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Tachyancistrocerus rhodensis in Italy, an expanding species?

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Abstract

Tachyancistrocerus rhodensis (de Saussure) (Hymenoptera: Vespidae: Eumeninae) is newly recorded from Continental Italy and Sardinia. The species was found in several localities ranging from Piedmont and Emilia-Romagna in the North to Calabria and Apulia in the South, widely expanding its distribution in Italy, previously limited to few records from Sicily. The possibly recent and invasive origin of the newly recorded populations is discussed.

Key words: solitary wasps, DNA barcoding, distribution, range expansion, *Tachyancistrocerus*.

Introduction

Tachyancistrocerus Giordani Soika is a small genus of Palearctic eumenine wasps, presently including 14 species ranging from the Maghreb, Sicily and Greece in the West to India and China in the East, with some species descending into the Arabian Peninsula (Giordani Soika, 1970; Gusenleitner, 1993; 2006; 2012; 2013; Li *et al.*, 2022). At the current state of knowledge, only *Tachyancistrocerus rhodensis* (de Saussure) has been reported for Italy (Selis, 2023), based on a few finds in Sicily and Lampedusa distributed over more than a century (André, 1883; DeStefani, 1889; Giordani Soika, 1942; Pagliano, 2003; Borsato and Turrisi, 2004). The study of material collected by the authors or received from other colleagues has, however, demonstrated the presence of *T. rhodensis* in numerous other Italian regions, all collected starting from 2013. These new findings are presented here, with hypotheses on the origin of these new populations.

Materials and methods

The specimens were identified using comparison material and taxonomic literature (Selis, 2023) under a Leica MZ6 stereoscopic microscope. Photos (figure 1) were acquired using a Canon EOS 1300D equipped with an inverted Canon EF-S 18-55 mm lens and extension tubes, stacked with CombineZP, processed, and assembled with Photoshop CC 2018.

The distribution maps (figure 2) were created using the online tool SimpleMappr (Shorthouse, 2010), including all data from examined material, reliable records from literature, and observations mined from iNaturalist.org, entomological Facebook groups and the "Forum Entomologi Italiani" (www.entomologiitaliani.net). Photographic records of living specimens are reported with "ph." indicating the author of the photos.

The following acronyms are used for museum collections: MIZT = Università di Torino, Italy; MSNVE = Museo Civico di Storia Naturale di Venezia, Italy; TUZ = Natural History Museum, University of Tartu, Estonia.

Molecular analysis and DNA barcode

Total DNA was extracted as previously reported by Selis *et al.* (2024a). Briefly, the right mid-leg was dissected from each wasp and was placed in 2 mL microtubes 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 DNA was 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 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). DNA sequences were deposited in GenBank (Accession Numbers PP472432-PP472460). The sequence JN934354, published by Turcinaviciene *et al.* (2014), was downloaded from GenBank.

The consensus sequences were processed with MEGA11 software (Tamura *et al.*, 2021): DNA alignment was made with Clustal Alignment, and intraspecific distances were calculated using the Kimura 2-parameter model (Kimura, 1980).

Figure 1. *Tachyancistrocerus rhodensis* (de Saussure). Habitus and face of both sexes, with detail of T1 (arrowhead pointing to the diagnostic post-carinal smooth area).

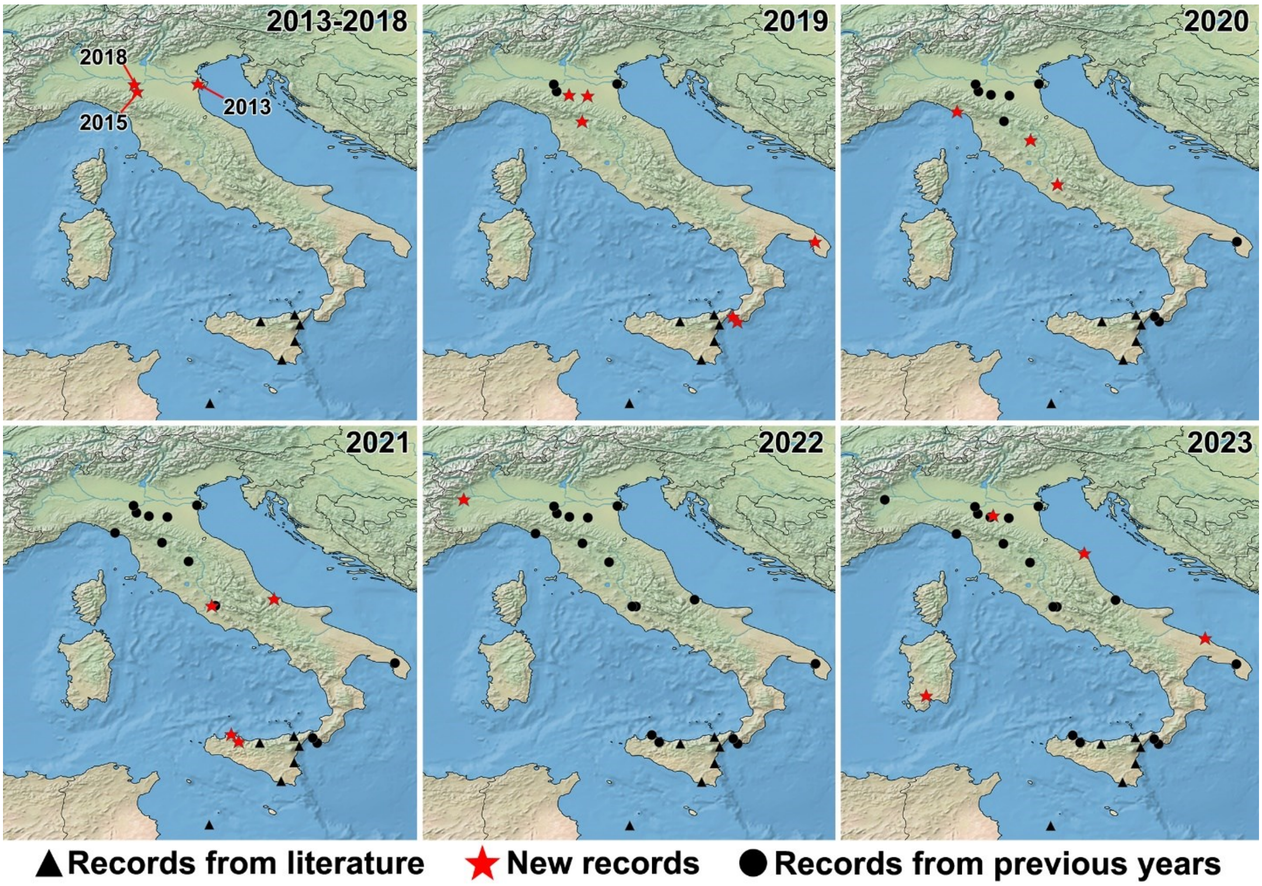

Figure 2. Distribution maps of the Italian records of *Tachyancistrocerus rhodensis* (de Saussure) divided by year.

Results

Examined specimens

IT, Apulia, BA, Cozze, 27.VII.2023, Cilia G. leg., Selis M. coll., 1♂ (Accession Number PP472455).

IT, Calabria, RC, Melito di Porto Salvo, 10 m, 37.9166N 15.7666E, 17.VIII.2019, 1♀, Femia A. leg. and coll.; same locality, 13-24.VIII.2020, 2♂ 2♀, Femia A. leg. and coll.

IT, Calabria, RC, Reggio Calabria, La Sorgente, 1 m, 38.0666N 15.6333E, 30.VIII.2019, Femia A. leg. and coll., 3♂.

IT, Emilia-Romagna, BO, Castel Maggiore, 9.VI.2020, Luthi F. leg., Selis M. coll., 2♂ (Accession Numbers PP472448, PP472449).

IT, Emilia-Romagna, FE, Mesola, 12.VII.2013, Maccapani D. leg., Selis M. coll., 1♂ (Accession Number PP472440).

IT, Emilia-Romagna, RE, Montecchio Emilia, 16.VI.2015, Violi M. leg., Selis M. coll., 1♂ (Accession Number PP472441).

IT, Emilia-Romagna, MO, Tabina, 70 m, 44.5994N 10.8155E, 22.VII-2.VIII.2019, 6♂ 1♀, Selis M. leg. and coll. (Accession Numbers PP472442, PP472443, PP472444); same locality, 19.VI-7.VII.2020, 6♂, Selis M. leg. and coll. (Accession Numbers PP472445, PP472446, PP472447).

IT, Lazio, RM, Guidonia Montecelio, Laghetto, 27.VI.2021, Pulvirenti E. leg., Selis M. coll., 1♂ (Accession Number PP472453).

IT, Lazio, RM, Tivoli, Lago di Favale, 19.VI.2020, Pulvirenti E. leg., Selis M. coll., 1♀ (Accession Number PP472454).

IT, Marche, AN, Ancona, Parco del Conero, 43.5403N 13.5808E, 14.VII.2023, Flaminio S. leg., Selis M. coll., 1♂ (Accession Number PP472452).

IT, Piedmont, TO, Torino, Falchera, 1♂, 5.VII.2022, MIZT; same locality, 27.VII.2022, 1♂, MIZT.

IT, Sardinia, CA, Decimoputzu, Rio Flumini Mannu, 15 m, 39.3458N 8.9302E, 25-31.VII.2023, Sechi D. leg., Selis M. coll., 2♂ 1♀ (Accession Numbers PP472457, PP472458, PP472459).

IT, Sicily, ME, Falcone, 24.VI.1941, Giordani Soika A. leg., MSNVE, 1♂ (Accession Number PP472460).

IT, Sicily, PA, Capaci, 26.VI.2021, Romano M. leg., Selis M. coll., 1♀ (Accession Number PP472456).

IT, Tuscany, AR, Cortona, Belvedere di Cortona, 495 m, 43.2666N 11.9833E, 13.IX.2020, Femia A. leg. and coll., 2♂ 1♀.

IT, Tuscany, FI, Sesto Fiorentino, Zambra, 46 m, 43.8166N 11.20E, 19.VII.2019, 3♂ 1♀, Femia A. leg., Femia A. and Selis M. coll. (Accession Numbers PP472450, PP472451); same locality, 29.VII-11.VIII.2019, 5♂ 8♀, Femia A. leg. and coll.; same locality, 20.VII.2020, 1♂, Femia A. leg. and coll.; same locality, 3.VIII.2020, 3♂, Femia A. leg. and coll.

GR, Calcidica, Vasilika, 7.VIII.2007, Fancello L. leg., Selis M. coll., 1♂ (Accession Number PP472435).

GR, Crete, Heraklion, 35.3368N 25.1546E, 5.X.2017, Soon V. leg., TUZ and Selis M. coll., 4♀ (Accession Number PP472437).

GR, Milos, Plathiena, 11.VIII.2019, Montanaro G. leg., Selis M. coll., 2♀ (Accession Number PP472432).

GR, Milos, Sarakiniko Beach, 10.VIII.2019, Montanaro G. leg., Selis M. coll., 1♂ (Accession Number PP472433).

GR, Rhodos, Soroni, 18-25.VI.2001, Fancello L. leg., Selis M. coll., 1♂ (Accession Number PP472434).

GR, Tessaglia, Trikala, Pili, 20.VI.2008, Fancello L. leg., Selis M. coll., 1♂ (Accession Number PP472436).

IL, Haifa, 20.VI.1965, Schlaefle W. Leg., Selis M. coll., 1♀.

IL, 10 km S of Kiryat Shmona, 370 m, 33.1166N 35.55E, 27.IV.2018, Kudrna A. leg., Selis M. coll., 1♀ (Accession Number PP472439).

IL, 20 km NNE of Acre, 40 m, 33.05N 35.15E, 28.IV.2018, Kudrna A. leg., Selis M. coll., 1♀ (Accession Number PP472438).

Citizen science records

IT, Abruzzo, CH, Paglieta, 31.V.2021, Ferrante S. ph., 1♂.

IT, Apulia, LE, Copertino, 40.2734N 18.0532E, 2.IX.2019, 1♀, Tarantino F. ph.; same locality, 27.V.2020, 1♂, Tarantino F. ph.

IT, Emilia-Romagna, BO, Castel Maggiore, 13-25.VI.2019, several specimens, Luthi F. ph., several specimens; same locality, 24.VII.2019, 1♀, Luthi F. ph.

IT, Emilia-Romagna, MO, Modena, 44.6545N 10.9009E, 28.VI.2023, "francesco_001" ph., 1♂.

IT, Emilia-Romagna, PR, Parma, 44.9081N 10.3647E, 13.VI.2018, "michidisperso" ph., 1♂ 1♀.

IT, Liguria, SP, La Spezia, 25.VII.2020, Scandurra L. ph., 1♀.

IT, Sicily, PA, Bolognetta, 37.9632N 13.4572E, 17.VI.2021, "nicolaaddelfio" ph., 1♀.

Identification

T. rhodensis, the only Italian species of the genus, is readily distinguished from all other Italian Eumeninae by the morphology of the first tergite, which is shaped like a truncated cone and presents a basal transverse carina followed by a smooth impunctate area (see detail in figure 1). The habitus and clypeus of both sexes are shown in figure 1 to allow an easier identification of the species.

DNA barcode

In total, 29 specimens were analysed using DNA barcoding, 21 from various Italian regions (PP472440-PP472460), six from Greece (PP472432-PP472437) and two from Israel (PP472438-PP472439), and the sequence JN934354 from Lesbos Island (Turcinaviciene *et al.*, 2014) was downloaded from GenBank. Two distinct groups were recovered, one from Italy and one from Greece and Israel, differentiated by a distance of 5.38-5.41%; similar intraspecific distances between Italian and Eastern Mediterranean populations are observed in other genera too, such as *Stenodynerus* (Selis *et al.*, 2024a) and *Euodynerus* (Selis *et al.*, 2024b). Only the Sicilian specimen from 1941 (PP472460) presented a higher distance (6.54%) from the Eastern group, attributable to the comparatively short sequence probably related to the DNA degradation that occurred in this individual sampled more than 80 years ago. The sequence from Lesbos Island was identical to those recovered from Italian specimens.

Distribution

Italy (figure 2): Piedmont, Emilia-Romagna, Liguria, Tuscany, Marche, Lazio, Abruzzo, Apulia, Calabria, Sardinia, Sicily (Andre, 1883; DeStefani, 1889; Giordani Soika, 1942; Pagliano, 2003; Borsato and Turrisi, 2004); Malta; Greece; Cyprus; Turkey; Azerbaijan; Syria; Israel; Iran (Giordani Soika, 1970; Fateryga *et al.*, 2021; Schmid-Egger *et al.*, 2021; Cassar *et al.*, 2022).

The simplified distribution reported by Selis (2023) is based on part of the data presented here.

Discussion and conclusions

The first extra-Sicilian findings of *T. rhodensis* date back to 2013-2018, with only three specimens coming from the municipalities of Mesola (2013), Montecchio Emilia (2015) and Torrile (2018), all in the Emilia-Romagna region in northern Italy. Since 2019, findings have increased significantly, with new locations in Emilia-Romagna and expansion into Calabria and Apulia in the South and Tuscany in the North, followed by findings in Liguria and Lazio in 2020, Abruzzo in 2021, Piedmont in 2022, and Marche and Sardinia in 2023. The temporal distribution of the Italian findings of this taxon is summarized in figure 2.

The latest dedicated papers on the distribution of the Eumeninae in Italy date back to 2004 and 2006, with the revision of the species present in Sicily (Borsato and Turrisi, 2004) and Sardinia (Borsato, 2006) respectively, while a paper focusing on the continental fauna was planned but was never published. Borsato and Turrisi (2004) cite *T. rhodensis* from three localities in Sicily, citing only Giordani Soika and Borsato (1995), which generically reported the species as present in Sicily, and the Lampedusa finding published by Pagliano (2003) as further evidence of its presence in Italy, without mentioning other Italian findings outside of Sicily, something done for other species mentioned in the same paper. It follows that Borsato was probably not aware of continental findings of the species despite his extensive work reviewing the distribution of the Italian Eumeninae, indicating the absence of findings in peninsular Italy at least until 2004. The study of museum and private collections conducted by us further confirms this, since it did not bring to light any specimen of *T. rhodensis*, even in entomological collections particularly rich in Eumeninae collected throughout the peninsula.

The discovery of a few scattered specimens between 2013 and 2018 in Emilia-Romagna, an area widely investigated by many entomologists and from which we studied abundant Eumeninae material, and the subsequent gradual expansion towards the West and South (Tuscany in 2019, Liguria and Lazio in 2020, Abruzzo in 2021, Piedmont in 2022) and the increase in findings in Emilia-Romagna, suggest that the northern population may have originated from anthropogenic importation of the species, probably from Sicily, due to the high similarity found from the COI gene analysis; the genetic distance between Italian and Eastern Mediterranean populations would exclude a trans-Adriatic importation. Some of the photographic records listed above showed *T. rhodensis* to be a cavity nester, which therefore uses tunnels of various kinds to nest, and it has been observed on several occasions nesting in artificial structures such as bee-hotels or reed-shades, implying the ease with which this species could be transported with goods; this route of introduction in Italy seems to be particularly efficient, being observed in allochthonous Hymenoptera with similar nesting styles, such as *Megachile disjunctiformis* Cockerell (Bortolotti *et al.*, 2018), *Megachile sculpturalis* (Smith) (Ruzzier *et al.*, 2020), and *Xylocopa aestuans* (L.) (Flaminio *et al.*, 2023a). The only findings that contrast with this hypothesis are those in Calabria and Apulia as early as 2019 and the discovery in the Marche only in 2023. The lack of previous findings in the Marche can be explained by the scarcity of hymenopterological research done in this region, which has always been characterized by a scarcity of resident entomologists interested in Hymenoptera, while the Calabrian and Apulian findings of 2019 could indicate that the species was already found in some areas of southern Italy but has escaped notice until now, indicating a continuity between the fauna of southern Italy and the Balkans, already observed in other groups of Hymenoptera such as solitary bees (Wood *et al.*, 2023; Cornalba *et al.*, 2024). The earlier findings in southern Italy could also result from a northward expansion of the Sicilian population following climate change, which led to the northward expansion of numerous species, including other vespids [e.g. *Vespa orientalis* L. (Graziani and Cianferoni, 2021)]; *T. rhodensis* seems to be a thermophilic species, found in hot environments or in inhabited centres that can act as heat islands.

Of particular interest are the findings in Sardinia and the JN934354 sequence from Lesbos, which could confirm the tendency of the species to be transported by anthropogenic means. Borsato (2006) published a review of the distribution of the Sardinian Eumeninae based on the examination of very abundant material collected in the last century without reporting any findings of *T. rhodensis*, and also the first author of the present contribution had the opportunity to study material from Sardinia collected in the last decade, finding the first Sardinian specimens of *T. rhodensis* only in 2023; given Sardinia's isolation from the Italian peninsula and Sicily, it is unlikely that the species arrived there naturally. The sequence from the island of Lesbos was identical to those obtained from the Italian specimens, differing from the other Greek specimens coming from nearby localities by 5.38%; considering that the island is an important tourist destination, it cannot be excluded that the resident population of *T. rhodensis* is the result of accidental importation.

To summarize, there are three possible scenarios: 1) the species has always been present in the whole Italian Peninsula but has escaped collections until now; 2) the peninsular findings derive from an anthropogenic importation into northern Italy; 3) the species spread northwards naturally, favoured by the increase in temperatures. While the first seems unlikely due to the complete lack of finds before 2013 in all the collections studied, the other two scenarios are likely to have occurred simultaneously given the expansion pattern evidenced in figure 2, with a natural northward expansion of the

Sicilian population together with the southward spread of the population introduced in the north, leading to the meeting of the two migratory flows in central/southern Italy.

The ecological consequences of the introduction of this species and the impact on other Hymenoptera are not known with certainty, due to the few findings not being accompanied by in-depth observations on its ecology, but it can be hypothesized that it may enter into competition with other cavity-nesting species. Photographic observations made in the municipality of Castel Maggiore (Emilia-Romagna) show how *T. rhodensis* nested inside a bee-hotel, competing for the smallest tunnels (2-4 mm diameter) with megachilids of the genus *Heriades*; the tunnels were then closed with a mud plug, characterized by a central cylindrical projection with a subspherical apex. Unfortunately, it was not possible for the authors to directly examine the nests of this species to identify the prey used and study the possible impact of *T. rhodensis* on their populations. Targeted sampling for this species will serve better to understand its current distribution in the Italian Peninsula and will provide further data on the impact of its introduction. Another useful tool that could help follow the distribution of *T. rhodensis* over time is citizen science, i.e. the involvement of the public in scientific research with the consequent collection of large data on a large scale. Citizen science has already proven to be particularly effective for monitoring wild bees in Italy, both native and invasive (Flaminio *et al.*, 2021), and has also been partly used for gathering the data used in this contribution, providing distributional data that would not otherwise have been available (e.g. reports for Liguria and Abruzzo are based exclusively on citizen science records).

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Conceptualization, MS; methodology, MS, GC; specimen collection and acquisition, MS, AF; laboratory work, GC; sequence analysis, MS, GC; taxonomic work, MS, AF; figure preparation, MS; original draft preparation, MS, GC.

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