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Integrating morphology with phylogenomics to describe four island endemic species of *Temnothorax* from Sicily and Malta (Hymenoptera, Formicidae)

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Abstract. *Temnothorax* (Myrmicinae, Crematogastrini) is one of the most diverse Holarctic ant genera, and new taxonomic advancements are still frequent worldwide. The Mediterranean region, a global biodiversity hotspot characterized by a complex geographic history, is home to a substantial portion of its described diversity. Sicily is the region's largest island and, as ongoing investigations are revealing, it is inhabited by a long-overlooked but highly diverse ant fauna that combines multiple biogeographic influences. We combined qualitative and quantitative morphology of multiple castes with phylogenomic analysis based on ultra-conserved elements (UCEs) to describe four species of *Temnothorax* endemic to Sicily and the neighboring Maltese Islands (Sicilian Channel). Three of these species, *T. marae* Alicata, Schifani & Prebus sp. nov., *T. poldii* Alicata, Schifani & Prebus sp. nov. and *T. vivianoi* Schifani, Alicata & Prebus sp. nov., are new to science, while a redescription clarifies the identity of *T. lagrecai* (Baroni Urbani, 1964). These descriptions highlight the current difficulties of delimiting monophyletic *Temnothorax* species groups based on morphological characters. The intra-insular endemicity patterns we revealed highlight the importance of Mediterranean paleogeography to contemporary ant diversity and distribution in the region.

Keywords. Maghreb, Italy, Mediterranean biogeography, island endemics, short-range endemic (SRE) invertebrates.

Introduction

The hyperdiverse genus *Temnothorax* Mayr, 1861 is mainly distributed across the Holarctic and northern Neotropical regions and is only marginally present in the northern Afrotropics (Prebus 2015; Janicki *et al.* 2016; Guénard *et al.* 2017). It contains more than 450 valid species, including a notable number of species with small geographic ranges (Bolton 2021). Part of the tribe Crematogastrini Forel, 1893 (see Ward *et al.* 2015), *Temnothorax* are mostly diminutive, inconspicuous, timid and cryptically colored ants, which form small colonies with only a few notable exceptions (Prebus 2017; Seifert 2018). Due to these characteristics, most species are easily overlooked during field surveys, yet many may play important ecological roles (e.g., Prebus 2017; Seifert 2018; Giannetti *et al.* 2022). In addition, while the vast majority of the species are free-living, several are social parasites, the correct naming of which has become a debated issue among myrmecologists (Seifert *et al.* 2016; Ward *et al.* 2016). Here, we adopt the nomenclatural system proposed by Ward *et al.* (2015).

During the last decades, efforts to describe the taxonomic diversity of *Temnothorax* have multiplied globally: out of the about 490 valid species and subspecies, some 150 new species were described during the last 20 years, mostly from Asia and North America (Bolton 2021). Moreover, a major advancement in the understanding of the genus' phylogenetic diversity was recently achieved thanks to the analysis of more than 100 described and undescribed morphospecies belonging to 37 species-groups, resulting in the discovery of 7 major clades (Prebus 2017, 2021b). In Europe, where three of these clades occur (Prebus 2017), the taxonomy of the genus has long been plagued by a lack of reliable diagnostic keys and descriptions of many so-called 'transitional forms' of uncertain taxonomic status, but has improved substantially during the last decades. The better investigated fauna is that of mainland Europe, where many of the recently conducted taxonomic investigations have uncovered cryptic diversity within the large geographic ranges of some species (e.g., Csősz *et al.* 2015, 2018; Seifert & Csősz 2015). For instance, the fauna of Central and Northern Europe consists of 24 species with relatively broad geographic distributions, and keys for their workers and queens are available (Seifert 2018). However, Europe and Asia Minor are expected to host some 150 species in total (Seifert 2018), and over 200 taxa are described from the Mediterranean region, which acts as a global biodiversity hotspot for the genus (Borowiec 2014). In this context, descriptions of non-cryptic species, usually with narrower geographic ranges, are not uncommon (e.g., Galkowski & Cagniant 2017; Salata *et al.* 2018; Salata & Borowiec 2019; Tinaut & Reyes-López 2020; Arcos 2021). Large Mediterranean islands may host a particularly rich and unique fauna. For instance, Crete is inhabited by 17 species, half of which are considered endemic (Salata *et al.* 2018, 2020). Such diversity and high levels of endemicity are unparalleled by any other ant genus of the region, but much of the local fauna of *Temnothorax* still remains undescribed or little-known, and some serious efforts in this direction are required in the face of the threats the regional biodiversity is facing (Cuttelod *et al.* 2009).

Sicily is the largest Mediterranean island, and its central position in the region allowed its long-neglected ant fauna to be shaped by different biogeographic influxes from the East and West, reaching it either from Europe through the Italian peninsula (now divided by the Strait of Messina) or from North Africa passing through the Eastern Maghreb (presently separated by the Sicilian Channel) (Schifani & Alicata 2018; Alicata & Schifani 2019; Schifani *et al.* 2020, 2021a, 2022). For these reasons, its ant fauna is characterized by some unique traits (Wang *et al.* 2022). There are currently 14 species of *Temnothorax* whose presence in Sicily has been recorded and was not later dismissed: *T. affinis* (Mayr, 1855), *T. clypeatus* (Mayr, 1853), *T. exilis* (Emery, 1869), *T. flavicornis* (Emery, 1870), *T. laestrygon* (Santschi,

1931), *T. lagrecai* (Baroni Urbani, 1964), *T. lichtensteini* (Bondroit, 1918), *T. mediterraneus* Ward *et al.*, 2015, *T. muellerianus* (Finzi, 1922), *T. nylanderi* (Foerster, 1850), *T. ravouxi* (André, 1896), *T. recedens* (Nylander, 1856), *T. rottenbergii* (Emery, 1870), and *T. unifasciatus* (Latreille, 1798) (see De Stefani 1889; Baroni Urbani 1964; Buschinger *et al.* 1988; Schifani & Alicata 2018; Schär *et al.* 2020; Schifani *et al.* 2021a, 2022; Schifani 2022). Of these, *T. laestrygon* and *T. lagrecai* are considered to be endemic, but the status of both appeared doubtful due to the lack of clearly defined diagnostic features.

Here, we present the descriptions of three new non-cryptic species of *Temnothorax* that we have found exclusively in Sicily or in the neighboring Aegadian and Maltese islands, as well as a redescription of *T. lagrecai*. We integrate qualitative and quantitative morphology of multiple castes with phylogenomic analysis of molecular data from ultra-conserved elements (UCEs), comment on the ecological and biogeographical features of these species, and illustrate their position within the global phylogeny of the genus. These descriptions allow us to present a complete key to the Sicilian light-colored species of *Temnothorax* with concolorous antennal clubs, and to comment on the current difficulties in species-group classification of *Temnothorax* based on morphology.

Material and methods

Species delimitation criteria

We focus on identifying non-cryptic species, here defined as species that differ one from another by multiple qualitative morphological characters in a consistent way (i.e., in >95% of the examined individuals), and we consider such differences to represent a reliable proxy indicating "separable clusters that have passed a threshold of evolutionary divergence" sensu Seifert (2020). To achieve a satisfactory species delimitation and a fully informative species description, we integrate the evidence provided by qualitative morphological characters with quantitative morphology as well as phylogenomic evidence in the form of an analysis of ultra-conserved element (UCE) locus data, which we integrate into the global phylogeny of the genus *Temnothorax* presented in Prebus (2017). Finally, we carefully interpret the results considering the available biogeographic data. Support from these different sources of information are then integrated and discussed to produce final species hypotheses.

Examined material

Sicilian specimens of *Temnothorax* were primarily obtained through field-collecting across the island by the authors and occasional collaborators as described in Schifani *et al.* (2021a) (Fig. 1), extending to the Italian Peninsula and the circum-Sicilian islands (including the Maltese islands). Sampling techniques consisted of direct sampling, soil sifting, pitfall traps, and Milieu Souterrain Superficiel (MSS) traps (Agosti *et al.* 2000; Mammola *et al.* 2016). These samples were compared either through pictures or direct investigation of other specimens of *Temnothorax* from our collections, specimens from museum collections (investigated directly or through photographs and belonging to: Basel Natural History Museum, Switzerland [NHMB], Museo Civico di Storia Naturale "G. Doria", Italy [MSNG], Museo Civico di Storia Naturale di Milano, Italy [MSNM], Museo Civico di Storia Naturale di Verona, Italy [MSNV], Natural History Museum of Vienna, Austria [NHMW]), specimens stored in private collections (David Misfud personal collection; Stephen Schembri personal collection) and specimens from museum and private collections whose high-quality images are available on the AntWeb.org archive (Bolton 2021).

Concerning the four taxa treated in this study, we examined 1180 workers, 8 males and 28 queens from 60 collecting sites, whose full list is given in Supp. file 1: Table S1. Holotype specimens of the species described in this study were deposited at the Museo Civico di Storia Naturale "G. Doria" (MSNG – Genoa, Italy).

Qualitative morphological characters

Directly available specimens were examined under stereoscopic microscopes at 60–180 × magnification. Qualitative morphological characters were defined as discrete characters (presence or absence of certain traits) easily observable by trained myrmecologists without the need of detailed numerical recording, and were investigated on all specimens examined during this study. The following characters were used in this study:

Antennal club pigmentation
Antennal clubs may be concolorous with the rest of the antenna, or, in light-colored individuals, they can also exhibit a darker pigmentation (Figs 2–3). This is considered a steady character in several (albeit not all) European species of *Temnothorax* (Seifert 2018).

Antennal segments
The standard number of segments in the Euro-Mediterranean species of *Temnothorax* is 12 in females (workers and queens) and 13 in males, but in at least one European free-living species, *T. flavicornis*, as well as in most members of the *gordiagini* group (i.e., of the former genus *Myrmoxenus* Ruzsky, 1902), this number is reduced to 11 in females and 12 in males (Emery 1870, 1895; Figs 2–3).

Eye size
The relative size of compound eyes is quite variable in *Temnothorax*, and is evaluated as a quantitative character in this study (see eye length [EL], eye width [EW] and their arithmetic mean [EYE]), but in
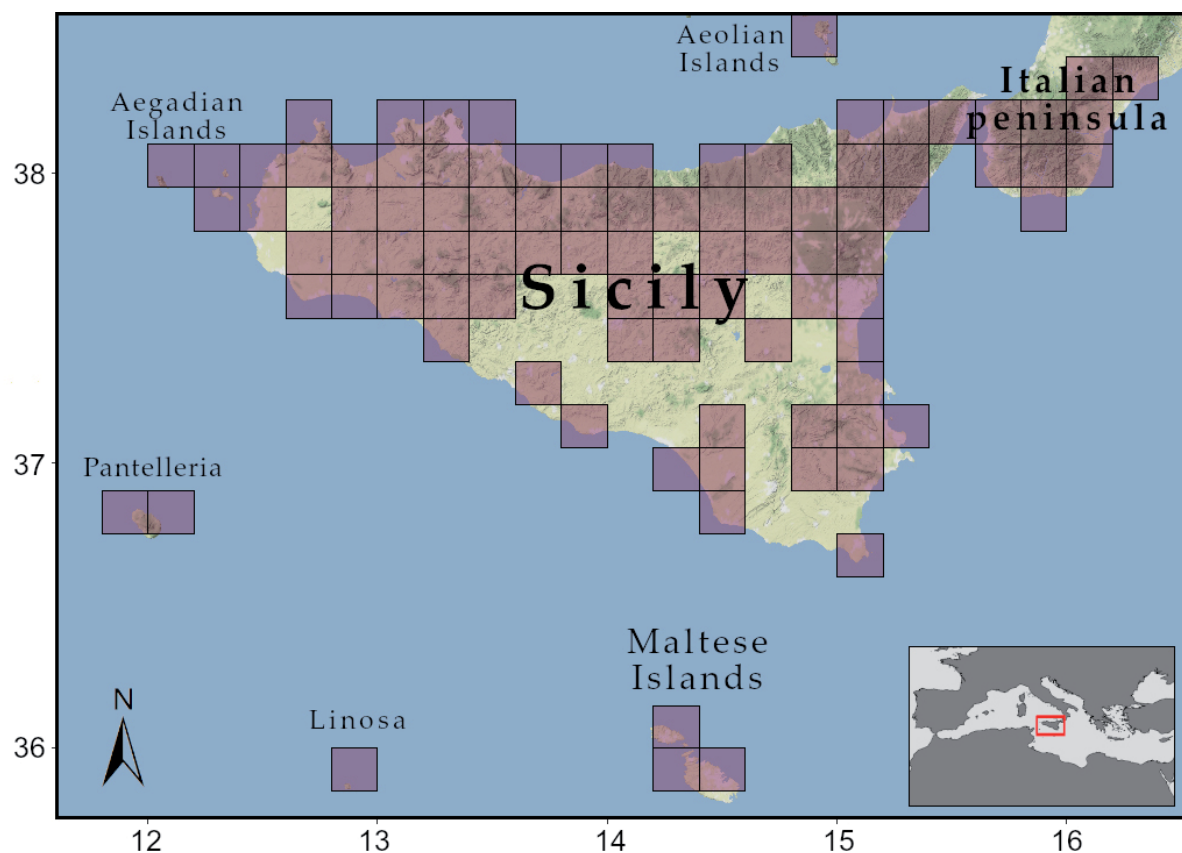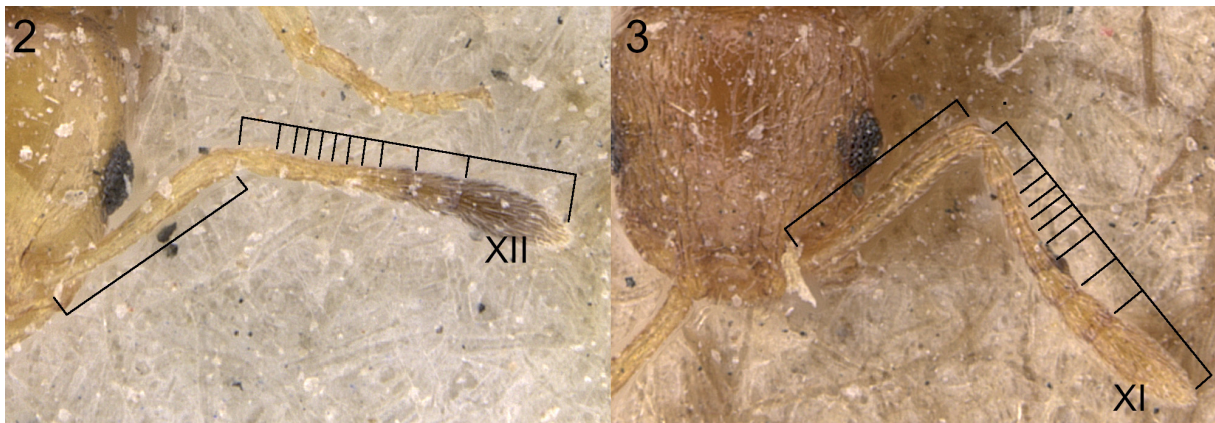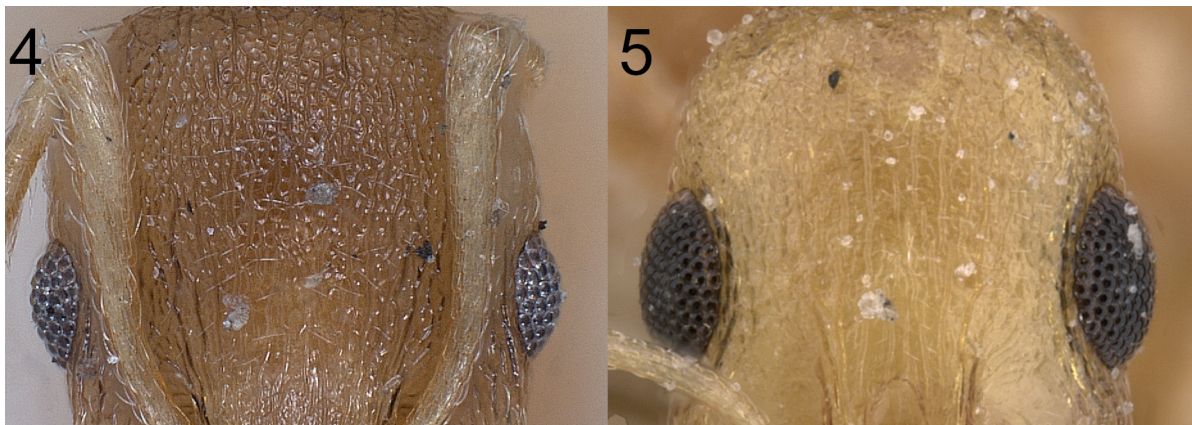

Fig. 1. Sampling efforts across Sicily and neighboring regions according to the geographic origin of the samples in the authors' collections.

Figs 2–3. Variation in antennae of workers of *Temnothorax* Mayr, 1861, photos from www.antweb.org, specimen identifiers in parentheses. **2**. *T. minozzii* (Santschi, 1922), type worker from Italy (CASENT0912964), photo by Will Ericson, antenna of 12 segments and darkened club. **3**. *T. flavicornis* (Emery, 1870), type worker from Italy (CASENT0904761), photo by Will Ericson, antenna of 11 segments and concolorous club.
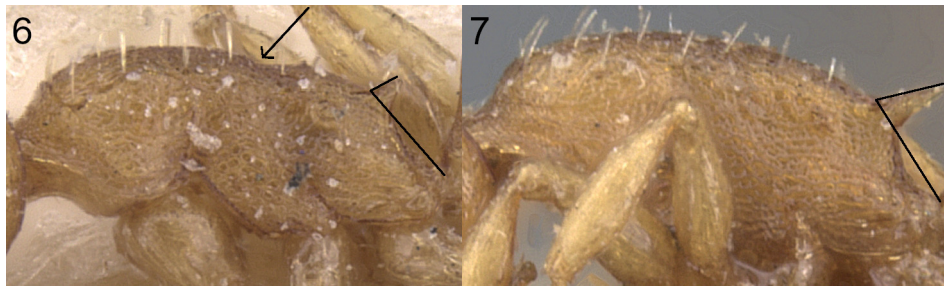

Figs 4–5. Eye size variation in workers of *Temnothorax* Mayr, 1861, photos from www.antweb.org, specimen identifiers in parentheses. **4**. *T. lichtensteini* (Bondroit, 1918), type specimen from France (ANTWEB1008441), photo by Roland Schultz, normal eye size. **5**. *T. arenarius* (Santschi, 1908), type specimen from Tunisia (CASENT0912903), photo by Will Ericson, large eye size.

the reference area there is a group of 'large-eyed' species distributed from the Maghreb to Iberia that represents a clear outlier. Outside this condition, eyes are defined 'normal', while finer differentiation is achieved by means of quantitative morphology (Figs 4–5).

Metanotal groove of workers

The metanotal groove (or depression) is generally either present or absent in a single species of *Temnothorax* (Figs 6–7), sometimes becoming a species group-defining character (Seifert 2018; Prebus 2021b). In dubious cases, to facilitate its observation the standard lateral view of the mesosoma may be angled slightly upwards.
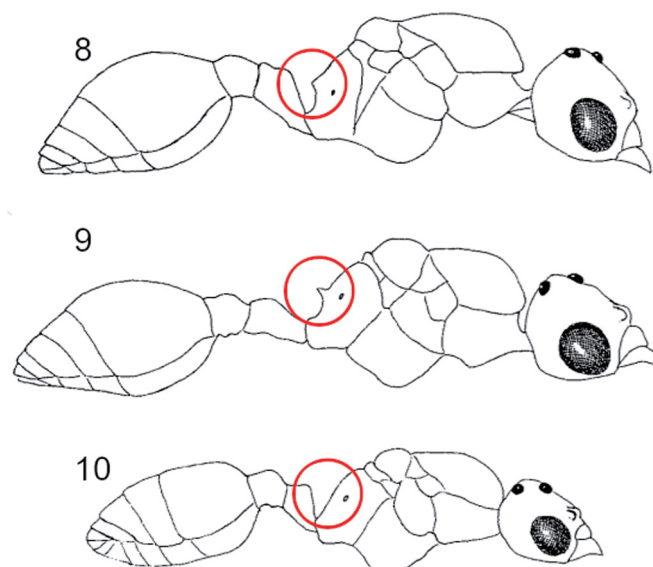
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Figs 6–7. Lateral view of the mesosoma of workers of *Temnothorax* Mayr, 1861 exhibiting different characters, photos by Will Ericson, available on www.antweb.org, specimen identifiers in parentheses. **6**. *T. curtulus* (Santschi, 1929), type specimen from Morocco (CASENT0912922), metanotal groove present (indicated by an arrow) and short propodeal spines. **7**. *T. luteus* (Forel, 1874), type specimen from France (CASENT0907603), no metanotal groove and long propodeal spines.

Propodeal spines

The length of propodeal spines is a quantitative character in this study (see the distance between the center of the propodeal spiracle and the spine tip [SPST]), but at the same time we also rely on simpler categorical definition for practical reasons, since the species treated in this study show a rather dichotomic division in long-spined and short-spined species. These categories refer to workers, and are based on the length of the propodeal spine visually compared with that of the propodeal declivity as in Prebus (2021b): short spines are those that are long about ⅓ or less of the propodeal declivity; long spines are those that are roughly long at least ½ of the propodeal declivity (Figs 6–7).

Concerning males, propodeal spines are entirely absent in most of the described W-Palearctic *Temnothorax*, but may be present in some cases, sometimes as normally-developed spines similar to those of workers and queens, sometimes as small dentiform protuberances (Figs 8–10).

Figs 8–10. Lateral view of male specimens of *Temnothorax* Mayr, 1861 from Algeria with a focus on the propodeum, drawings from Cagniant (1970). **8**. *T. curtulus* (Santschi, 1929), dentiform propodeal spines. **9**. *T. gentilis* (Santschi, 1923), well-developed propodeal spines. **10**. *T. tebessae* (Forel, 1890), no propodeal spines.

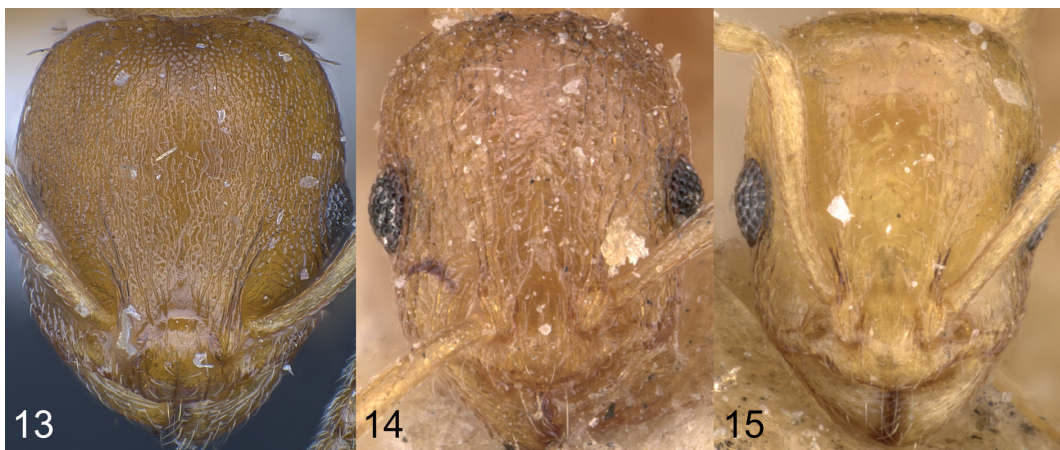**Petiole shape**

In species of *Temnothorax*, the petiole shape exhibits a remarkable interspecific but in some cases also intraspecific variation, making it at the same time a very useful character and one that requires extra vigilance from the investigator. Two parts of the petiole are important in our revision and should be observed in lateral view (Figs 11–12): i) its dorsal profile, characterized by the way the anterior and posterior margins meet may form a single edge with no horizontal component or two edges delimiting a clear horizontal section; ii) the subpetiolar process, which may develop downwards in correspondence to the anterior part of the petiole, forming a tooth-like protuberance, or may be simply represented by a ventral carina to the petiole.

Figs 11–12. Petiole shape variation in workers of *Temnothorax* Mayr, 1861, photos from www.antweb.org, specimen identifiers in parentheses. **11**. *T. atomous* (Cagniant & Espadaler, 1997), type specimen from Morocco (CASENT0915391), photo by Will Ericson, petiole dorsal profile with a single edge and no horizontal component, subpetiolar process carina-like. **12**. *T. alienus* Schulz, Heinze & Pusch, 2007, type specimen from Italy (ANTWEB1041297), photo by Roland Schultz, petiole dorsal profile with two edges and a horizontal component, subpetiolar process with a tooth-like protuberance.

Figs 13–15. Head sculpture variation in workers of *Temnothorax* Mayr, 1861, photos from www.antweb.org, specimen identifiers in parentheses. **13**. *T. alienus* Schulz, Heinze & Pusch, 2007, type specimen from Italy (ANTWEB1041297), photo by Roland Schultz, weak longitudinal striae on a background of dense alveolate sculpture. **14**. *T. flavicornis* (Emery, 1870), type specimen from Italy (CASENT0904761), photo by Will Ericson, strong longitudinal striae with a shiny background. **15**. *T. tebessae* (Forel, 1890), type specimen from Algeria (CASENT0909034), photo by Will Ericson, extremely weak and few longitudinal striae on a shiny background.
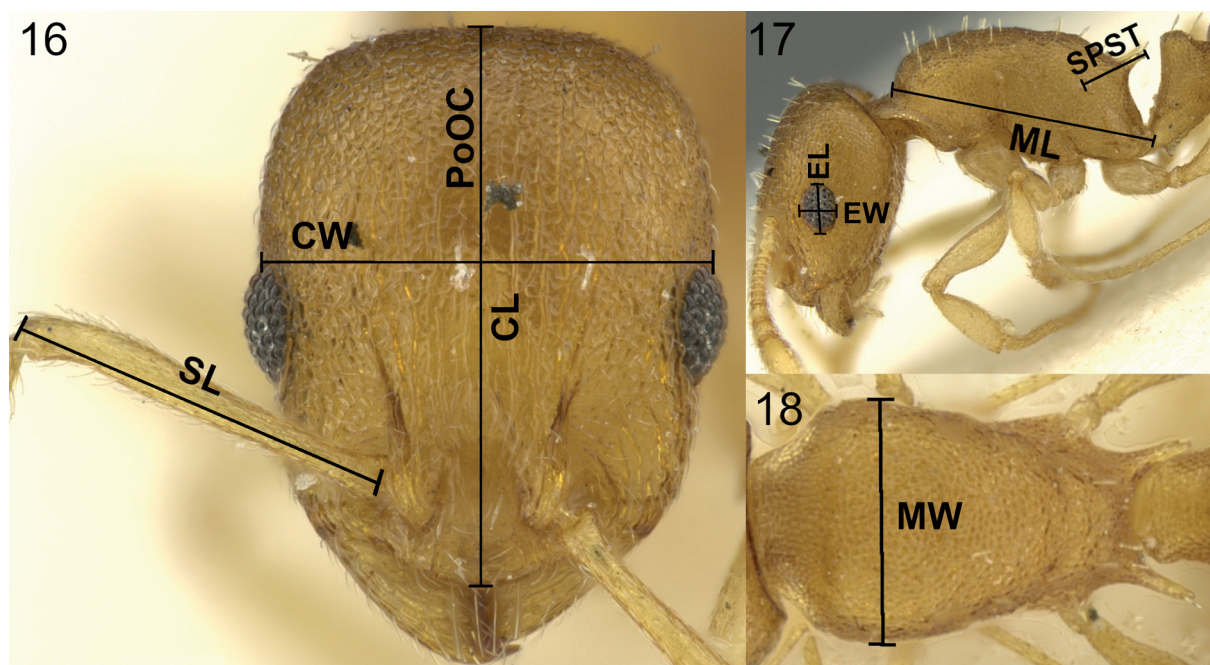
Surface sculpturing

Only two types of surface sculpturing play an important role in this paper, and our terminology is here a strong simplification of what is described by Harris (1979): we use the term alveolate sculpture whenever a light sculpture divides the surface in numerous small, relatively regular spaces; we use the term striae to describe parallel or subparallel lineations, more or less markedly raised, that may be linked by anastomoses. Finally, the term 'shiny' is used to indicate the apparent lack of sculpturing at the given magnifications, which produces a shiny and glossy appearance. Rugae may occur above a weaker background of alveolate or shiny sculpture. See Figs 13–15.

Quantitative morphological characters

Morphometric measurements were taken using high-quality pictures of specimens photographed with a Canon 1300D reflex and Canon MP-E 65mm f/2.8 1–5× Macro Photo lens and the software ImageJ (Schneider *et al.* 2012). To analyze differences between the species, Principal Component Analysis (PCA) was employed using the software R and R Studio and the function princomp from the package stats (R Core Team 2021; Rstudio Team 2021). In these analyses, each species was represented by a set of workers about equally divided between multiple nests and sites, thus resulting in a similarly distributed number of pseudoreplicates. A total of 648 morphometries were recorded from 52 workers, 10 males and 10 queens by recording the following nine morphometric characters on each specimen (acronyms and definitions follow Seifert 2018; see Figs 16–18):

CL   =   cephalic length, measured as the maximum distance between the occipital margin of the head to the lowermost margin of the clypeus. Head in full face view

CW   =   cephalic width, measured as the maximum width of the head, right above the level of the eyes. Head in full face view

EL   =   eye length, the longest diameter of the compound eye (including unpigmented marginal ommatidia)

Figs 16–18. Morphometric characters recorded from workers of *Temnothorax* Mayr, 1861 in this study. The background image is a paratype worker of *T. lagrecai* (Baroni Urbani, 1964) from the Natural History Museum of Vienna, photos by Anna Pal available from www.antweb.org, specimen identifier CASENT0919741. **16**. Head view. **17**. Lateral profile view. **18**. Dorsal view of the mesosoma.

EW   =   eye width, the shortest diameter of the compound eye (including unpigmented marginal ommatidia)

ML   =   mesosoma length, measured as the maximum diagonal between the point where the pronotum meets the cervical shield and the posterior basal angle of the propodeal lobe. Measured in profile view of the specimen

MW   =   mesosoma width, measured as the maximum width of the pronotum. Measured in dorsal view of the specimen

PoOC   =   post-ocular distance, measured as the distance between the occipital margin of the head and the central point of the imaginary horizontal line of the CW measurement. Head in full face view

SL   =   scape length, measured as the maximum length of the scapus but excluding the basal constriction or neck that occurs just distal of the condylar bulb

SPST   =   maximum distance between the center of the propodeal spiracle and the distalmost tip of the spine. Measured in profile view of the specimen (not measured in spineless male specimens)

All measurements are given in µm as mean ± standard deviation (minimum, maximum). Based on these nine characters, we computed CS (cephalic size) as the arithmetic mean between CL and CW and EYE as the arithmetic mean between EL and EW (Seifert 2018). Furthermore, we calculated indices to weight each of the nine characters based on the relative size of the specimen. To achieve this, we divided them by CS (Seifert 2018) with the following exceptions: SL and PoOC were divided by CL, while CL was divided by CW. Concerning the SL and PoOC indices, the aim was to provide a ratio more associated with the visual comparison of antennal scapes and post-ocular distance with cephalic length in frontal view. Concerning CL, dividing it by CW allowed us to describe of the shape of the cephalic capsule more clearly. All morphometric data are provided in Supp. file 1: Table S3.

Sequencing and analysis of Ultra-Conserved Elements (UCEs)

The sequence dataset used in this study was compiled from previously published datasets (Branstetter *et al.* 2017; Prebus 2017, 2021a; Blaimer *et al.* 2018) as well as newly generated data (NCBI BioProject PRJNA770978). See Supp. file 1: Table S2 for NCBI accession numbers.

The sequenced taxa for this study were selected based on the sampling used in Prebus (2017), with the addition of populations of the four focal taxa.

For the newly generated data, adult worker ants were prepared for nondestructive DNA extraction by using a flame-sterilized size 2 stainless steel insect pin to pierce the cuticle of the head, mesosoma, and gaster on the right side of the specimens. These prepared specimens were then extracted using the DNeasy Blood & Tissue Kit (Qiagen, Inc.) following the manufacturer's protocols, using a 12-hour digestion in proteinase-K solution on a shaking heat block. The extracted specimens were stored in 95% ethanol prior to being mounted as molecular vouchers. Up to 50 ng of DNA was used as input, sheared to a target fragment size of 400–600 bp into a genomic DNA library preparation protocol for targeted enrichment of ultraconserved elements (UCEs) following Faircloth *et al.* (2015) as modified by Branstetter *et al.* (2017) using a unique combination of iTru barcoding adapters for each sample (Glenn *et al.* 2019; see Supp. file 1: Table S2 for a list of the adapters used). Enrichments were performed on pooled libraries using the custom version of the Hym 2.5Kv2A ant-specific RNA probes (Branstetter *et al.* 2017; ArborBiosciences, Ann Arbor, MI), which target 2524 UCE loci in the Formicidae Latreille, 1809. The library enrichment procedures for the probe kit were followed, except that the RNA probe concentration was reduced to 0.1X (note that this step is only necessary for the custom kit; the currently available catalog kit is already diluted to 0.1X concentration), custom adapter blockers were used instead of the standard blockers, and enriched DNA was left bound to the streptavidin beads during PCR, as described in Faircloth *et al.* (2015). Following post-enrichment PCR, the resulting pools were purified using SpeedBead magnetic carboxylate beads (Rohland & Reich 2012; Sigma-Aldrich) and adjusted their volume to 22 µL.

Enrichment success was verified and measured size adjusted DNA concentrations of each pool with qPCR using a SYBR® FAST qPCR kit (Kapa Biosystems) and a Bio-Rad CFX96 RT-PCR thermal cycler (Bio-Rad Laboratories) and combined all pools into an equimolar final pool. The final pool was sequenced as a single lane at the High Throughput Genomics Facility at the University of Utah on an Illumina HiSeq 2500 (125 cycle paired end sequencing v4).

Following sequencing, raw reads were trimmed of adapter contamination, Illumina sequencing artefacts, and low-quality bases using the program illumiprocessor, which is included in PHYLUCE ver. 1.7.1 (Faircloth 2016). Cleaned reads were assembled denovo with PHYLUCE using SPAdes ver. 3.12.0 (Bankevich *et al.* 2012). All newly generated raw sequence reads have been submitted to the National Center for Biotechnology Information (NCBI) Sequence Reads Archive (BioProject PRJNA770978).

The standard PHYLUCE protocol was followed for processing UCEs in preparation for phylogenomic analysis, aligning the monolithic unaligned fasta file with the phyluce_align_seqcap_align command, using MAFFT (Katoh & Standley 2013) as the aligner (–aligner mafft) and opting not to edge-trim the alignment (–no-trim). The resulting alignments were trimmed with the phyluce_align_get_gblocks_ trimmed_alignments_from_untrimmed command in PHYLUCE, which uses GBlocks ver. 0.91b (Castresana 2000), using the following settings: b1 0.5, b2 0.5, b3 12, b4 7. After removing UCE locus information from taxon labels using the command phyluce_align_remove_locus_name_from_nexus_ lines, the alignment statistics were examined using the command phyluce_align_get_align_summary_ data, and a dataset was generated in which each locus contains a minimum of 85% of all taxa using the command phyluce_align_get_only_loci_with_min_taxa.

Because the assumption that the evolutionary rates of sequence data are homogenous is often violated in empirical data (Buckley *et al.* 2001), we partitioned our UCE loci into sets of similarly evolving sites. To achieve this, we used the command phyluce_align_format_nexus_files_for_raxml which concatenates loci into a single alignment, and generates a partition file for input into the SWSC-EN method (Tagliacollo & Lanfear 2018). The resulting datablocks were used as input for partitioning in IQTREE ver. 2.1.2 (Nguyen *et al.* 2015), using the command -m TESTNEWMERGEONLY. The substitution model was set to 'general time reversible' (-mset GTR), and the rate heterogeneity models were set to a subset of rate heterogeneity models that includes everything except the combination of gamma and proportion of invariable sites (-mrate E, I, G). The combination of gamma and proportion of invariable sites (+I+G) has been demonstrated to result in anomalies in likelihood estimation (Sullivan & Swofford 2001; Yang 2006). The search algorithm was set to -rclusterf 10. The resulting partitioned dataset was used as input for maximum likelihood tree inference in IQ-TREE, using 1000 ultrafast bootstrap replicates (-bb 1000).

Results

Taxonomy

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Class Insecta Linnaeus, 1758
Order Hymenoptera Linnaeus, 1758
Suborder Apocrita Latreille, 1810
Family Formicidae Latreille, 1809
Subfamily Myrmicinae Lepeletier de Saint-Fargeau, 1835
Tribe Crematogastrini Forel, 1893
Genus *Temnothorax* Mayr, 1861

Temnothorax lagrecai (Baroni Urbani, 1964)
Figs 16–33

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Diagnostic character combination

Antennal clubs concolorous yellowish, antennae 12-segmented in females and 13 in males, eyes normal, metanotal groove very weak or absent, worker propodeal spines long, male propodeal spines dentiform, petiole upper profile usually with a short horizontal component, subpetiolar process tooth-like, sculpturing mostly areolate and weak.

Etymology

Baroni Urbani (1964) dedicated this species to the Italian entomologist Marcello La Greca (1914–2001).

Material examined

We investigated 56 colony samples from 23 localities, consisting in a total of 212 ☿☿, 8 ♀♀, 2 ♂♂ from our collections, one colony under rearing in our possession, holotype queen and paratype workers from the MSNV and NHMW collections, and additional non-type workers from the personal collections of David Misfud and Stephen Schembri and from the MSNM. A detailed list is provided in the Supp. file 1: Table S1.

Redescription

Worker (Figs 19–27)

MEASUREMENTS AND INDICES (13 specimens, 6 localities, 6 colonies). CL: 547.15 ± 33.51 (488–592); CW: 447.54 ± 31.37 (401–495); CS: 497.35 ± 31.84 (444–543); PoOC: 231.69 ± 14.9 (212–257); SL: 387.77 ± 24.95 (352–435); ML: 603.15 ± 57.6 (527–709); MW: 295.92 ± 26.65 (260–347); EL: 116.85 ± 16.1 (100–153); EW: 87.92 ± 11.19 (78–112); EYE: 102.38 ± 13.28 (89–132); SPST: 147.6 ± 17.97 (120–175); CL/CW: 1.22 ± 0.03 (1.18–1.29); PoOC/CL: 0.42 ± 0.01 (0.40–0.45); SL/CL: 0.71 ± 0.01 (0.68–0.73); ML/CS: 1.21 ± 0.05 (1.15–1.33); MW/CS: 0.59 ± 0.02 (0.56–0.65); EL/CS: 0.23 ± 0.02 (0.20–0.29); EW/CS: 0.18 ± 0.02 (0.15–0.21); EYE/CS: 0.20 ± 0.01 (0.18–0.25); SPST/CS: 0.30 ± 0.02 (0.27–0.32).

COLORATION. Entirely yellowish with the exception of a variably large and often not clearly demarcated black transverse band on the gaster.

HEAD. Subrectangular with rounded margins near the four angles, clypeus and mandibles rounded. Antennae of 12 segments, antennal clubs of 3 segments, antennal scapes relatively short (SL/CL: 0.71 ± 0.01). Compound eyes relatively small, ovoidal (EYE/CS: 0.20 ± 0.01).

MESOSOMA. May present a slight metanotal depression, its dorsal profile being from almost straight to definitely rounded in lateral view. Propodeal spines are relatively long (SPST/CS: 0.30 ± 0.02), usually proportionally thin in minor worker but sometimes considerably thick in large ones.

METASOMA. The petiole in profile view usually presents a short but visible horizontal section. A subpetiolar process is normally visible, consisting of a small tooth angled down at up to 90°. Postpetiole ordinarily roundish in lateral profile, subrectangular in dorsal view.

SURFACE SCULPTURING. Most of the body is characterized by a very fine areolate-rugose sculpture, the longitudinal component of which tends to be notably more marked on the frontal side of the head only in larger specimens. The gaster and appendages are smooth, as well as the clypeus and a variable area extending around the frons. A central longitudinal stria visible in the lower portion of the clypeus. Very sparse, occasionally suberect but usually erect setae all over the body; dense, fine, and mostly adpressed pilosity on all appendages, especially abundant on the antennal flagelli.

Queen (Figs 28–30)

MEASUREMENTS AND INDICES (2 specimens, 2 localities). CL: 649–627; CW: 531–548; CS: 579–598; PoOC: 239–256; SL: 457–469; ML: 932–989; MW: 585–585; EL: 190–195; EW: 157–161; EYE: 173–178; SPST: 216–237; CL/CW: 1.08–1.08; PoOC: 0.37–0.41; SL/CL: 0.72–0.73; ML/CS: 1.65–1.61; MW/CS: 0.97–1.00; EL/CS: 0.32–0.33; EW/CS: 0.27–0.27; EYE/CS: 0.30–0.30; SPST/CS: 0.37–0.39.

COLORATION. Entirely yellowish with the main exception of parts of the gaster: at least the first tergite characterized by a blackish transverse band. Moreover, the mesoscutellar disk is darkened caudally.

HEAD. Subrectangular, with rounded margins near the four angles, clypeus and mandibles rounded. Antennae of 12 segments, antennal clubs of 3 segments, antennal scapes relatively short (SL/CL: 0.72–0.73). Compound eyes large and ovoidal (EYE/CS: 0.30); ocelli circular.

MESOSOMA. Propodeal spines relatively long (SPST/CS: 0.37–0.39).

METASOMA. Dorsum of petiole in profile view without a horizontal section. Subpetiolar process usually visible, consisting of a small tooth. Postpetiole ordinarily roundish in profile, subrectangular in dorsal view.

SURFACE SCULPTURING. Most of the body is characterized by a variably fine areolate-rugose sculpture, strong longitudinal rugae on the head. Clypeus. gaster and appendages smooth; a variable area extending around the frons, most of the pronotum, anepisterna, katepisterna, mesoscutellar disk and mesoscutum. A central longitudinal stria visible in the lower portion of the clypeus. Very sparse, occasionally suberect but usually erect setae all over the body; dense, fine, and mostly adpressed pilosity on all appendages, especially abundant on the antennal flagelli.

Male (Figs 31–33)

MEASUREMENTS AND INDICES (3 specimens, 2 localities). CL: 443 ± 13.11 (429–445); CW: 405.67 ± 3.51 (402–409); CS: 424.33 ± 7.84 (415–430); PoOC: 189.67 ± 2.51 (187–192); SL: 203 ± 4 (199–207); ML: 836.67 ± 5.13 (832–841); MW: 498.67 ± 3.78 (496–503); EL: 186.67 ± 6.03 (181–193); EW: 165.67 ± 3.78 (163–170); EYE: 176.16 ± 3.62 (172–178); SPST: 126.33 ± 4.51 (122–131); CL/CW: 1.04 ± 0.01 (1.03–0.06); PoOC/CS: 0.43 ± 0.01 (0.42–0.43); SL/CL: 0.45 ± 0.01 (0.45–0.46); ML/CS: 1.97 ± 0.04 (1.93–2.01); MW/CS: 1.17 ± 0.03 (1.15–1.21); EL/CS: 0.44 ± 0.01 (0.42–0.45); EW/CS: 0.39 ± 0.02 (0.38–0.41); EYE/CS: 0.41 ± 0.01 (0.40–.043); SPST/CS: 0.30 ± 0.02 (0.28–0.31).

COLORATION. Whole body yellowish, head slightly darker, appendages whitish, gaster dark and mesoscutellar disk caudally darkened.

HEAD. Subrectangular, rounded especially above the eyes; clypeus and mandibles rounded. Antennae of 13 segments, antennal clubs of 4 segments. Compound eyes large and ovoidal (EYE/CS: 0.41 ± 0.01); ocelli circular.

MESOSOMA. Propodeum armed with very short and thick spines (SPST/CS: 0.30 ± 0.02).

METASOMA. Postpetiole ordinarily roundish in profile, subrectangular in dorsal view.

SURFACE SCULPTURING. Sculpture relatively strong on the head, with well-developed longitudinal striae on its frontal side, very light all over the propodeum, petiole, postpetiole and in some areas near the mesosoma lateral sutures, all the remaining parts smooth. A central longitudinal stria visible in the lower portion of the clypeus. Sparse, erect setae all over the body; fine and mostly adpressed pilosity over the appendages.

Phylogeny (Fig. 79)

Sister species of *T. marae* Alicata, Schifani & Prebus sp. nov., which together are closely related to *T. flavicornis*.

Distribution and biogeography (Figs 80–81)

Occurs almost all over Sicily and is also found in the Maltese Islands. In the past, Sicily and the Maltese Islands have been connected via an extensive land bridge covering a large area of currently shallow waters, and the last time such a connection existed was during the Last Glacial Maximum (Foglini *et al.* 2016).

Figs 19–33. Specimens of *Temnothorax lagrecai* (Baroni Urbani, 1964) in lateral (left), dorsal (center) and head view (right). Photos available on www.antweb.org, specimen identifiers in parentheses. **19–21**. Worker from Monte Pellegrino (Palermo mountains, Sicily) (ANTWEB1041536), photo by Enrico Schifani. **22–24**. Paratype worker from Bosco di Santo Pietro (Hyblaean Sicily) (ANTWEB1041537), photo by Elia Nalini. **25–27**. Worker from Bosco di Linera (Etna, Sicily) (ANTWEB1041538), photo by Enrico Schifani. **28–30**. Queen from Bosco di Santo Pietro (type locality, Hyblaean Sicily) (ANTWEB1041539), photo by Enrico Schifani. **31–33**. Male from Bosco di Santo Pietro (type locality, Hyblaean Sicily) (ANTWEB1041540), photo by Enrico Schifani. Scale bars = 0.5 mm.

Ecology and conservation

Relatively thermophilous, collected from 5 to 840 m a.s.l. (see Fig. 82). *Temnothorax lagrecai* inhabits Mediterranean shrublands and relatively open forests like native and artificial forests of *Pinus halepensis* Mill., sometimes found under trees of *Quercus ilex* L., but was also collected in artificial gardens in the leaf litter of trees of *Citrus* L. Due to the wide ecological niche and distribution it may face a relatively positive situation in conservationist terms, but further assessment would be interesting.

Nesting

Nests are found in the soil, probably opportunistically exploiting several kinds of microhabitats when available. On Monte Etna entire nests were found several times under moss.

Biology

Monogynous in all documented cases.

Social parasites

One colony was found to be hosting *T. muellerianus* (Finzi, 1922) in the R.N.O. Pino d'Aleppo (Vittoria).

Phenology

Flying queens and males (often attracted by artificial lights) and mating were observed in Mondello (Palermo) from the early days of July to late August at least.

Notes

Baroni Urbani (1964) assembled the type series of this species from an unspecified number of worker specimens and a single queen collected in Bosco di S. Pietro (Hyblaean Plateau, SE Sicily) by Giovanni Sichel. No additional information on this taxon was published after its description, and its name was mentioned only on very few occasions (Baroni Urbani 1971; Salata & Borowiec 2019). Baroni Urbani decided to define the only queen he had as the holotype, despite the specimen being damaged (missing both antennae) and with the taxonomy of *Temnothorax* being mostly built around workers. He based the worker caste description on a specimen he defined as the 'ergatotype', but this definition has no legal value anymore according to the ICZN code, therefore that specimen must be considered as a simple paratype. We examined the holotype along with several paratypes preserved at the Natural History Museums of Verona and Vienna, but our efforts to find the ergatotype failed. Over 20 years ago, it was briefly observed at the home of Bruno Poldi (1920–2002) by one of us (AA), but it appears to be absent from the MNHM where Poldi's collection is kept, as well as from the MSNV, NHMB and NHMW where other material from Baroni Urbani and specimens of *T. lagrecai* are stored. According to its description, the ergatotype shows several characters deviating from all type and non-type workers of *T. lagrecai* that we have found; however, it is unclear whether this is due to the actual features of this lost specimen or an imprecise description. *Temnothorax marae* sp. nov. co-occurs with *T. lagrecai* in the Bosco di S. Pietro, yet it is not characterized by the sharp petiole and mesoepinotal furrow of the drawing of the ergatotype of *T. lagrecai* found in Baroni Urbani's original description. In any case, the holotype of *T. lagrecai*, although partly damaged, is fully coherent with the morphology of the queens collected alongside workers with the same characters of *T. lagrecai* that we present here, so that we consider the issue over the identity of *T. lagrecai* to be resolved.

Maltese records under *Leptothorax rabaudi* Bondroit, 1918 by Schembri & Collingwood (1981) are partly based on misidentified *T. lagrecai* according to an investigation of their voucher specimens.

Temnothorax marae Alicata, Schifani & Prebus sp. nov.
urn:lsid:zoobank.org:act:CDBACBF1-90EE-47D0-B250-9B4F7D84D465
Figs 34–45

Diagnostic character combination

Antennal clubs concolorous yellowish, antennae 12-segmented in females and 13 in males, eyes normal, metanotal groove absent, worker propodeal spines short, male propodeal spines absent, petiole upper profile usually with a short horizontal component but no well-defined edges, subpetiolar process carina-like, sculpturing mostly areolate and weak.

Etymology

The species is dedicated to Mara La Rocca, wife of Antonio Alicata.

Material examined

We investigated 17 colony samples from 11 localities, consisting in a total of 41 ☿☿, 2 ♀♀, 4 ♂♂ from our collections, one colony under rearing in our possession, and additional workers from the personal collections of David Misfud and Stephen Schembri. A detailed list is provided in the Supp. file 1: Table S1.

Type material

Holotype
ITALY • ☿; Vendicari; 36.7814° N, 15.0894° E; 21 Sep. 2019; A. Alicata leg.; MSNG.

Paratypes (7 ☿☿, 2 ♀♀, 3 ♂♂)
ITALY • 1 ☿; same collection data as for holotype; MSNG • 2 ♂♂; Isola di Capo Passero; 36.6872° N, 15.1493° E; 10 Jul. 1997; A. Alicata leg.; MSNG • 6 ☿☿; Pineta di Vittoria; 36.8879° N, 14.4828° E; 30 Mar. 2001; A. Alicata and A. Adorno leg.; MSNG • 1 ♀ (damaged); Pineta di Vittoria; 36.8927° N, 14.4745° N; 25 Mar. 2019; A. Alicata and E. Schifani leg.; MSNG • 1 ♂, 1 ♀; Bosco di S. Pietro; 2 Jun. 2019; A. Alicata and S. Csősz leg.; MSNG.

Description

Worker (Figs 34–39)
Measurements and indices (7 specimens, 6 colonies, 5 localities). CL: 565.86 ± 35.45 (512–616); CW: 449 ± 34.46 (406–515); CS: 507.42 ± 34.31 (459–565); PoOC: 239.43 ± 11.73 (223–262); SL: 398.14 ± 23.5 (360–434); ML: 605.86 ± 43.15 (540–664); MW: 298.86 ± 22.28 (264–334); EL: 106.43 ± 6.7 (97–115); EW: 82.71 ± 7.43 (72–93); EYE: 94.57 ± 6.4 (84–99); SPST: 117.57 ± 13.24 (102–142); CL/CW: 1.26 ± 0.03 (1.19–1.30); PoOC/CL: 0.42 ± 0.02 (0.39–0.43); SL/CL: 0.70 ± 0.02 (0.67–0.72); ML/CS: 1.19 ± 0.02 (1.17–1.22); MW/CS: 0.59 ± 0.01 (0.57–0.61); EL/CS: 0.21 ± 0.01 (0.19–0.23); EW/CS: 0.16 ± 0.01 (0.15–0.17); EYE/CS: 0.19 ± 0.01 (0.17–0.20); SPST/CS: 0.23 ± 0.01 (0.21–0.25).

Coloration. Entirely yellowish with the exception of the gaster, which is often entirely blackish (but in some colonies the black part forms a transverse band).

Head. Subrectangular, with rounded margins near the four angles; clypeus and mandibles rounded. Antennae of 12 segments, antennal clubs of 3 segments, antennal scapes relatively short. Compound eyes relatively small and ovoidal (EYE/CS: 0.19 ± 0.01).

Mesosoma. Without a metanotal depression, straight or slightly rounded in profile. Propodeal spines notably short and thick (SPST/CS: 0.23 ± 0.01).

Metasoma. The petiole in profile view rather blunt dorsally, lacking well-defined edges; on its antero-ventral part, a small carina weakly emerges in profile view. Postpetiole ordinarily roundish in profile, subrectangular in dorsal view.

Surface sculpturing. Most of the body characterized by a variably fine areolate-rugose sculpture, the longitudinal component of which tends to be more marked on the frontal side of the head. Clypeus, gaster, and appendages smooth; a variable area extending around the frons. Very sparse, occasionally suberect but usually erect setae all over the body; dense, fine, mostly adpressed pilosity on all appendages, especially abundant on the antennal flagelli.

Queen (Figs 40–42)

Measurements and indices (1 individual). CL: 636; CW: 557; CS: 596.5; PoOC: 243; SL: 468; ML: 1032; MW: 618; EL: 174; EW: 151; EYE: 162.5; CL/CW: 1.07; PoOC/CS: 0.38; SL/CL: 0.74; ML/CS: 1.73; MW/CS: 1.04; EL/CS: 0.29; EW/CS: 0.25; EYE/CS: 0.27; SPST/CS: 0.28.

Coloration. Entirely yellowish with the main exception of parts of the gaster: all tergites are blackish with the exception of their yellowish caudalmost part, and the first tergite is yellowish near the postpetiole. Moreover, the mesoscutellar disk is caudally darkened.

Head. Subrectangular, with rounded margins near the four angles; clypeus and mandibles rounded. Antennae of 12 segments, antennal clubs of 3 segments, antennal scapes relatively short (SL/CL: 0.74). Compound eyes large and ovoidal (EYE/CS: 0.27); ocelli circular.

Mesosoma. Propodeal spines very short and thick (SPST/CS: 0.28).

Metasoma. Petiole in profile view sharp, and does not include a horizontal component. On its antero-ventral part, a small carina weakly emerges in profile view. Postpetiole ordinarily roundish in profile, subrectangular in dorsal view.

Surface sculpturing. Most of the body is characterized by a variably fine areolate-rugose sculpture, the longitudinal component of which tends to be more marked on the frontal side of the head. Clypeus, gaster, appendages, a variable area extending around the frons, anepisterna, katepisterna, mesoscutellar disk, and mesoscutum smooth. A central longitudinal stria visible in the lower portion of the clypeus. Very sparse, occasionally suberect but usually erect setae all over the body; dense, fine, and mostly adpressed pilosity on all appendages, especially abundant on the antennal flagelli.

Male (Figs 43–45)

Measurements and indices (3 specimens, 1 colony). CL: 472.67 ± 16.2 (454–483); CW: 419.33 ± 20.01 (396–437); CS: 445.83 ± 18.42 (425–460); PoOC: 197 ± 6.08 (190–201); SL: 252 ± 1.73 (251–254); ML: 845.67 ± 27.8 (814–866); MW: 467.66 ± 20.55 (448–489); EL: 185 ± 7.21 (179–193); EW: 157.66 ± 4.16 (153–161); EYE: 171.33 ± 4.16 (168–176); CL/CW: 1.06 ± 0.01 (1.05–1.07); PoOC/CS: 0.42 ± 0.02 (0.41–0.42); SL/CL: 0.53 ± 0.02 (0.52–0.55); ML/CS: 1.89 ± 0.03 (1.86–1.91); MW/CS: 1.08 ± 0.03 (1.03-1.06); EL/CS: 0.41 ± 0.01 (0.40–0.43); EW/CS: 0.35 ± 0.02 (0.33–0.38); EYE/CS: 0.38 ± 0.02 (0.36–0.40).

Coloration. Whole body yellowish, appendages whitish, gaster dark.

HEAD. Subrectangular, rounded especially above the compound eyes; clypeus and mandibles rounded. Antennae of 13 segments, antennal clubs of 4 segments, antennal scapes relatively long (SL/CL: 0.53 ± 0.02). Compound eyes large and ovoidal (EYE/CS: 0.38 ± 0.02); ocelli circular.

MESOSOMA. Propodeum spineless and rounded, most of the length of its lateral profile composed by the declivious posterior margin.

METASOMA. Petiole ordinarily low and blunt. Postpetiole ordinarily roundish in lateral profile, subrectangular in dorsal view.

SURFACE SCULPTURING. Sculpture relatively strong on the head, with well-developed longitudinal striae on its frontal side, very light all over the propodeum, petiole, postpetiole and in some areas near the

Figs 34–45. Specimens of *Temnothorax marae* Alicata, Schifani & Prebus sp. nov. in lateral (left), dorsal (center) and head view (right). Photos by Enrico Schifani, available on www.antweb.org, specimen identifiers in parentheses. **34–36**. Holotype worker from Vendicari (Hyblaean Sicily) (ANTWEB1041541). **37–39**. Paratype worker from Vendicari (Hyblaean Sicily) (ANTWEB1041542). **40–42**. Queen from Bosco di Santo Pietro (Hyblaean Sicily) (ANTWEB1041543), note that the lateral image was edited to compensate for the fact that the petiole and gaster plus postpetiole were accidentally disarticulated. **43–45**. Male from Bosco di Santo Pietro (Hyblaean Sicily) (ANTWEB1041544). Scale bars = 0.5 mm.

mesosoma lateral sutures, all the remaining parts are smooth. A central longitudinal stria visible in the lower portion of the clypeus. Sparse, erect setae all over the body; fine and mostly adpressed pilosity over the appendages.

Phylogeny (Fig. 79)

Sister species of *T. lagrecai*, which together are closely related to *T. flavicornis*.

Distribution and biogeography (Figs 80–81)

Apparently restricted to SE Sicily (Hyblaean Plateau) and the Maltese islands. The Hyblean Pleateau may be the earliest sector of Sicily to have emerged from the Mediterranean Sea and has a strong biogeographic characterization (Guarino & Pasta 2018).

Ecology and conservation

More thermophilous than any other species treated in this study, it was found at altitudes between 5 and 175 m a.s.l. (see Fig. 82). Mainly found in the leaf litter of phryganas or shrublands, but also within a native *P. halepensis* forest and under riparian vegetation. Its habitat was likely severely reduced by strong anthropization of coastal areas and agricultural activities.

Biology

Small and monogynous colonies in all documented cases.

Social parasites

One colony was found to be hosting *T. muellerianus* (Finzi, 1922) in the R.N.O. Vendicari.

Nesting

Nests were always found on dead twigs, either on bushes or laying on the ground, although nesting directly on the soil or in other microhabitats on the ground may also occur.

Phenology

Males were produced under rearing conditions during July.

Notes

Maltese records under *Leptothorax rabaudi* Bondroit, 1918 by Schembri & Collingwood (1981) are partly based on misidentified *T. marae* sp. nov. according to an investigation of their voucher specimens. *Temnothorax aveli* (Bondroit, 1918) (the senior synonym of *L. rabaudi*, see Casevitz-Weulersse & Galkowski 2009), a mainly arboreal-nesting species found in SW Europe may be absent from Malta as it is from Sicily (see also under *T. lagrecai*).

Temnothorax poldii Alicata, Schifani & Prebus sp. nov.
urn:lsid:zoobank.org:act:4A0D0DEF-4DCF-4D95-8D4D-43C56CBAC9D8
Figs 46–63

Diagnostic character combination

Antennal clubs concolorous and ferruginous, 12-segmented in females and 13 in males, eyes normal, metanotal groove absent, worker propodeal spines long, male propodeal spines absent, petiole upper profile usually with a clear horizontal component, subpetiolar process tooth-like, sculpturing characterized by distinct rugae, especially in larger workers, and a shiny area at the center of the frons.

Etymology

This species is dedicated to the Italian physician and amateur myrmecologist Bruno Poldi (1920–2002), which has also been a mentor and friend to Antonio Alicata in his early approach to the study of ants.

Material examined

We investigated 62 colony samples from 21 localities, consisting in a total of 820 ☿☿, 6 ♀♀, 2 ♂♂ from our collections. A detailed list is provided in the Supp. file 1: Table S1.

Type material

Holotype
ITALY • ☿; Monte Arso; 37.7564° N, 14.9103° E, 14 Aug. 1992; A. Alicata leg.; MSNG.

Paratypes (30 ☿☿, 2 ♀♀, 1 ♂)
ITALY • 8 ☿☿, 1 ♀; same collection data as for holotype; MSNG • 17 ☿☿, 1 ♂; Monte Ruvolo; 37.7514° N, 14.8907° E; 18 Aug. 1992; A. Alicata leg.; MSNG • 5 ☿☿, 1 ♀; Monte Manfrè; 37.6735° N, 14.9814° E; 24 May 1992; A. Alicata leg.; MSNG.

Description

Worker (Figs 46–57)
MEASUREMENTS AND INDICES (11 specimens, 5 localities, 6 colonies). CL: 694.91 ± 54.72 (636–771); CW: 626.18 ± 62.94 (529–724); CS: 660.54 ± 57.86 (585–747); PoOC: 291 ± 21.32 (268–322); SL: 538.91 ± 46.76 (481–614); ML: 784.36 ± 77.51 (671–905); MW: 384.91 ± 16.16 (324–460); EL: 137.82 ± 16.16 (111–159); EW: 102 ± 11.54 (87–120); EYE: 119.91 ± 13 (99–138); SPST: 192 ± 29.76 (148–239); CL/CW: 1.11 ± 0.04 (1.06–1.21); PoOC/CL: 0.42 ± 0.01 (0.40–0.43); SL/CL: 0.77 ± 0.02 (0.75–0.81); ML/CS: 1.18 ± 0.05 (1.10–1.28); MW/CS: 0.58 ± 0.03 (0.54–0.64); EL/CS: 0.21 ± 0.01 (0.18–0.23); EW/CS: 0.15 ± 0.01 (0.14–0.16); EYE/CS: 0.18 ± 0.01 (0.16–0.19); SPST/CS: 0.29 ± 0.02 (0.24–0.32).

COLORATION. Entirely ferruginous with the possible exception of the gaster, which varies from slightly darker than the rest of the ant to substantially black in a minority of examined colonies.

HEAD. Subrectangular with rounded margins near the four angles; clypeus and mandibles rounded. Antennae of 12 segments, antennal clubs of 3 segments, antennal scapes relatively long (SL/CL: 0.77 ± 0.02). Compound eyes relatively small and ovoidal (EYE/CS: 0.18 ± 0.01).

MESOSOMA. Without a metanotal depression, its dorsal profile being from usually rounded in lateral view. Propodeal spines relatively long and moderately thick (SPST/CS: 0.29 ± 0.02).

METASOMA. Petiole in profile view relatively high, usually presenting a clear dorsal horizontal component in profile view. On its antero-ventral part, the subpetiolar process is usually visible, consisting of a small tooth. Postpetiole ordinarily roundish in lateral profile, subrectangular in dorsal view.

SURFACE SCULPTURING. Body sculpture relatively strong, with well-marked longitudinal rugae on the mesosoma, head sides, more variably on the dorsal surfaces of the waist segments. Development of anastomoses and variably finer areolate-rugose areas between the strong longitudinal rugae is quite variable in many areas, but usually absent in the lateral sides of the pronotum. Completely smooth areas are often observed between the larger rugae. The waist segments and some areas of the mesosoma may be entirely characterized by a finer areolate-rugose sculpture not interrupted by strong rugae. The gaster, appendages, and a long area from the clypeus to the occiput through the frons are smooth. Very sparse,

occasionally suberect but usually erect setae all over the body; dense, fine, and mostly adpressed pilosity on all appendages, especially abundant on the antennal flagelli.

Queen (Figs 58–60)

MEASUREMENTS AND INDICES (2 specimens, 2 localities). CL: 823–841; CW: 771–784; CS: 797–812; PoOC: 325–335; SL: 635–655; ML: 1368–1408; MW: 860–904; EL: 229–239; EW: 177–179; EYE: 204–208; SPST: 280–298; CL/CW: 1.03–1.03; PoOC/CL: 0.39–0.41; SL/CL:0.77–0.78; ML/CS: 1.71–1.73; MW/CS: 1.08–1.11; EL/CS: 0.29–0.29; EW/CS: 0.22–0.22; EYE/CS: 0.25–0.26; SPST/CS: 0.34–0.37.

COLORATION. Entirely ferruginous, gaster darker.

HEAD. Subrectangular with rounded margins near the four angles; clypeus and mandibles rounded. Antennae of 12 segments, antennal clubs of 3 segments, antennal scapes relatively long (SL/CL:0.77–0.78). Compound eyes large and ovoidal (EYE/CS: 0.25–0.26); ocelli circular.

MESOSOMA. Propodeal spines relatively long (SPST/CS: 0.34–0.37).

METASOMA. The petiole in profile view is sharp, lacking a horizontal component dorsally. The subpetiolar process is usually visible as a small tooth. Postpetiole ordinarily roundish in lateral profile, subrectangular in dorsal view.

SURFACE SCULPTURING. Most of the body is covered with relatively fine parallel rugae with almost no anastomoses except for parts of the head, a fine areolate sculpture is visible in parts of the propodeum and nodes. Clypeus, gaster, and appendages, a variable area extending around the frons, the lower areas of the katepisterna and partly the mesoscutellar disk smooth. A central longitudinal stria visible in the lower portion of the clypeus. Very sparse, occasionally suberect but usually erect setae all over the body; dense, fine, and mostly adpressed pilosity on all appendages, especially abundant on the antennal flagelli.

Male (Figs 61–63)

MEASUREMENTS AND INDICES (3 specimens, 2 localities). CL: 574.67 ± 14.01 (559–586); CW: 554.33 ± 8.38 (549–564); CS: 564.5 ± 9.26 (554–571); PoOC: 237.67 ± 3.05 (235–241); SL: 207.67 ± 3.05 (205–211); ML: 1024 ± 14.42 (1008–1036); MW: 608 ± 15 (593–623); EL: 235.33 ± 3.78 (231–238); EW: 191.67 ± 5.03 (187–197); EYE: 213.5 ± 1.32 (212–214); CL/CW: 1.02 ± 0.01 (1.01–1.03); PoOC/CS: 0.41 ± 0.01 (0.40–0.43); SL/CL: 0.36 ± 0.01 (0.35–0.37); ML/CS: 1.81 ± 0.01 (1.80–1.82); MW/CS: 1.07 ± 0.04 (1.04–0.12); EL/CS: 0.42 ± 0.00 (0.41–0.42); EW/CS: 0.34 ± 0.01 (0.32–0.35); EYE/CS: 0.37 ± 0.00 (0.37–0.38).

COLORATION. Whole body dark reddish-brownish, appendages whitish.

HEAD. Subrectangular, rounded especially above the eyes; clypeus and mandibles rounded. Antennae of 13 segments, antennal clubs of 4 segments, antennal scapes relatively short (SL/CL: 0.36 ± 0.01). Compound eyes large and ovoidal (EYE/CS: 0.37 ± 0.00); ocelli circular.

MESOSOMA. Propodeum spineless and rounded.

METASOMA. Petiole ordinarily low and blunt. Postpetiole ordinarily roundish in profile, subrectangular in dorsal view.

SURFACE SCULPTURING. Rugulose-areolate sculpture on head and propodeum, everything else smooth. Sparse erect setae all over the body; fine and mostly adpressed pilosity over the appendages.

Figs 46–63. Specimens of *Temnothorax poldii* Alicata, Schifani & Prebus sp. nov. in lateral (left), dorsal (center) and head view (right). Photos by Enrico Schifani, available on www.antweb.org, specimen identifiers in parentheses. **46–48**. Holotype worker from Monte Arso (Etna, Sicily) (ANTWEB1041545). **49–51**. Worker from Vallone Madonne degli Angeli (Madonie, Sicily) (ANTWEB1041546). **52–54**. Paratype worker from Monte Ruvolo (Etna, Sicily) (ANTWEB1041547). **55–57**. Worker from Monte Manfrè (Etna, Sicily) (ANTWEB1041548), with ergatogynes characters in the mesosoma. **58–60**. Paratype queen from Monte Arso (Etna, Sicily) (ANTWEB1041549). **61–63**. Paratype male from Monte Ruvolo (Etna, Sicily) (ANTWEB1041550). Scale bars = 0.5 mm.

Phylogeny (Fig. 79)

Appearing as the sister taxon of *Temnothorax ibericus* (Menozzi, 1922) among the sequenced species. *Temnothorax ibericus* is a mountain species like *T. poldii* sp. nov. and is endemic to Iberia (Espadaler *et al.* 2017), but the two species are not particularly similar in broad morphological terms, and close relatedness appears unlikely.

Distribution and biogeography (Figs 80–81)

Occurs almost all over Sicily (although its ecological requirements clearly make it less widespread than *T. lagrecai*, e.g., absence from the westernmost section may be due to aridity and lack of suitable forests). An apparently similar niche is occupied by *T. alienus* in the southern Italian Peninsula (Aspromonte, Calabria).

Ecology and conservation (Fig. 82)

Collected between 340 and 1612 m a.s.l., mostly in hilly to mountainous conditions, always under tree coverage, usually consisting of different oak species. Massive historic deforestation of Sicily likely resulted in a huge habitat loss and fragmentation into isolated populations at least outside the mountain chains along the Tyrrhenian coast (Sicilian Apennines).

Nesting

Nests are found in the soil, probably opportunistically exploiting several kinds of microhabitats when available.

Biology

Monogynous in all documented cases.

Phenology

Males inside nests of *T. poldii* sp. nov. were found in August, but the time when nuptial flights occur is unknown.

Temnothorax vivianoi Schifani, Alicata & Prebus sp. nov.
urn:lsid:zoobank.org:act:F873BCD2-E771-48D1-9871-F23CFA6C7EE6
Figs 64–74

Diagnostic character combination

Antennal clubs concolorous yellowish, antennae 12-segmented in females and 13 in males, eyes normal, metanotal groove absent, worker propodeal spines short, male propodeal spines absent, petiole upper profile usually without a horizontal component, subpetiolar process carina-like, sculpturing mostly areolate and weak.

Etymology

The species is dedicated to our friend Roberto Viviano, who donated to us most of the material of this species we had the opportunity to examine, collected by him during malacological surveys.

Material examined

We investigated 20 colony samples from 11 localities, consisting in a total of 111 ☿☿, 14 ♀♀, 1 ♂ from our collections. A detailed list is provided in the Supp. file 1: Table S1.

Type material

Holotype
ITALY • 1 ☿; Monte Pellegrino; 38.1866° N, 13.3539° E; 9 Oct. 2016; R. Viviano leg.; MSNG.

Paratypes (30 ☿☿, 2 ♀♀)
ITALY • 10 ☿☿; same collection data as for holotype; MSNG • 10 ☿☿, 1 ♂; Monte Pellegrino; 38.1580° N, 13.3664° E; 15 Mar. 2016; R. Viviano leg.; MSNG • 5 ☿☿, 1 ♀; Monte Pellegrino; 38.1641° N, 13.3466° E; 20 May 2016; R. Viviano leg.; MSNG • 5 ☿☿; Monte Pellegrino, 38.1558° N, 13.3544 ° E; 6 May 2018; R. Viviano leg.; MSNG.

Description

Worker (Figs 64–69)
Measurements and indices (21 specimens, 6 colonies, 2 localities). CL: 610.25 ± 26.66 (546–642); CW: 509.27 ± 27.02 (449–554); CS: 559.2 ± 26.09 (498–598); PoOC: 255.5 ± 11.6 (229–270); SL: 451.05 ± 30.02 (395–496); ML: 643.33 ± 36.03 (548–683); MW: 323.5 ± 23.74 (281–361); EL: 110.75 ± 6.64 (97–124); EW: 84.05 ± 7.49 (71–98); EYE: 97.4 ± 6.8 (84–110); SPST: 114.19 ± 12.55 (92–145); CL/CW: 1.19 ± 0.02 (1.11–1.23); PoOC/CL: 0.42 ± 0.01 (0.39–0.44); SL/CL: 0.74 ± 0.02 (0.70–0.79); ML/CS: 1.14 ± 0.03 (1.08–1.18); MW/CS: 0.58 ± 0.02 (0.54–0.61); EL/CS: 0.19 ± 0.01 (0.18–0.21); EW/CS: 0.15 ± 0.01 (0.13–0.17); EYE/CS: 0.17 ± 0.01 (0.16–0.18); SPST/CS: 0.20 ± 0.01 (0.18–0.24).

Coloration. Entirely yellowish with the exception of a black transverse band on the first gaster tergite.

Head. Subrectangular, with rounded margins near the four angles; clypeus and mandibles rounded. Antennae of 12 segments, antennal clubs of 3 segments, antennal scapes relatively short (SL/CL: 0.74 ± 0.02). Compound eyes relatively small and ovoidal (EYE/CS: 0.17 ± 0.01).

Mesosoma. Without a metanotal depression, straight or slightly rounded in profile. Propodeal spines notably short and thick (SPST/CS: 0.20 ± 0.01).

Metasoma. The petiole in profile view usually presents no dorsal horizontal component. Subpetiolar process carina-like. Postpetiole ordinarily roundish in profile, subrectangular in dorsal view.

Surface sculpturing. The body is almost entirely meticulously covered with a relatively fine areolate-rugose sculpture, the longitudinal component of which tends to slightly more marked on the frontal side of the head. Clypeus, gaster, and appendages smooth; a variable area extending around the frons. Very sparse, occasionally suberect but usually erect setae all over the body; dense, fine, and mostly adpressed pilosity on all appendages, especially abundant on the antennal flagelli.

Queen (Figs 70–72)
Measurements and indices (5 specimens, 4 colonies, 1 locality). CL: 732.4 ± 36.48 (672–769); CW: 646.6 ± 33.98 (588–670); CS: 689.83 ± 34.80 (630–719); PoOC: 283.8 ± 13.6 (264–301); SL: 527.8 ± 28.2 (482–552); ML: 1150.2 ± 53.66 (1062–1204); MW: 693.6 ± 37.15 (630–723); EL: 190.8 ± 14.16 (171–210); EW: 154.8 ± 6.38 (144–160); EYE: 172.8 ± 10.02 (157–184); SPST: 212.6 ± 9.61 (200–227); CL/CW: 1.06 ± 0.01 (1.05–1.07); PoOC/CL: 0.39 ± 0.00 (0.38–0.39); SL/CL: 0.72 ± 0.02 (0.71–0.73); ML/CS: 1.67 ± 0.02 (1.63–1.70); MW/CS: 1 ± 0.00 (1.00–1.01); EL/CS: 0.28 ± 0.01 (0.26–0.29); EW/CS: 0.22 ± 0.00 (0.22–0.23); EYE/CS: 0.25 ± 0.00 (0.24–0.26); SPST/CS: 0.31 ± 0.01 (0.29–0.33).

Coloration. Entirely yellowish with the exception of black transverse bands on each gaster tergite.

Head. Subrectangular, with rounded margins near the four angles; clypeus and mandibles rounded. Antennae of 12 segments, antennal clubs of 3 segments, antennal scapes relatively short (SL/CL: 0.72 ± 0.02). Compound eyes large and ovoidal (EYE/CS: 0.25 ± 0.00); ocelli circular.

Mesosoma. Propodeal spines very short and thick (SPST/CS: 0.31 ± 0.01).

Metasoma. The petiole in profile view sharp dorsally and rather high; on its antero-ventral part, a small carina weakly emerges in profile view. Postpetiole ordinarily roundish in lateral profile, subrectangular in dorsal view.

Figs 64–74. Specimens of *Temnothorax vivianoi* Schifani, Alicata & Prebus sp. nov. in lateral (left), dorsal (center) and head view (right). Photos by Enrico Schifani, available on www.antweb.org, specimen identifiers in parentheses. **64–66.** Holotype worker from Monte Pellegrino (Palermo Mountains, Sicily) (ANTWEB1041551). **67–69.** Paratype worker from Monte Pellegrino (Palermo Mountains, Sicily) (ANTWEB1041552). **70–72.** Paratype queen from Monte Pellegrino (Palermo Mountains, Sicily) (ANTWEB1041553). **73–74.** Damaged male from Palermo Mountains (Sicily) (ANTWEB1041554). Scale bars = 0.5 mm.

SURFACE SCULPTURING. Most of the body is covered with moderately marked longitudinal rugae, which are stronger on head. Very fine areolate sculpture is also present on propodeum and waist segments. Clypeus, gaster, and appendages smooth; a variable area extending around the frons, anepisterna, katepisterna, mesoscutellar disk and mesoscutum. Central longitudinal stria visible in the lower portion of the clypeus. Very sparse, occasionally suberect but usually erect setae all over the body; dense, fine, and mostly adpressed pilosity on all appendages, especially abundant on the antennal flagelli.
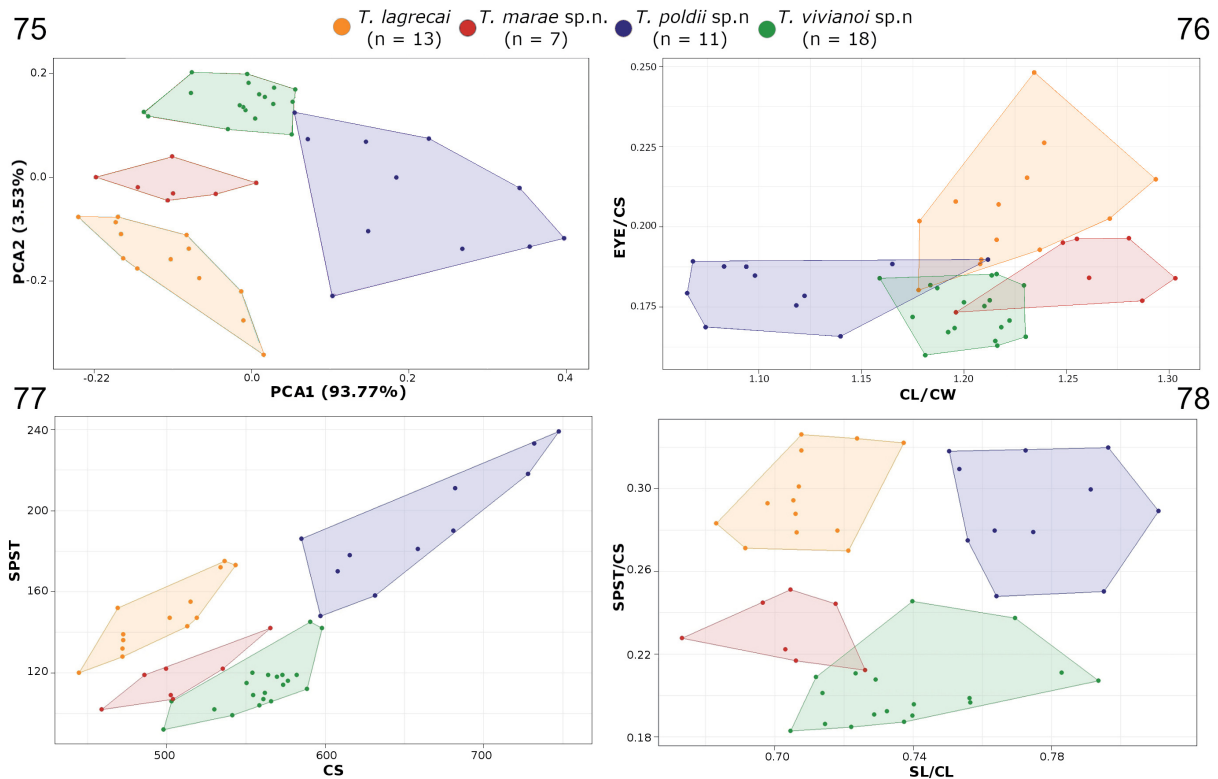
Male (Figs 73–74)

MEASUREMENTS AND INDICES (1 individual - unfortunately, only a single damaged specimen of this species was found so far, solely consisting of the mesosoma, wings and legs). ML: 765; MW: 385.

COLORATION. Entirely yellowish.

MESOSOMA. Propodeum spineless and rounded, in profile view propodeal declivity clearly shorter than the propodeal dorsum.

SURFACE SCULPTURING. Sculpture very weak, most areas smooth with exception of a fine areolate-rugose sculpture on the propodeum. Few sparse erect setae.

Figs 75–78. Morphometric differentiation of the four taxa treated in this study based on the worker caste. **75**. Principal component analysis of all nine morphometric characters (excluding indices). **76**. Scatter plot of eye size index (EYE/CS) against cephalic length index (CL/CW). **77**. Scatter plot of absolute spines length (SPST) against cephalic size (CS). **78**. Scatter plot of spine length index (SPST/CL) against scape length index (SL/CS).

Phylogeny (Fig. 79)

Sister taxon to all species in 'Palearctic subclade IV' sensu Prebus (2017), which consists of a large radiation of species with Mediterranean, Middle Eastern and northern Afrotropical distributions.

Distribution and biogeography (Figs 80–81)

Restricted to NW Sicily and neighboring Aegadian Islands in the drepano-panormitan biogeographic sector that is constituted by a very old carbonatic platform. The area is known to have a significant
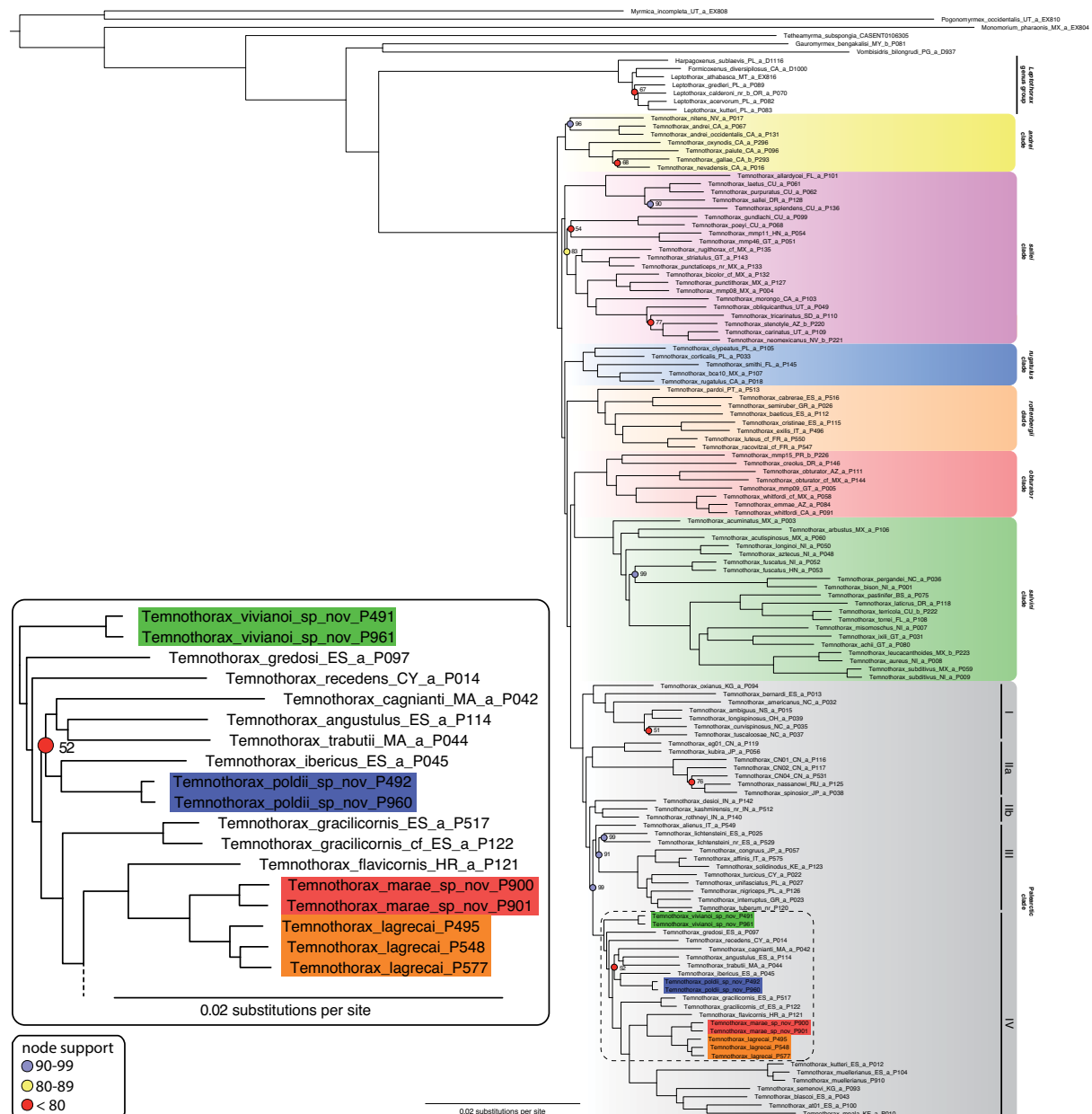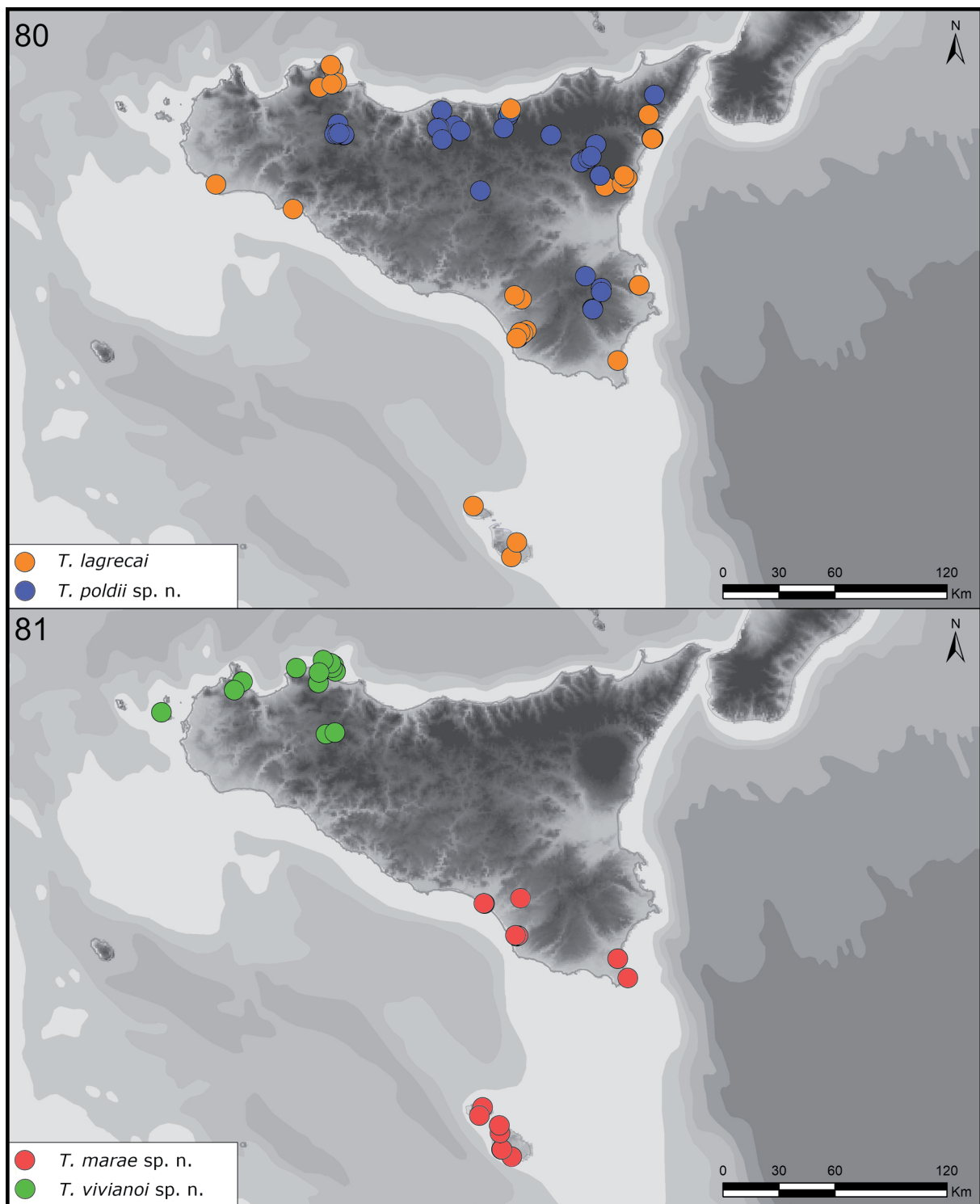

Fig. 79. Maximum likelihood phylogeny inferred with IQTREE ver. 2.1.2. The major clades found in Prebus (2017) are highlighted, and the focal species of the current study (all within the 'Palearctic clade IV') are evidenced as in Figs 75–78. Maximum likelihood bootstrap support for all nodes are 100, except where indicated.

Figs 80–81. Geographic distribution of the four taxa treated in this study. Note that the shallow water area (light grey) between Sicily and the Maltese Islands roughly corresponded to a land bridge during glaciations.
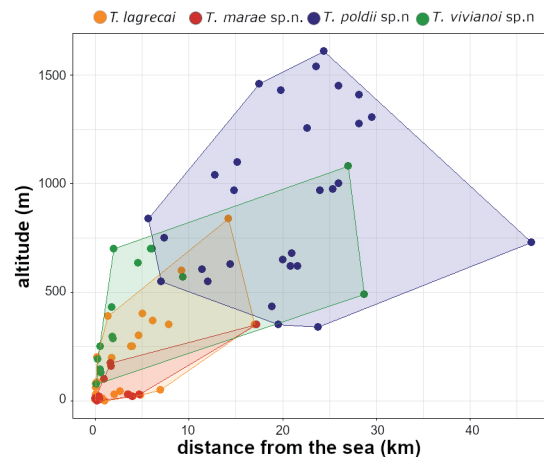
Fig. 82. Spatial characterization of the sites where the four species treated in this study were found.

zoogeographic relevance for ants, hosting some species that are either unique or absent from the rest of Sicily, as discussed by Schifani *et al.* (2020).

Ecology and conservation

Found from 75 to 1080 m a.s.l. (see Fig. 82). Most of our sites are characterized by forest or shrubs of *Quercus ilex*/*Fraxinus ornus* L. growing on rocky landslides or very rocky substrates, usually at the base of hundred meters-high north-facing cliffs hosting relatively wet and cool conditions compared to the surroundings. At least twice it was found in more open habitats characterized by rocky outcrops with sparse shrubs, in one case surrounded by agricultural areas. Populations may be fragmented due to anthropogenic fragmentation of forested areas and shrublands, but further assessment are required.

Biology

Small and monogynous colonies in all documented cases.

Nesting

Nests are found in the soil, probably opportunistically exploiting several kinds of microhabitats when available. The large number of the samples we examined consisted of colonies living entirely within abandoned shells of gastropods. In Monte Pellegrino, on several occasions entire colonies were found inside the abandoned shells of *Marmorana* (*Murella*) *sicana* (Férussac, 1822), a short-range endemic species which is locally very abundant. In Monte Pecoraro, a colony was found under a stone inside an abandoned shell of another short-range endemic of Palermo mountains, *Chilostoma macrostoma* (Rossmässler, 1837). It has also been found within shells of *Rumina decollata* (Linnaeus, 1758). However, these repeated findings may not indicate a true specialization in the use of abandoned gastropod shells, but a reflection of the nesting opportunities offered by many of the collecting sites, as well as the malacological nature of the sampling efforts during which it was found.

Phenology

Nuptial flights observed in late August in Mondello (Palermo), where queens were attracted by artificial lights.

Notes

Specimens from Favignana (Aegadian Islands) mentioned as unidentified or related to *T. lagrecai* by Mei (1995) and Scupola (2009) represent *T. vivianoi* sp. nov. based on our investigation of vouchers at the MSNG.

Worker-based key to the Sicilian species of Temnothorax with light-colored pigmentation and concolorous antennal clubs

Note that recently emerged workers and queens of dark species (e.g., *T. exilis*) may temporarily present a yellowish pigmentation. The key is meant to be used for species in which the final pigmentation of females is naturally light-colored. High-quality images of all the species are available on AntWeb.org. Quantitative and qualitative morphological characters mentioned in the key are defined in the Material and methods section of this paper.

1. Antennal segments reduced to 11 2
– 12 antennal segments 3

2. Extreme development of the subpetiolar process, short spines .... social parasite species of the *gordiagini* group, of which only *T. ravouxi* (André, 1896) is confirmed for Sicily. However, the possible presence of *T. kraussei* (Emery, 1915) cannot be discarded, see Seifert (2018) for their differentiation
– Subpetiolar process falling within the variation described in the materials and methods of this study; free-living species ....***T. flavicornis*** (Emery, 1870)

3. Long erect setae on the dorsum of the body; metanotal groove very deep; propodeal spines very short ....***T. recedens*** (Nylander, 1856)
– Setae shorter; metanotal groove absent or weak; if present, propodeal spines longer 4

4. Relatively large arboreal species; most of the body ferruginous; petiole bulky and short, unlike any other species of the key; propodeal spines long ....***T. clypeatus*** (Mayr, 1853)
– Character combination deviating from above 5

5. Petiole narrow; metanotal groove present; head often darker than the mesosoma; subpetiolar process limited to a carina-like shape ....***nylanderi*** group (also see Csősz *et al.* 2015)   6
– Character combination deviating from above 7

6. Propodeal spines very long, deviating very little from the mesosoma axis (20–25°); more thermophilous .... ***T. lichtensteini*** (Bondroit, 1918)
– Propodeal spines shorter, deviating more from the mesosoma axis; usually higher altitude sites .....
....***T. nylanderi*** (Foerster, 1850)

7. Color ferruginous; sculpture striate, particularly strong in larger individuals; frons with a shiny area; propodeal spines long; size large (normally CS > 600) ....
....***T. poldii*** Alicata, Schifani & Prebus sp. nov.
– Color yellowish, sculpture weaker, size normally smaller (CS < 600) 8

8. Propodeal spines long; subpetiolar process tooth-like .... ***T. lagrecai*** (Baroni Urbani, 1964)
– Propodeal spines short; subpetiolar process with a carina-like form 9

9. Upper profile of petiole rather blunt, forming a weakly delimited horizontal plane; SE Sicily ....
....***T. marae*** Alicata, Schifani & Prebus sp. nov.
– Upper profile of petiole very sharp, usually not forming an horizontal plane; NW Sicily ....
.... ***T. vivianoi*** Schifani, Alicata & Prebus sp. nov.

UCE sequence processing and phylogenomic inference

Following assembly and UCE extraction, the mean number of loci per specimen was 2242, with a mean contig length of 885 bp, and a mean coverage score of 44.3x (see Supp. file 1: Table S2). Following alignment, trimming and filtering of the full UCE dataset to loci with ≥85% taxon presence, the dataset had 2039 loci, with a mean locus alignment length of 793 bp. The concatenated matrix was 1 617 690 bp in length, in which 819 040 sites (50.6%) were variable, 533 245 sites (33%) were parsimony informative, with 19% missing data.

The partitioning analysis of the dataset resulted in a 260-partition scheme (see Dryad data repository; https://doi.org/10.25338/B8K63Z). The tree resulting from the IQTREE analysis (Fig. 80) had strong overall maximum likelihood bootstrap support. The phylogenetic placements of the species described in the current study, all of which fell within the subclade 'Palearctic clade IV' (sensu Prebus 2017), were unambiguous with respect to their closest relatives. As a side note, 'Palearctic clade II' (sensu Prebus 2017), which was composed of taxa from eastern and southern Asia, was recovered as paraphyletic in the current study. It now appears to consist of two clades, which are renamed 'Palearctic clade IIa' and 'Palearctic clade IIb' (see Fig. 79).

Discussion

The ideal context for descriptive alpha-taxonomic studies is often that of revisions carried out at the species group or species complex level. If the species of a genus clearly cluster into multiple species groups and complexes of easily definable boundaries, describing new taxa within the context of well-delimited groups is a safe way to build a stable taxonomic framework and avoid missing synonymies. However, while a clear morphological definition of monophyletic species groups may be easily achieved in some ant genera, for the time being this is rarely the case within the highly diverse W-Palearctic fauna of *Temnothorax*. This impediment clearly conditioned our investigation, whose results further suggest caution in future studies. We could not detect any described species closely resembling *T. poldii* sp. nov. and we were unable to define a species group hosting this species even after obtaining phylogenetic data on its account. Its (relative) phylogenetic proximity with *T. ibericus* is interesting. The latter was lately proposed to be a member of the *sordidulus* group (Arcos 2021), yet *T. sordidulus* (Müller, 1923) and the species complex named after it were defined as a part of the *nylanderi* group (Csősz *et al.* 2015), which is unrelated to either *T. ibericus* or *T. poldii* according to our phylogenomic analysis. Molecular data on *T. sordidulus* to settle this issue are not yet available. The situation was even more complex for the other three species treated in this study. For instance, *T. lagrecai* was previously thought to belong to the *luteus* complex (Lebas *et al.* 2016), and *T. flavicornis* was thought to belong to the *nylanderi* group (Csősz *et al.* 2015). Our phylogenomic evidence shows that the *luteus* complex belongs to the *rottenbergii* clade and is close to the *exilis* group, while *T. lagrecai* and *T. flavicornis* cluster together within the Palearctic clade, but far from the *nylanderi* group. On the other hand, some authors claimed the existence of a Ibero-Maghrebian species complex ('superspecies') composed by taxa such as *T. atomus* (Cagniant & Espadaler, 1997), *T. curtulus*, *T. gentilis*, *T. monjauezi* (Cagniant, 1968) and *T. tebessae* spp. and belonging to the *tuberum* group (Cagniant & Espadaler 1997; also see Reyes-López & Carpintero-Ortega 2013). Following the key of Cagniant & Espadaler (1997), *T. lagrecai*, *T. marae* sp. nov. and *T. vivianoi* sp. nov. could also belong to the same group, which would also be biogeographically credible. While we are inclined to think that the closest relatives of these three species treated in this study may be found within the *tebessae* complex sensu Cagniant & Espadaler (1997), we doubt it can represent a monophyletic entity anyway: not only even *T. luteus* would key out as a member of it, but *T. vivianoi* is not closely related to *T. lagrecai* and *T. marae*. Also, none of them belongs to the *tuberum* group anyway. And while *T. lagrecai* and *T. marae* clustered closely together, there was no easily identifiable morphological character that could have led us to believe, prior to the phylogenomic results, that *T. marae* was closer to *T. lagrecai* compared to *T. vivianoi*. In other words, the taxonomy of

Palearctic *Temnothorax* currently suffers from an absence of clearly defined and reliable morphological characters to delimit the boundaries of species groups at least for a large proportion of species. It is possible to speculate on whether such characters may be hidden in the often undescribed male or even queen castes of *Temnothorax* (although queens often share many characters with workers). In fact, male morphology may, at least occasionally, be a powerful source of information on species group delimitation in some other ant genera (e.g., Alicata & Schifani 2019). Moreover, dispersing sexuals can occasionally prove very important to detect the presence of certain ant species (Espadaler & López-Soria 1991; Schifani *et al.* 2021b; Menchetti *et al.* 2022). However, less than 20% of the descriptions of *Temnothorax* of the last 20 years included the male caste (Bolton 2021), and even for well-studied regions like Central Europe no keys based on males are available yet (Seifert 2018). Integrating morphological descriptions with molecular data proves especially important to understand the diversification patterns and evolutionary relationships within Mediterranean *Temnothorax*.

Further investigation should also focus on the ecology and possible conservation issues of the four species treated in this study. Certain morphological traits here described for their workers may offer some first speculative hypotheses over their lifestyle based on possible functional implications: greater absolute size variation and relatively wider heads of *T. poldii* sp. nov. compared to the other species may indicate slightly more developed task subdivision and stronger mandible force (e.g., Retana & Cerdá 1994; Gronenberg *et al.* 1997), while relatively larger eyes in *T. lagrecai* may be linked to different light conditions associated with foraging activities (e.g., Weiser & Kaspari 2006). These and several other biological aspects of these four species remain to be explored.

Acknowledgments

Data availability

The input alignment files, partition files, and output from the IQTREE analyses are available on Dryad https://doi.org/10.25338/B8K63Z). All raw reads from newly generated sequence data are available from the NCBI Sequence Read Archive (SRA) (BioProject PRJNA770978). Morphometric and geographic distribution data are available in the Supp. file 1 of this paper.

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Supp. file 1. List of investigated samples and their geographic origin, as well as genetic and morphometric data used in this study. **Table S1** – Material examined. **Table S2** – Sequence data. **Table S3** – Morphometric data. https://doi.org/10.5852/ejt.2022.833.1891.7485