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First report of *Xylocopa aestuans* in Italy: a new species for Europe?

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Abstract

Xylocopa (*Koptortosoma*) *aestuans* (L. 1758) (Hymenoptera Apoidea), a species native to Southeast Asia, is reported for the first time in Europe, following the collection of seven individuals in Italy (Ragusa, Sicily). The barcode of COI region confirmed the identification of the specimens. Since it is not yet clear whether the species can reproduce outside its range of origin, monitoring the area is strongly recommended.

Key words: carpenter bees, *Xylocopa*, invasive species, Apoidea, Sicily, barcoding.

Introduction

The spread of species beyond their native range can lead to invasions that generate significant damage to local ecosystems and native species, with ecological and economic impacts (Vilà *et al*., 2009; Bellard *et al*., 2016; Early *et al*., 2016; Diagne *et al*., 2021; Najberek *et al*., 2021; Lanner *et al*., 2022). The introduction of not native species is strictly related to globalisation and the increase in transport between different geographic areas (Seebens *et al*., 2015). Besides, climate change may accelerate and favour the establishment of introduced species (Walther *et al*., 2009; Huang *et al*., 2011; Bellard *et al*., 2018; da Silva *et al*., 2020).

The genus *Xylocopa* Latreille 1802 is present in Europe with 9 species, of which two, *Xylocopa* (*Xylocopoides*) *virginica* (L. 1771) (Falk and Lewington, 2015) and *Xylocopa* (*Afroxylocopa*) *nigrita* (F. 1775) are non-native for Europe (Rasmont *et al*., 2017). A third species, *Xylocopa* (*Koptortosoma*) *pubescens* Spinola 1838, with a distribution from Senegal, North Africa, as far as Pakistan and South India has been reported from Greece and Cyprus (Rasmont *et al*., 2017) but also reported for other European countries, specifically Canary Islands (Ruiz *et al*., 2020), Spain (Ortiz-Sanchez and Pauly, 2016) and France (le Divelec *et al*., 2022). Also, *Xylocopa* (*Koptortosoma*) *caffra* (L. 1767) was reported in Zakynthos (Greece), based on specimens collected in the 19th century (Vicidomini, 2007). No other *X. caffra* individuals were observed afterwards, thus suggesting a case of mislabelling or single introduction.

Only three species are native to Italy: *Xylocopa* (*Copoxyla*) *iris* (Christ 1791), *Xylocopa* (*Xylocopa*) *valga* Gerstaecker 1872 and *Xylocopa* (*Xylocopa*) *violacea* L. 1758. During a survey carried out in Ragusa (Sicily, Italy) from August to December 2022, seven *Xylocopa* specimens with a different habitus to the species present in Italy were noticed and collected and turned out to be *Xylocopa* (*Koptortosoma*) *aestuans* (L. 1758), a species never been reported in Europe before.

We, therefore, report for the first time the presence of *X. aestuans* in Italy and, consequently, Europe.

Materials and methods

Examined specimens

2 ♀, Italy, Sicily, Ragusa, 36°54'06"N 14°25'24.5"E, 18.VIII.2022, in flight, Cilia G. legit (figure 1).

2 ♀, Italy, Sicily, Ragusa, 36°54'06"N 14°25'24.5"E, 17.IX.2022, *Lantana camara*, Cilia E. legit.

2 ♀, Italy, Sicily, Ragusa, 36°54'06"N 14°25'24.5"E, 6.XI.2022, in flight, Cilia E. legit.

1 ♂, Italy, Sicily, Ragusa, 36°54'06"N 14°25'24.5"E, 23.XII.2022, in flight, Cilia G. legit (figure 2).

The specimens were collected during an entomological survey in Sicily, in Ragusa province (figure 3), in coastal areas approximately 25 km west of the City of Ragusa, and subsequently brought to the laboratory and identified using comparison material and taxonomic literature (Lieftinck, 1964; Hurd and Moure, 1963). Besides, a DNA analysis was performed to confirm the morphological identification.

Molecular analysis and DNA Barcode

Two different methods were used to generate DNA barcodes.

Total DNA was extracted from the right hind leg (Villalta *et al*., 2021), dissected from one female and one male individuals. At the same time, a sterile microbiological swab soaked with digestion buffer was gently rubbed over the sternites of each *Xylocopa* specimen, following a specific protocol (Cilia *et al*., 2022). Finally, the individuals were air-dried. A yellow label with the code "XAI_001" and "XAI_002" was placed under the voucher specimens.

Legs and swabs were placed in a 2 mL microtube filled with 1 mL digestion buffer and incubated for 18 hours at 56 °C. Total DNA purification was performed using a phenol:chloroform extraction (UltrapureTM Phenol:Chloroform:Isoamyl Alcohol, ThermoFisher Scientific, Waltham, MA, USA), as reported (Cilia *et al*., 2022).

The obtained DNAs were quantified using the spectrophotometer Infinite 200 PRO NanoQuant™ (TECAN Life Technologies, Männedorf, Switzerland) and stored

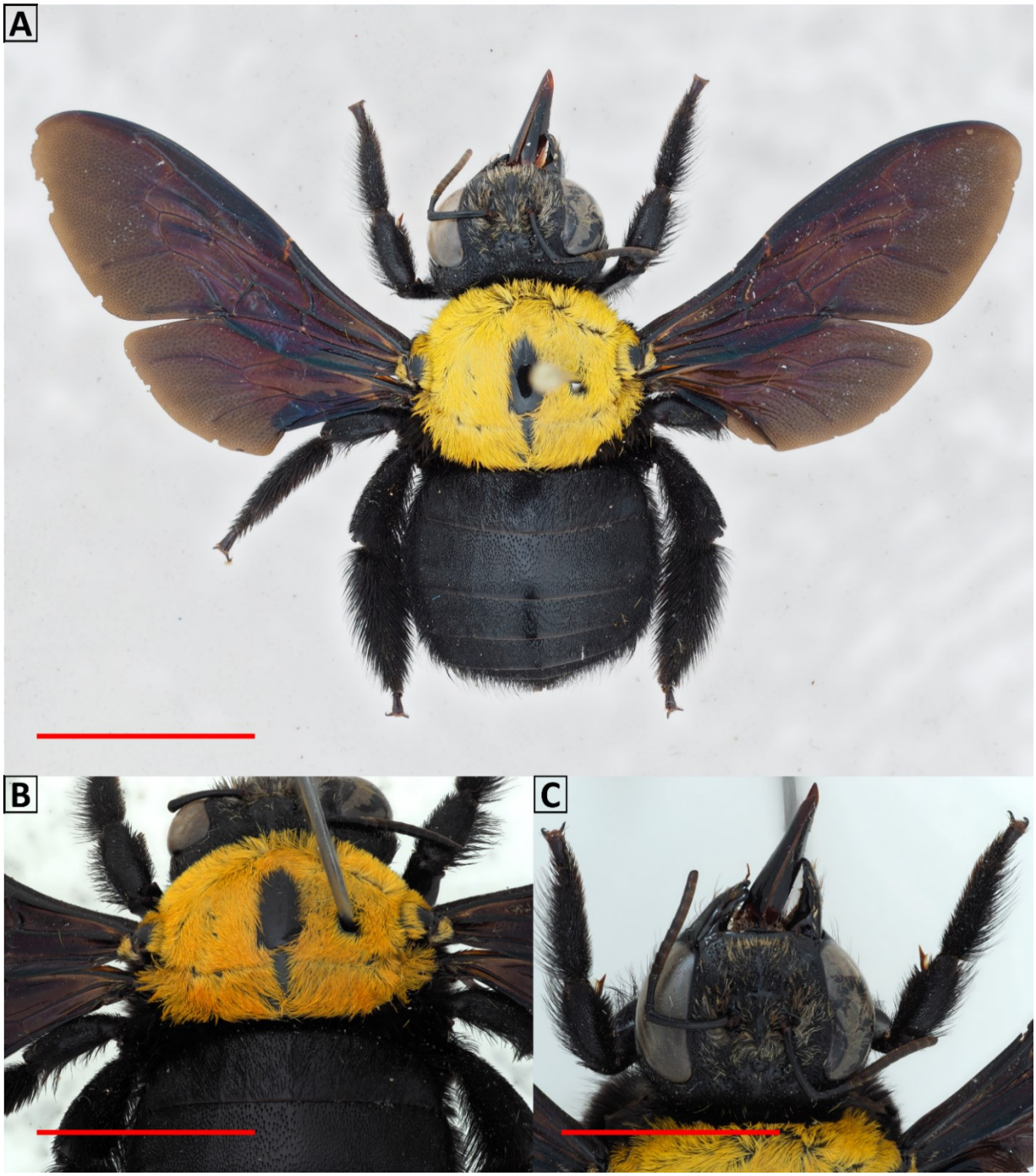

Figure 1. *X. aestuans*, Sicily (Italy), female. Dorsal habitus (**A**), scutum (**B**) and head from dorsal (**C**). Scale bar 5 mm in red.

at −20 °C until the analysis. Double-distilled Rnase-Dnase-free water was used as a negative control for all of these processes.

Amplification of mitochondrial DNA (mtDNA) was performed using primer pairs able to amplify a 710-bp fragment within the highly conserved region coding for the Cytochrome C oxidase subunit I (COI) gene: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3) (Folmer *et al*., 1994). The PCR was performed in 25 µl of volume using HotStarTaq Polymerase (Qiagen, Hilden, Germany) following the manufacturer's instructions using 5 µl of DNA, forward and reverse probes (500 nM). The PCR assay was performed on Applied Biosystems® 2720 Thermal Cycler (ThermoFisher Scientific) and samples were amplified, after an initial activation at 95 °C for 15 minutes, through 35 cycles (1 minute at 95 °C, 1 minute at 40 °C, and 1.5 minutes at 72 °C), followed by a final extension at 72 °C for 7 minutes. All amplicons were visualized on a 1.5% agarose gel. The obtained amplicons were purified using ExoSAP-IT Express (ThermoFisher Scientific) and they were sequenced through

Figure 2. *X. aestuans*, Sicily (Italy), male. Dorsal habitus (**A**), head from dorsal (**B**) and aedeagus in dorsal view (**C**). Scale bar: 5 mm in red; 1 mm in black.

the standard Sanger methodology (BMR Genomics, Padua, Italy). 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).

Results

Identification

The collected specimens were identified as *Xylocopa* (*Koptortosoma*) due to the posterior margin of the scutellum projecting beyond the metanotum and the mandibles bidentate at the apex (the posterior margin of the scutellum does not project beyond the metanotum and tridentate mandibles in the subgenus *Copoxyla* Maa 1954 and *Xylocopa* Latreille 1802). The specimens were identified as *X. aestuans* due to the impunctate and raised line on the clypeus, the head being distinctly broader than long and the interocellar distance being less than the ocellocular distance, distinguishing it from *X. pubescens* which has no impunctate raised line on the clypeus and the interocellular distance being at least ¾ of the ocellular distance (Lieftinck, 1964). The male is morphologically characterized by yellowish hairiness covering the body, first tergum with the dorsal surface abruptly and angled

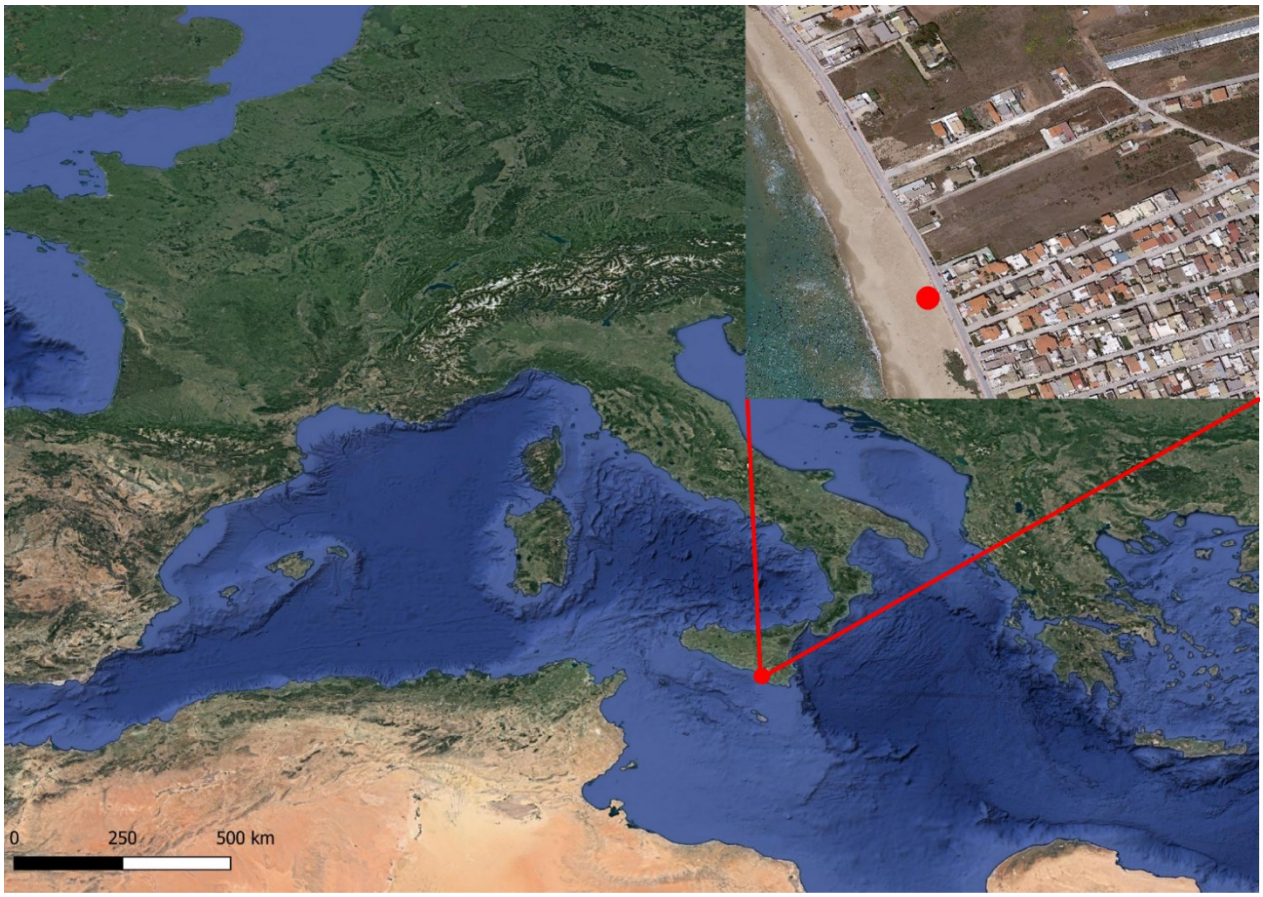

Figure 3. Point of occurrence in Italy of *X. aestuans* (in red). Source: Google Earth®.

separated from the declivous surface (subhorizontal dorsal surface of first tergum sloping or rounding into declivous anterior surface in *Copoxyla* and *Xylocopa*) and gradulus of first tergum transverse (gradulus of T1 curved posteriorly at the side in *Copoxyla* and *Xylocopa*) (Michener, 2007). Furthermore, *X. aestuans* males can be separated from *X. pubescens* by observing the genitalia (Lieftinck, 1964).

DNA barcode

The mtDNA was successfully amplified and sequenced from the analysed specimens. The same sequence was obtained using the two different methods from the investigated *Xylocopa* specimens.

The BLAST analysis confirmed the similarity to *X. aestuans* (sequence deposited using the synonym *X. confusa*) [Query cover: 98%; E-value: 3e-172; Percentage of identity: 94.17%] sampled in 2016 in Laos (Kawazoe *et al*., 2008).

The sequence was deposited in GenBank (OP894630 and OQ473440).

Ecology

X. aestuans is a subsocial species. Like all species of the *Xylocopa* species, it nests in dead wood such as hollow stems, trunks, old xylophagous insect tunnels or even old *Xylocopa* nests (Ng Kia Yi, 2014). *X. aestuans* is polylectic. The plants present around the collection site were several blooming specimens of *Lantana* L. (Verbenaceae), commonly used in southern Italy as an ornamental plant, and a few specimens belonging to the genus *Carduus* L., *Cirsium* Mill. and *Sylibum* Adanson. Given that the specimens were collected in flight and only two in *Lantana camara*, no interactions with local flora could be reported.

Distribution

The native distribution of the species is Southeast Asia, with the main focus in the Malayan subregion like Java, Malaysia, and Singapore eastwards to Lombok.

Discussion

Five not native bee species are present to date in Europe: *Megachile scultpuralis* Smith 1853 (Lanner *et al*., 2022), *Megachile disjunctiformis* Cockerell 1911 (Bortolotti *et al*., 2018), *X. pubescens* (Ortiz-Sanchez and Pauly, 2016; Ruiz *et al*., 2020), *X. nigrita* (Rasmont *et al*., 2017) and *X. virginica* (Falk and Lewington, 2015). The finding of the seven specimens in Sicily is the first report of *X. aestuans* for Italy and the first report outside its native range. Previously, one specimen of *X. aestuans* seemed to have been reported for Germany (Witt, 2022) but, from the taxonomic characters and the photos in the article, it can be deduced that the specimen has been misidentified. In particular, the basitibial plate does not correspond to the subgenus *Koptortosoma* Gribodo 1894 and the dense yellowish hair on the mesopleurae rather leads to the species *X. virginica*.

The not native species was probably introduced to Italy accidentally, within woods or plants, as reported in other cases for aboveground nesting bees (Okabe *et al*., 2010; Dahlberg *et al*., 2013; Russo, 2016; Ruiz *et al*., 2020; le Divelec *et al*., 2022). The area around the point of discovery is characterised by strong floricultural activities, resulting in a strong import of plants, substrates and wood. Also, this part of Sicily is characterised by a high density of greenhouses for vegetables and timber manipulation for agricultural products and related transports. Besides, 40 km south of the sampled point is the Port of Pozzallo (36°43'20"N 14°50'85"E), which handles approximately 1,000,000 tons of goods annually.

Given the casual observation of the specimens collected, it is not possible to know whether the species has actually been established in the area, even if the presence of males could generate further generations. Considering the Mediterranean sub-tropical climate of coastal Sicily (Fantappiè *et al*., 2015), and the tolerance that *X. aestuans* seems to show in its range of origin (Ng Kia Yi, 2014), it cannot be ruled out that the species, may establish in Italy, specifically in Sicily. It is therefore recommended to monitor the area of discovery and, more generally, the province of Ragusa, in order to ascertain the size of a possible established population.

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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: 403-410.

BELLARD C., LEROY B., THUILLER W., RYSMAN J. F., COURCHAMP F., 2016.- Major drivers of invasion risks throughout the world.- *Ecosphere*, 7 (3): e01241.

BELLARD C., JESCHKE J. M., LEROY B., MACE G. M., 2018.- Insights from modeling studies on how climate change affects invasive alien species geography.- *Ecology & Evolution*, 8: 5688-5700.

BORTOLOTTI L., LUTHI F., FLAMINIO S., BOGO G., SGOLASTRA F., 2018.- First record of the Asiatic bee *Megachile disjunctiformis* in Europe.- *Bulletin of Insectology*, 71: 143-149.

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

DAHLBERG L., HAUSER M., YANEGA D., 2013.- Japanese carpenter bee, *Xylocopa appendiculata* Smith 1852 (Hymenoptera: Apidae) potentially established in Santa Clara County, first record for North America.- *Pan-Pacific Entomologist*, 89: 226-229.

DA SILVA C. R. B., BEAMAN J. E., DOREY J. B., BARKER S. J., CONGEDI N. C., ELMER M. C., GALVIN S., TUIWAWA M., STEVENS M. I., ALTON L. A., SCHWARZ M. P., KELLERMANN V., 2020.- Climate change and invasive species: a physiological performance comparison of invasive and endemic bees in Fiji.- *Journal of Experimental Biology*, 224: jeb230326.

DIAGNE C., LEROY B., VAISSIÈRE A. C., GOZLAN R. E., ROIZ D., JARIĆ I., SALLES J. M., BRADSHAW C. J. A., COURCHAMP F., 2021.- High and rising economic costs of biological invasions worldwide.- *Nature*, 592: 571-576.

EARLY R., BRADLEY B. A., DUKES J. S., LAWLER J. J., OLDEN J. D., BLUMENTHAL D. M., GONZALEZ P., GROSHOLZ E. D., IBAÑEZ I., MILLER L. P., SORTE C. J. B., TATEM A. J., 2016.- Global threats from invasive alien species in the twenty-first century and national response capacities.- *Nature Communications*, 7: 12485.

FALK S., LEWINGTON R., 2015.- *Field guide to the bees of Great Britain and Ireland*.- Bloomsbury, London, UK.

FANTAPPIÈ M., PRIORI S., COSTANTINI E. A. C., 2015.- Soil erosion risk, Sicilian Region (1:250,000 scale).- *Journal of Maps*, 11: 323-341.

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 invertebrates.- *Molecular Marine Biology and Biotechnology*, 3: 294-299.

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.

HANNAN M. A., ALQARNI A. S., OWAYSS A. A., ENGEL M. S., 2012.- The large carpenter bees of central Saudi Arabia, with notes on the biology of *Xylocopa sulcatipes* Maa (Hymenoptera, Apidae, Xylocopinae).- *Zookeys*, 201: 1-14.

HUANG D., HAACK R. A., ZHANG R., 2011.- Does global warming increase establishment rates of invasive alien species? A centurial time series analysis.- *PLoS ONE*, 6: e24733.

HURD P. D., MOURE J. S., 1963.- *A classification of the large carpenter bees (Xylocopini) (Hymenoptera : Apoidea)*.- University of California Publications in Entomology 29, University of California Press, Berkeley, USA.

KAWAZOE K., KAWAKITA A., KAMEDA Y., KATO M., 2008.- Redundant species, cryptic host-associated divergence, and secondary shift in *Sennertia* mites (Acari: Chaetodactylidae) associated with four large carpenter bees (Hymenoptera: Apidae: Xylocopa) in the Japanese island arc.- *Molecular Phylogenetics and Evolution*, 49: 503-513.

LANNER J., DUBOS N., GESLIN B., LEROY B., HERNÁNDEZ-CASTELLANO C., DUBAIĆ J. B., BORTOLOTTI L., CALAFAT J. D., ĆETKOVIĆ A., FLAMINIO S., LE FÉON V., MARGALEF-MARRASE J., ORR M., PACHINGER B., RUZZIER E., SMAGGHE G., TUERLINGS T., VEREECKEN N., MEIMBERG H., 2022.- On the road: anthropogenic factors drive the invasion risk of a wild solitary bee species.- *Science of the Total Environment*, 827: 154246.

LE DIVELEC R., SEMAL L., SZUBA M., ROME Q., 2022.- Sur la présence de *Xylocopa* (*Koptortosoma*) *pubescens* Spinola, 1838, en France métropolitaine (Hymenoptera, Apidae).- *Bulletin de la Société Entomologique de France*, 127: 205-212.

LIEFTINCK M. A., 1964.- The identity of *Apis aestuans* Linné, 1758, and related Old World carpenter-bees (*Xylocopa* Latr.).- *Tijdschrift voor Entomologie*, 107: 137-158.

MICHENER C. D., 2007.- *The bees of the world*, 2nd edition.- The Johns Hopkins University Press, Baltimore, USA.

NAJBEREK K., KOSIOR A., SOLARZ W., 2021.- Alien balsams, strawberries and their pollinators in a warmer world.- *BMC Plant Biology*, 21: 500.

NG KIA YI, 2014.- *Xylocopa aestuans* - carpenter bee.- Taxo4254 - Wiki.nus. [online] URL: https://wiki.nus.edu.sg/display/TAX/Xylocopa+aestuans+-+Carpenter+Bee#XylocopaaestuansCarpenterBee-[2.1]Xylocopaaestuans (accessed November 27, 2022).

OKABE K., MASUYA H., KAWAZOE K., MAKINO S., 2010.- Invasion pathway and potential risks of a bamboo-nesting carpenter bee, *Xylocopa tranquebarorum* (Hymenoptera: Apidae), and its micro-associated mite introduced into Japan.- *Applied Entomology and Zoology*, 45: 329-337.

ORTIZ-SANCHEZ F. J., PAULY A., 2016.- Primera cita de *Xylocopa* (*Koptortosoma*) *pubescens* Spinola, 1838 (Hymenoptera, Apidae) en Europa occidental.- *Boletín de la Asociación Española de Entomología*, 40 (3-4): 499-501.

RASMONT P., DEVALEZ J., PAULY A., MICHEZ D., RADCHENKO V. G., 2017.- Ajouts à la liste de l'UICN des Abeilles sauvages d'Europe (Hymenoptera : Apoidea).- *Annales de la Société Entomologique de France*, 53: 17-32.

RUIZ C., SUÁREZ D., NARANJO M., DE LA RÚA P., 2020.- First record of the carpenter bee *Xylocopa pubescens* (Hymenoptera, Apidae) in the Canary Islands confirmed by DNA barcoding.- *Journal of Hymenoptera Research*, 80: 169-175.

RUSSO L., 2016.- Positive and negative impacts of non-native bee species around the world.- *Insects*, 7 (4): 69.

SEEBENS H., ESSL F., DAWSON W., FUENTES N., MOSER D., PERGL J., PYŠEK P., VAN KLEUNEN M., WEBER E., WINTER M., BLASIUS B., 2015.- Global trade will accelerate plant invasions in emerging economies under climate change.- *Global Change Biology*, 21: 4128-4140.

VICIDOMINI S., 2007.- Distribuzione della tribù Xylocopini (Hymenoptera: Apidae: Xylocopinae) in Grecia: specie africane segnalate sull' Isola di Zante.- *Annales Musei Goulandris*, 11: 311-314.

VILÀ M., BASNOU C., GOLLASCH S., JOSEFSSON M., PERGL J., SCALERA R., 2009.- One hundred of the most invasive alien species in Europe, pp. 265-268. In: *Handbook of alien species in Europe*.- Springer, Dordrecht, The Netherlands.

VILLALTA I., LEDET R., BAUDE M., GENOUD D., BOUGET C., CORNILLON M., MOREAU S., COURTIAL B., LOPEZ-VAAMONDE C., 2021.- A DNA barcode-based survey of wild urban bees in the Loire Valley, France.- *Scientific Reports*, 11: 4770.

WALTHER G.-R., ROQUES A., HULME P. E., SYKES M. T., PYŠEK P., KÜHN I., ZOBEL M., BACHER S., BOTTA-DUKÁT Z., BUGMANN H., 2009.- Alien species in a warmer world: risks and opportunities.- *Trends in Ecology and Evolution*, 24: 686-693.

WITT R., 2022.- Nachweis von *Xylocopa aestuans* (Linné, 1758) in Deutschland (Hymenoptera: Apiformes).- *Ampulex*, 13: 68-69.

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