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Rediscovery of *Symmorphus declivis* in Italy

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Abstract

Symmorphus declivis Harttig 1932 (Hymenoptera: Vespidae: Eumeninae) is discovered in two localities in central Italy after 80 years since the first and only Italian record. The global distribution is revised and summarized, and ecological notes and DNA barcoding are provided.

Key words: solitary wasps, Lazio, DNA barcoding, distribution.

Introduction

Symmorphus Wesmael 1836 is a genus of eumenine wasps distributed in the Palearctic, Oriental and Nearctic regions and the northernmost Neotropical region. Its phylogenetic affinities are uncertain since various phylogenetic analyses placed it in different parts of the evolutionary tree of the Eumeninae: a morphological approach by Carpenter and Cumming (1985) placed it as the sister-group of Eumeninae Odynerini with rounded tegula (*Odynerus* + *Pterocheilus* group) due to the similar structure of cephalic foveae, but Cumming and Leggett (1985) speculated the cephalic foveae morphology to have independently evolved, while recent molecular evidence (Bank *et al.*, 2017; Piekarski *et al.*, 2018; Luo *et al.*, 2022) place *Symmorphus* as a relatively isolated branch at the base of a large group of "Odynerini" (clade C *sensu* Bank *et al.*, 2017).

It currently includes more than 50 species, 10 of which occur in Europe and Italy (Giordani Soika and Borsato, 1995; Gusenleitner, 1999; Selis, 2023). Among them, *Symmorphus declivis* Harttig 1932 is one of the rarest species, distributed in the northeastern Mediterranean region from Corsica and Italy in the West to Turkey and Cyprus in the East, with the only Italian record being a female specimen collected in May 1940 in Castel Porziano, near Rome (Giordani Soika, 1942). The species was not collected or recorded in Italy until recently, when the first author collected it in two different localities in Lazio region. The new data are here presented with a revision of the known global distribution, together with information on the ecology and the first DNA barcodes for the species.

Materials and methods

The specimens were collected by sweep net and pinned. The specimens were identified using comparison material and taxonomic literature (Cumming, 1989; Gusenleitner, 1999). Examined material is preserved in the private collections of the authors.

Molecular analysis and DNA barcode

Total DNA was extracted as previously reported in Selis *et al.* (2024). Briefly, the right mid-leg was dissected from each wasp and was placed in 2 mL micro-tubes with 1 mL of the specific digestion buffer (Cilia *et al.*, 2022) and incubated for 36 hours at 56 °C. The total DNA purification was performed using a phenol-chloroform extraction (UltrapureTM Phenol:Chloroform:Isoamyl Alcohol, ThermoFisher Scientific, Waltham, MA, USA) (Flaminio *et al.*, 2023b). The obtained DNAs were quantified using the spectrophotometer Infinite 200 PRO NanoQuant™ (TECAN Life Technologies, Männedorf, Switzerland) and stored at −20 °C until the analysis. As a negative control, double-distilled Rnase-Dnase-free water was used for all of these processes.

The amplification of high conserved region Cytochrome C oxidase subunit I (COI) gene was performed using LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') primer pairs able to amplify a 710-bp fragment (Folmer *et al.*, 1994).

The PCR was performed in 25 µL of volume using HotStarTaq Polymerase (Qiagen, Hilden, Germany) on Applied Biosystems® 2720 Thermal Cycler (ThermoFisher Scientific), as reported in Flaminio *et al.* (2023a). The obtained amplicons were visualized on a 1.5% agarose gel, purified using ExoSAP-IT Express (ThermoFisher Scientific) and they were sequenced through the SeqStudio™ (ThermoFisher Scientific) using standard Sanger methodology. The obtained sequences were analysed using BioEdit (Hall, 1999) to create the consensus one aligning forward and reverse sequences and BLAST (using megablast algorithm) (Altschul *et al.*, 1990).

The consensus sequences were processed on MEGA11 software (Tamura *et al.*, 2021): DNA alignment was made with Clustal Alignment, Neighbor-Joining (NJ) phylogenetic analysis (Saitou and Nei, 1987) was performed with bootstrap values determined from 10.000 replications (Felsenstein, 1985), and intra- and interspecific distances were calculated using the Kimura 2-parameter model (Kimura, 1980), with rate variation modelled with a gamma distribution. The genera *Alastor* Lepeletier 1841 and *Odynerus* Latreille 1802 were chosen to root the tree following the phylogeny proposed by

Piekarski *et al.* (2018), and 12 other species of *Symmorphus* were used as outgroups, with sequences downloaded from the Barcode of Life Data System and GenBank. All the sequences analysed in the present contribution are listed below:

Alastor atropos Lepeletier: GBACU3135-13.

Alastor mocsaryi (Andre): GBICH609-17.

Odynerus spinipes (L.): MZ628673.

Symmorphus albomarginatus (de Saussure): KR782819, KR795019.

Symmorphus allobrogus (de Saussure): HQ563014, HQ947738, HQ947739, HQ947740, JN934292, JN934293, JN934294, JN934295, KC460738, KC460739, KC460740, KC460741, MZ626819, MZ624204.

Symmorphus angustatus (Zetterstedt): MZ625422.

Symmorphus apiciornatus (Cameron): LC492875.

Symmorphus bifasciatus (L.): HQ947741, HQ947742, JN270116, JN934296, JN934297, JN934298, JN934299, JN934300, JN934301, JN934302, JN934303, JN934304, JN934305, JN934306, JN934307, JN934308, JN934309, JN934310, JN934311, JN934312, JN934313, JN934314, JN934315, JN934316, JN934317, JN934318, JN934319, JN934320, KR882448, MZ626578, MZ627615, MZ656436.

Symmorphus canadensis (de Saussure): EU649666, HQ929273, HM422977, JN293020, KR783228, KR783446, KR784566, KR785751, KR786187, KR794003, KR794199, KR798270, KR798611, KR801224, KR802705, KR803952, KR804813, KR805721, KR892764, KR894844, MG374323, MG375034, MG375333, MG378278, MG380897, MG381420, MG382355, MG382650, MG497200.

Symmorphus connexus (Curtis): HQ947743, HQ947744, JN270117, MZ627458.

Symmorphus crassicornis (Panzer): HQ947745, HQ947746, HQ947747, JN934321, JN934322, KC460742, KC460743, KC460744, KC460745, KC460746, KC460747, KC460748, MZ627596.

Symmorphus cristatus (de Saussure): EU649667, HQ552248, JN292384, KM555882, KM557559, KM558325, KM559297, KM563274, KM566573, KM567354, KR374025, KR933327.

Symmorphus debilitatus (de Saussure): HQ947748, HQ947749, HQ947750, HQ947751, JN934323, JN934324, KC460749, KC460750, KC460751.

Symmorphus declivis Harttig: PP190334, PP190335, PP190336, PP190337, PP190338.

Symmorphus gracilis (Brulle): HQ947752, HQ947753, HQ947754, JN934325, JN934326, JN934327, KC460752, KC460753, KC460754, KC460755, KC460756, KC460757, KC460758, MZ628574.

Symmorphus murarius (L.): HQ947755, HQ947756, HQ947757, JN934328, JN934329, JN934330, KC460759, KC460760, KC460761, KC460762, KC460763, MZ623410, MZ625217.

Results

Examined specimens

22 ♀, IT, Lazio, RM, Santa Severa, Valle di Rio Fiume, 60 m a.s.l., 42.0628N 11.9531E, 22.IV.2023, Selis M. leg.

1 ♀, IT, Lazio, VT, Viterbo, Necropoli etrusca di Norchia, 135 m a.s.l., 42.3369N 11.9482E, 17.IV.2021, Selis M. leg.

1 ♀, IT, Lazio, VT, Viterbo, Necropoli etrusca di Norchia, 135 m a.s.l., 42.3369N 11.9482E, 29.IV.2023, Selis M. leg.

Identification

S. declivis can be immediately distinguished from all other European species of *Symmorphus* by the peculiar morphology of the metasoma, with the second tergite being basally angled and the second sternite having a basal transverse carina delimiting a vertical anterior face (figure 1A). All other European species of the genus have evenly rounded second tergite and second sternite. This peculiar metasomal morphology prompted Blüthgen (1943) to create a dedicated monotypic subgenus for *S. declivis*, *Koptodynerus* Bluthgen, later synonymized by Cumming and van der Vecht (1986) after observing that the same metasomal morphology is found in various other *Symmorphus* species unrelated to *S. declivis*.

DNA barcode

Five specimens were barcoded, one from the type locality of Podčetrtek in Slovenia (GenBank Accession Number: PP190335), one from Cyprus (PP190336), and three from the new Italian localities of Norchia (PP190334) and Santa Severa (PP190337, PP190338). No genetic distance was found between the five barcoded specimens, which share the same COI sequence. The closest species is *S. murarius* with a genetic distance of 16.00-17.07% (mean 16.34%), while all other available species differ by a greater distance, with a range of mean distances of 37.21-45.27% (figure 2). The genetic data place *S. declivis* clearly within the nominal subgenus, further confirming the synonymy of the former subgenus *Koptodynerus* (Cumming et van der Vecht 1986).

Ecology

Little or nothing is known about the natural history of *S. declivis*, but examination of old and new data allows some considerations to be made. Blüthgen (1961) assumed the genus *Symmorphus* to be univoltine, but Gusenleitner (1969) stated that this could not be the case of *S. declivis*, bringing the finding of the species in September in Cyprus as proof. Looking at all the data published by Cumming (1989), we agree that *S. declivis* should have two generations, at least in some parts of its range.

Arens (2012) noted that this species in Peloponnese is usually found in densely wooded areas. This applies to the three Italian localities too, all characterized by dense woods with scarce or absent human activity and abundant dead wood, which may serve as nesting sites. The 2021 specimen from Norchia was collected sweeping branches of *Sambucus* sp. at short distance from two large fallen trees (figure 1C), while the series from Santa Severa was mostly collected on a path crossing a forest with large quantities of dead wood (mostly *Acer* sp., *Quercus* spp. and *Cercis siliquastrum* L.), flying few centimetres from the ground and collecting mortar for cell partitions (figure 1D).

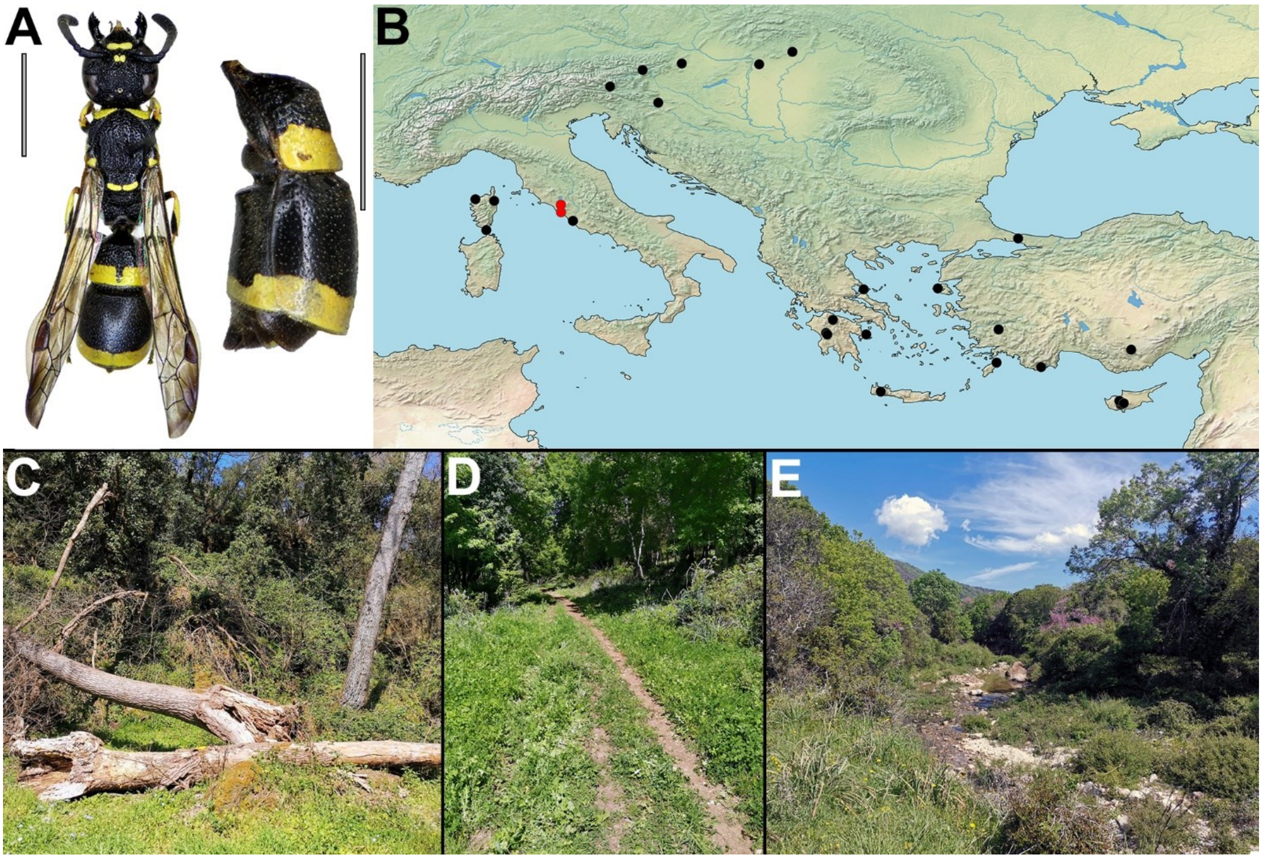

Figure 1. *S. declivis*. **(A)** female habitus in dorsal view and metasoma in lateral view (scale bars: 2 mm); **(B)** distribution map (black dots: literature data; red dots: new data); **(C)** collecting site in Norchia; **(D-E)** collecting site in Santa Severa.

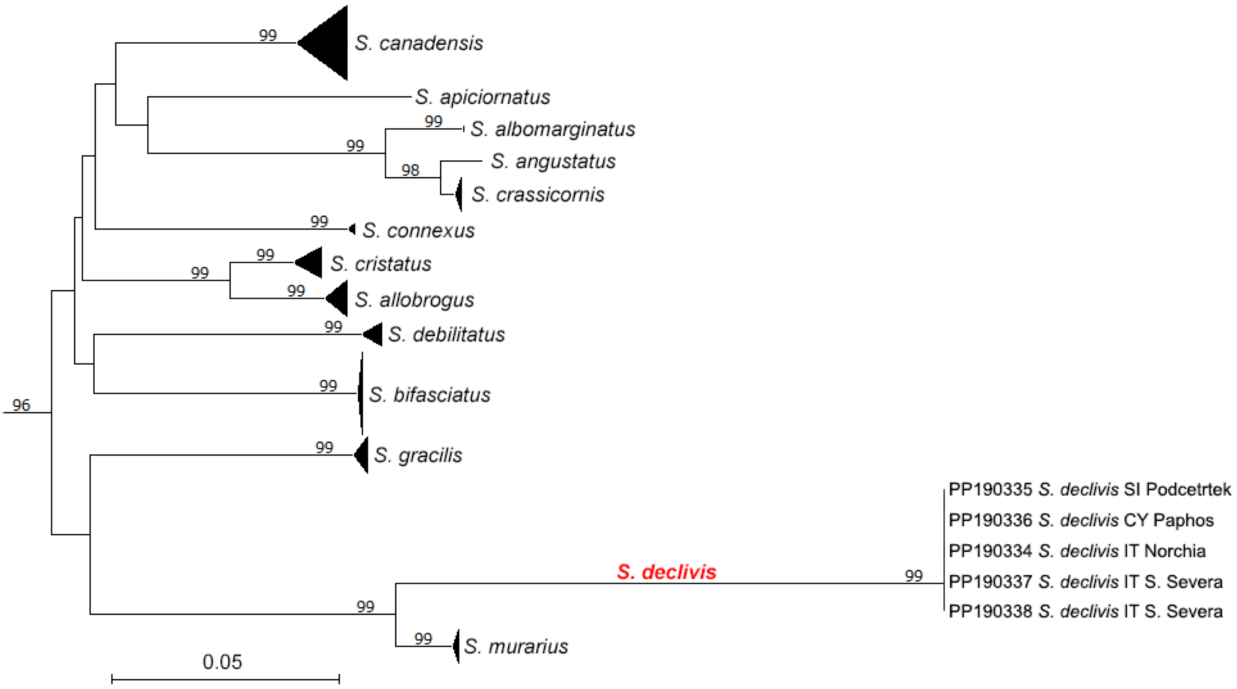

Figure 2. Phylogenetic tree (NJ) of *Symmorphus* based on the mitochondrial gene COI. Numbers above branches indicate posterior probabilities, omitting values below 75. Except for *S. declivis*, branches of each species are compressed for better visualization of the tree, and outgroup taxa are not shown. All the GenBank and BOLD accession numbers are reported in the material and methods section.

Only one of the females from Santa Severa was found flying along a small stream (Rio Fiume), visiting young *Salix* sp. bushes infested by first instar larvae of *Chrysomela populi* L. (figure 1E). *Symmorphus* species are known to provision nests with Chrysomelid larvae, therefore we can assume the female was hunting the small larvae for provisioning. Interestingly, the same locality was visited in May, with the same bushes infested by fully grown *C. populi* larvae and constantly visited by females of two larger *Symmorphus* species, *S. crassicornis* and *S. gracilis*, but no specimens of *S. declivis* were collected. Probably, *S. declivis* flies earlier than other *Symmorphus* species, taking advantage of the preyed larvae being first instar and of adequate size. The 2023 specimen from Norchia was collected on flowers of *Quercus* sp.

Distribution

Austria, Cyprus, France (Corsica), Greece (including Crete), Hungary, Italy, Slovenia, Turkey (Harttig, 1932; Giordani Soika, 1942; 1963; 1970; Blüthgen, 1961; Gusenleitner, 1969; Cumming, 1989; Standfuss and Standfuss, 2004; Borsato, 2006; Castro and Dvorak, 2010; Arens, 2012).

The available data (figure 1B) show a disjointed distribution with three recognizable populations: the first occurring in central Italy and Corsica, the second one in the Pannonian Basin and surrounding mountains, and the last one ranging from Greece in the West to Cyprus and central Turkey in the East. Comparison of females from these three populations (Italy, Slovenia, Rhodes and Cyprus) did not allow to find any morphological difference, and the conspecificity is confirmed by the genetic data too. Given the rarity and the ecological needs of the species, it is possible that it has gone unnoticed in the areas connecting the three known populations; targeted research in well-preserved forest environments in those areas could lead to the discovery of additional populations.

Discussion and conclusions

The study of the subfamily Eumeninae in Italy has always been somewhat neglected in comparison to other European countries, with the first reliable data being published by Giordani Soika and Borsato (1995) in the context of the checklist of the Italian fauna but reporting only the presence of each species in four macro-areas (North Italy, South Italy, Sardinia, and Sicily) without providing precise locality data. Borsato started publishing updates on the Italian fauna of Eumeninae, with two contributions providing several new data on the Sicilian (Borsato and Turrisi, 2004) and Sardinian (Borsato, 2006) species, and mentioning an upcoming contribution to the species occurring in peninsular Italy (corresponding to South Italy in the checklist of the Italian fauna) that was never published. The current knowledge of the peninsular species of Eumeninae is therefore very poor and fragmentary, with the few historical data being substantially unusable due to the important changes made in the taxonomy of the Eumeninae in recent times (Giordani Soika and Borsato, 1995).

Given this historical background and the absence for a long time of targeted research for Eumeninae in central Italy, it is not surprising that the presence of *S. declivis* has been unnoticed until now, for more than 80 years since its only record. In addition to the mentioned factors, another possible cause of the apparent rarity of this species are its ecological needs, as it seems to be strictly linked to well-preserved forest environments with abundant dead wood, an association already observed by Arens (2012) and supported by our findings. The collection of over 20 specimens over the course of an hour on a short stretch of trail supports the hypothesis that the species is not intrinsically rare as previously thought, but rather linked to increasingly rare and degraded environments in peninsular Italy.

Further faunistic research on the Eumeninae of peninsular Italy, preferably concentrated in environments with reduced anthropic impact, will certainly lead to the discovery of further finds of *S. declivis* and other species currently considered rare or absent in Italy.

References

ALTSCHUL S. F., GISH W., MILLER W., MYERS E. W., LIPMAN D. J.,1990.- Basic local alignment search tool.- *Journal of Molecular Biology*, 215 (3): 403-410.

ARENS W., 2012.- Die solitären Faltenwespen der Peloponnes (Hymenoptera: Vespidae: Raphiglossinae, Eumeninae) – 1. Teil.- *Linzer Biologische Beiträge*, 44 (1): 481-522.

BANK S., SANN M., MAYER C., MEUSEMANN K., DONATH A., PODSIADLOWSKI L., KOZLOV A., PETERSEN M., KROGMANN L., MEIER R., ROSA P., SCHMITT T., WURDACK M., LIU S., ZHOU X., MISOF B., PETERS R. S., NIEHUIS O., 2017.- Transcriptome and target DNA enrichment sequence data provide new insights into the phylogeny of vespid wasps (Hymenoptera: Aculeata: Vespidae).- *Molecular Phylogenetics and Evolution*, 116: 213-226.

BLÜTHGEN P., 1943.- Taxonomische und biologische Notizen über paläarktische Faltenwespen (Hym. Vespidae).- *Stettiner Entomologische Zeitung*, 104: 149-158.

BLÜTHGEN P., 1961.- Die Faltenwespen Mitteleuropas (Hymenoptera, Diploptera).- *Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin, Klasse für Chemie, Geologie und Biologie*, 2: 1-251.

BORSATO W., 2006.- Contributo alla conoscenza degli Eumenidae di Sardegna e note sulla fauna corsa (Hymenoptera, Vespoidea).- *Bollettino del Museo Civico di Storia Naturale di Venezia*, 57: 123-147.

BORSATO W., TURRISI G. F., 2004.- Contributo alla conoscenza degli Eumenidae di Sicilia (Hymenoptera, Vespoidea).- *Bollettino del Museo Civico di Storia Naturale di Venezia*, 55: 127-150.

CARPENTER J. M., CUMMING J. M., 1985.- A character analysis of the North American potter wasps (Hymenoptera: Vespidae; Eumeninae).- *Journal of Natural History*, 19: 877-916.

CASTRO L., DVORAK L., 2010.- New and noteworthy record of vespid wasps (Hymenoptera: Vespidae) from the Palaearctic region (III).- *Acta Musei Moraviae, Scientiae Biologicae (Brno)*, 95 (2): 37-53.

CILIA G., FLAMINIO S., QUARANTA M., 2022.- A novel and non-invasive method for DNA extraction from dry bee specimens.- *Scientific Reports*, 12: 11679.

CUMMING J. M., 1989.- Classification and evolution of the eumenine wasp Genus *Symmorphus* Wesmael (Hymenoptera: Vespidae).- *Memoirs of the Entomological Society of Canada*, 148: 1-168.

CUMMING J. M., LEGGETT F. L., 1985.- Cephalic foveae of eumenine wasps (Hymenoptera: Vespidae).- *Journal of Natural History*, 19: 1197-1207.

CUMMING J. M., VAN DER VECHT J., 1986.- New Oriental species of *Symmorphus* Wesmael, with description of a new subgenus (Hymenoptera: Vespidae; Eumeninae).- *Entomologische Berichten*, 46: 23-32.

FELSENSTEIN J., 1985.- Confidence limits on phylogenies: an approach using the bootstrap.- *Evolution*, 39: 783-791.

FLAMINIO S., BORTOLOTTI L., CILIA G., 2023a.- First report of *Xylocopa aestuans* in Italy: a new species for Europe?- *Bulletin of Insectology*, 76 (2). 161-166.

FLAMINIO S., CILIA G., VELLA A., QUARANTA M., LO VERDE G., 2023b.- *Melitta schmiedeknechti* (Hymenoptera Apoidea, Melittidae), a new species for the fauna of Italy.- *Bulletin of Insectology*, 76 (2): 315-319.

FOLMER O., BLACK M., HOEH W., LUTZ R., VRIJENHOEK R., 1994.- DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrated.- *Molecular Marine Biology and Biotechnology*, 3 (5): 294-299.

GIORDANI SOIKA A., 1942.- Vespidi mediterranei nuovi o poco noti.- *Bollettino della Società Entomologica Italiana*, 74 (5): 51-61.

GIORDANI SOIKA A., 1963.- Notulae vespidologicae XIX. *Symmorphus* paleartici nuovi o poco noti.- *Bollettino della Società Entomologica Italiana*, 93: 122-126.

GIORDANI SOIKA A., 1970.- Contributo alla conoscenza degli Eumenidi del Medio Oriente. Missione Giordani Soika in Iran 1965, III.- *Bollettino del Museo Civico di Storia Naturale di Venezia*, 20-21: 27-183.

GIORDANI SOIKA A., BORSATO W., 1995.- Hymenoptera Vespoidea, pp. 1-9. In: *Checklist delle specie della fauna italiana*, 103 (MINELLI A., RUFFO S., LA POSTA S., Eds).- Calderini, Bologna, Italy.

GUSENLEITNER J., 1969.- Faunistische Nachrichten aus der Steiermark (XV/5): *Symmorphus* (*Koptodynerus*) *declivis* (Harttig, 1932). Zweitfund für Österreich (Insects, Hymenoptera).- *Mitteilungen des Naturwissenschaftlichen Vereins für Steiermark*, 99: 213-214.

GUSENLEITNER J., 1999.- Bestimmungstabellen mittel- und südeuropäischer Eumeniden (Vespoidea, Hymenoptera) Teil 12. Die Gattung *Symmorphus* Wesmael 1836.- *Linzer Biologische Beiträge*, 31 (2): 585-592.

HALL T. A. A., 1999.- BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.- *Nucleic Acids Symposium Series*, 41: 95-98.

HARTTIG G., 1932.- Eine neue paläarktische *Symmorphus*-art (Hym. Vesp.).- *Mitteilungen der Deutschen Entomologischen Gesellschaft*, 3: 73-75.

KIMURA M., 1980.- A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences.- *Journal of Molecular Evolution*, 16: 111-120.

LUO L., CARPENTER J. M., CHEN B., LI T., 2022.- First comprehensive analysis of both mitochondrial characteristics and mitogenome-based phylogenetics in the subfamily Eumeninae (Hymenoptera: Vespidae).- *Insects*, 13 (6): 529.

PIEKARSKI P. K., CARPENTER J. M., LEMMON A. R., LEMMON E. M., SHARANOWSKI B. J., 2018.- Phylogenomic evidence overturns current conceptions of social evolution in wasps (Vespidae).- *Molecular Biology and Evolution*, 35 (9): 2097-2109.

SAITOU N., NEI M., 1987.- The neighbor-joining method: a new method for reconstructing phylogenetic trees.- *Molecular Biology and Evolution*, 4: 406-425.

SELIS M., 2023.- Illustrated key to the genera and a checklist of Italian Vespidae (Hymenoptera).- *Fragmenta Entomologica*, 55 (1): 63-88.

SELIS M., CILIA G., WOOD T. J., SOON V., 2024.- Taxonomic revision of the *Stenodynerus fastidiosissimus* species-group in Western Europe and North Africa (Hymenoptera: Vespidae: Eumeninae).- *Zootaxa*, 5418 (1): 34-56.

STANDFUSS K., STANDFUSS L., 2004.- Zum aktuellen Vorkommen solitärer Faltenwespen der Ölbaumzone in Südost-Thessalien/Griechenland (Hymenoptera: Vespoidea: Eumenidae).- *Entomofauna*, 25 (15): 249-260.

TAMURA K., STECHER G., KUMAR S., 2021.- MEGA: Molecular Evolutionary Genetics Analysis version 11.- *Molecular Biology and Evolution*, 38: 3022-3027.

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