from brood cells of two *Celonites* nests from Morocco (see text for details).

Acknowledgements

Annette Rosenbauer kindly identified the collected plants. Comments by Alexander Fateryga and James Carpenter improved the manuscript.

References

AM ONLINE Project (2017) Climate-Data https://climate-data.org [retrieved 2 October 2017]

Bellmann H (1984) Beobachtungen zum Brutverhalten von *Celonites abbreviatus* Villers (Hymenoptera, Masaridae). Zoologischer Anzeiger 212: 321–328.

Bellmann H (1995) Bienen, Wespen, Ameisen – Hautflügler Mitteleuropas. Kosmos Naturführer, Franckh-Kosmos Verlags-GmbH, Stuttgart, 336 pp.

Blüthgen P (1961) Die Faltenwespen Mitteleuropas (Hymenoptera, Diploptera). Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin Klasse für Chemie, Geologie und Biologie 2: 1–249. https://doi.org/10.1515/9783112537084

Brauns H (1913) Dritter Beitrag zur Kenntnis der Masariden (Hym.) von Südafrika. Entomologische Mitteilungen 2: 193–209. https://doi.org/10.5962/bhl.part.14992

Erlandsson S (1972) Hymenoptera Aculeata from the European parts of the Mediterranean Countries. Eos 48: 11–93.

Ferton C (1901) Notes détachées sur l'instinct des Hyménoptères mellifères et ravisseurs avec la description de quelques espèces. Annales de la Société Entomologique de France 70: 83–148.

Ferton C (1910) Notes détachées sur l'instinct des Hyménoptères mellifères et ravisseurs. 6e Série. Annales de la Société Entomologique de France 79: 145–178.

Gess SK (1996) The pollen wasps – Ecology and natural history of the Masarinae. Harvard University Press, Cambridge, Massachusetts, 340 pp. https://doi.org/10.4159/harvard.9780674281684

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences 101: 14812–14817. https://doi.org/10.1073/pnas.0406166101

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Lichtenstein J (1869) No title. Annales de la Société Entomologique de France (4) 9 Bulletin entomologique: XXIX.

Lichtenstein J (1875) No title. Annales de la Société Entomologique de France (5) 5 Bulletin entomologique: CCX, CCXI.

Linsenmaier W (1999) Die Goldwespen Nordafrikas (Hymenoptera, Chrysididae). Entomofauna, Zeitschrift für Entomologie Supplement 10: 1–281.

Mauss V, Fateryga AV, Prosi R (2016) Taxonomy, distribution and bionomics of *Celonites tauricus* Kostylev, 1935, stat. n. (Hymenoptera, Vespidae, Masarinae). Journal of Hymenoptera Research 48: 33–66. https://doi.org/10.3897/JHR.48.6884

Mauss V, Müller A (2014) First contribution to the bionomics of the pollen wasp *Celonites fischeri* Spinola, 1838 (Hymenoptera, Vespidae, Masarinae) in Cyprus. Journal of Hymenoptera Research 39: 119–153. https://doi.org/10.3897/JHR.39.7841

Müller A, Kuhlmann M (2008) Pollen hosts of western palaearctic bees of the genus *Colletes* (Hymenoptera: Colletidae): the Asteraceae paradox. Biological Journal of the Linnean Society 95(4): 719–733. https://doi.org/10.1111/j.1095-8312.2008.01113.x

Pauli T, Castillo-Caja RF, Rosa P, Kukowka S, Berg A, van den Berghe E, Fornoff F, Hopfenmüller S, Niehuis M, Peters R, Staab M, Strumio F, Tischendorf S, Schmitt T, Niehuis O (2019) Phylogenetic analysis of cuckoo wasps (Hymenoptera: Chrysididae) reveals a partially artificial classification at the genus level and a species-rich clade of bee parasitoids. Systematic Entomology 44: 322–335. https://doi.org/10.1111/syen.12323

Richards OW (1962) A revisional study of the masarid wasps (Hymenoptera, Vespoidea). British Museum (Natural History), London, 294 pp.

Swofford DL (2002) PAUP 4.0 Phylogenetic Analysis using Parsimony (and other Methods). Version 4. Sinauer Associates, Sunderland.

Westrich P, Schmidt K (1986) Methoden und Anwendungsgebiete der Pollenanalyse bei Wildbienen (Hymenoptera, Apoidea). Linzer biologische Beiträge 18: 341–360. https://doi.org/10.1051/apido:19870209