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SHORT NOTE

Social Parasite Ants in the Alps: a New Site of the Vulnerable *Myrmica myrmicoxena* and New Uppermost Altitudinal Limit for *M. microrubra*

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Abstract

We conducted a survey on the Alpine fauna of one of the largest Natural Park of the Italian Alps (Stelvio National Park) in the framework of a broad ecological monitoring of Alpine biodiversity. A two-years standardized sampling employing pitfall traps along a 1200 m altitudinal gradient led to the discovery of the inquiline social parasite ants *Myrmica myrmicoxena* Forel, 1895 and *M. microrubra* Seifert, 1993. *Myrmica myrmicoxena*, which is classified as Vulnerable according to the IUCN Red List, was so far known from only three sites across a narrow geographic range between Italy and Switzerland. Our data support the previous hypothesis over its ecology and host association. *Myrmica microrubra* is considered an incipient species of high evolutionary interest, sometimes regarded as an intraspecific form of *M. rubra*. While having a wide distribution in Europe, its presence in Italy was hitherto known only from a single site. Our record extends its altitudinal distribution limit in Europe upwards by about 600m.

Social parasitism has evolved several times and in different forms among ants, showing amazing morpho-functional, chemical and behavioral adaptations to this peculiar habit (Visicchio et al., 2001; Buschinger, 2009). Inquilinism is considered the most extreme of such forms: inquiline social parasites produce few to no workers while they concentrate their efforts on the production of reproducers which their hosts raise (Hölldobler & Wilson, 1990; Buschinger, 2009; Degueldre et al., 2021). As a result, inquiline ant species are rarely detected during field surveys. There is an underdeveloped understanding of their biology, ecology, and conservation status (López-Soria, 1991; Schifani, 2017). However, a large number of the European inquiline ants were classified as Vulnerable on the IUCN Red List (Social Insects Specialists Group, 1996).

The genus *Myrmica* Latreille, 1804 counts about 190 species distributed across the Holarctic region (Bolton, 2021), and it includes a remarkable variety of inquiline social parasites: in the West-Palearctic region, over 30 *Myrmica* species are recognized (Radchenko & Elmes, 2010; Seifert, 2018),

one quarter of which is made of obligate or facultative social parasites of other congeneric species (Radchenko & Elmes, 2003; 2010). Most of them are inquilines, which evolved multiple times, independently in the genus (Radchenko & Elmes, 2003; Jansen et al., 2010; Seifert, 2018). Unlike in other ant genera (e.g., *Plagiolepis*, see Degueldre et al., 2021), most parasite *Myrmica* spp. do not follow the strict version of Emery's rule (Emery, 1909), as their host species are not always their closest evolutionary relatives (Jansen et al., 2010). In particular, parasite *Myrmica* species with a long evolutionary history seemed to have developed the ability to exploit a wider and less closely related set of congeneric species as hosts (Jansen et al., 2010; Seifert, 2018).

Some of the free-living species of the *M. scabrinodis*-complex are the hosts for the overwhelming majority of the West-Palearctic social parasite *Myrmica* (Radchenko & Elmes, 2003; 2010; Seifert, 2018): these range from the widespread facultative parasite *M. vandeli* Bondroit, 1920, to more specialized species with narrower geographic ranges (*M. bibikoffi* Kutter, 1963, *M. hirsuta* Elmes, 1978, and *M. laurae* (Emery, 1907)), to forms of extreme morpho-functional adaptation to inquilinism (*M. lesmanei* Bernard, 1967, *M. kabylica* Cagniant, 1970, and *M. karawajevi* Arnol'di, 1930) (Radchenko & Elmes, 2010; Seifert, 2018). Host species range from only one to several, with *M. karawajevi* exploiting the widest amount of different hosts, probably due to a longer evolutionary history (Jansen et al., 2010; Seifert, 2018).

Outside the *M. scabrinodis*-complex, only two parasite *Myrmica* species are found in the West-Palearctic. The Alpine endemic *M. myrmicoxena* is a workerless inquiline collected only in three sites within a narrow altitudinal range for 150 years. *M. lobulicornis* (from the *M. lobicornis*-group) is considered to be likely its only host species (Forel, 1895; Glaser et al., 2010). On the other hand, *M. microrubra* Seifert, 1993, widely distributed in Europe, is either considered as an incipient species or as an intraspecific parasitic form of *M. rubra* (Linnaeus, 1758) (Pearson, 1980; 1981; Seifert, 1994; 2018; Seppä & Pamilo, 1995; Steiner et al., 2006; Schär & Nasch, 2014; Leppänen et al., 2011; 2015; 2016). Here we treat it as a good species following the latest review of the European ant fauna (Seifert, 2018).

From 2018 to 2020, terrestrial arthropods were sampled by 150 pitfall traps in 30 sites across an altitudinal and habitat gradient in the Lombardy Sector of the Stelvio National Park. The traps were built with plastic glasses of c. 6 cm of diameter and c. 7 cm of height, buried in the ground, filled with c. 150 ml cc of an attractive and a preserving mixture of white vinegar, sodium chloride, and a drop of detergent as a surfactant, as described by Gobbi (2020). A total of 1800 samples were taken during six sampling sessions per year (150 x 6 x 2 = 1800). Ants were identified under a Zeiss Stemi 508 stereoscopic microscope and measured with the aid of an Axiocam Erc 5 s and Zeiss Zen Core Software according to the keys provided by Radchenko & Elmes (2010) and Seifert (2018). This effort led to a significant database of over 1700 Alpine ant records and interesting incidental discoveries, such as the first finding of ergatandromoph individuals of *M. lobulicornis* in 2018 (Schifani et al., 2020). In 2019, two rare social parasite *Myrmica* species of particular interest were collected, namely *M. microrubra* and *M. myrmicoxena* (Fig 1). Both were previously known from only one locality in Italy (Glaser, 2003; Glaser et al., 2011), and for both, the new occurrence sites yielded new important ecological or distributional information. Collecting data of the two species are as follows:

- *M. microrubra* (1♀) alongside *M. rubra* (103☿☿ 1♀): Valle Messi, Sondrio, 46.296930, 10.503609, 1588 m asl, south-facing slope, peat bog, 24.IX.2019 (plot 6.1.4).
- *M. myrmicoxena* (1♀) alongside *Formica lemani* Bondroit, 1917 (5☿☿), *F. lugubris* Zetterstedt, 1838 (13☿☿), *M. lobulicornis* (12☿☿): Sobretta-Valle del Gavia, Sondrio, coordinates hidden in conformity with Glaser et al. (2011), 2175 m asl, north-facing slope, pasture/shrubland, 04.X.2019 (plot 4.5.2).

The geographic range of *M. microrubra* is relatively wide but does not cover the entire distribution of its host *M. rubra* (Seifert, 2018). Records of *M. microrubra* around the Alps often come from sites below 700 m asl (e.g., Wagner, 2020). In Italy, *M. rubra* records are mostly distributed North of the Apennines and especially common in the Po Plain and Alps (Baroni Urbani, 1971; Mei, 1984; Le Moli & Zaccone, 1995; Glaser, 2003; 2004; Sielezniew et al., 2010; Glaser et al., 2012; Scupola, 2018; Castracani et al., 2020). The first discovery of *M. microrubra* in Italy was published by Glaser (2003) and made in South Tyrol (980 m asl). Since our record also comes from the Alpine region, it is still uncertain where the species' southernmost distribution limit stands. During our survey, *M. rubra* was detected in four sites, mostly occurring from 1400 to 1600 m asl, with a single finding at 1900 m asl. A total of 1848 *M. rubra* workers were found in 113 samples from pastures or peat bogs. Ecological data on *M. microrubra* are relatively few and scattered; however, to the best of our knowledge, our record significantly extends its altitudinal limit upwards by about 600 m.

On the other hand, ecological data over *M. myrmicoxena* mostly come from only two sites (Glaser et al., 2011), as very little information is contained in its original description (Forel, 1896). Our data are consistent with these previous two findings regarding altitudinal preferences (*M. myrmicoxena* was previously found at 1700 and 2213 m asl), habitat selection (Alpine pastures), North-facing slopes, and host species. The new site is geographically placed between the two Swiss sites and the Italian site reported by Glaser et al. (2011) (Fig 2). We collected *M. lobulicornis*, possibly the exclusive host of *M. myrmicoxena*, on 151 samples distributed in 15 sites from 1600 to 2400 m asl, resulting in 962 workers. Sites above 1900 m mostly represented open habitats, while a few sites at lower altitudes were coniferous and deciduous forests or peat bogs.

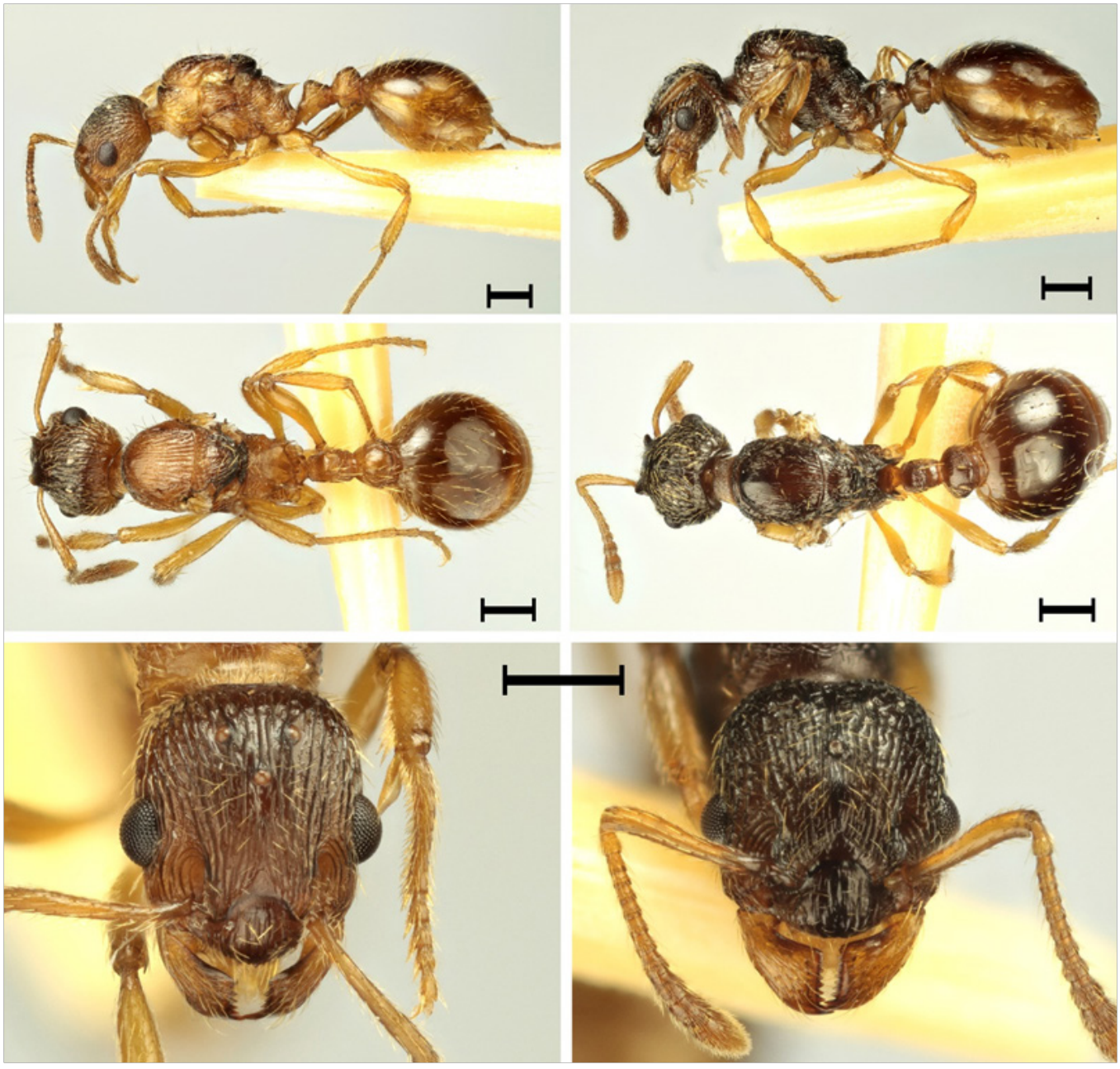

Fig 1. *Myrmica microrubra* (left) and *M. myrmicoxena* (right) from the Stelvio National Park. Scale bars: 0.5 mm.

In comparison, the related *M. lobicornis*, which appears to be the only other species that could potentially serve as a host for *M. myrmicoxena* (Glaser et al., 2011), was collected between 1400 (the lowest sampled altitude) and 2000 m asl in 12 sites. Given the extreme scarcity of data on *M. myrmicoxena* and the conservation concerns expressed by previous authors, this additional discovery significantly reinforces the ecological and biological hypotheses formulated so far (Glaser et al., 2011). The Italian distribution of *M. lobulicornis* currently appears poorly documented, and future investigations may contribute to either confirming or dismissing *M. myrmicoxena* as an Alpine endemic ant (a rare condition only shared with *F. paralugubris* Seifert, 1996 – see Seifert, 2018).

Myrmica microrubra and *M. myrmicoxena* were caught only in 0.7-0.9% of the samples in which their respective host species were found. During the two-years survey, only a single specimen of another inquiline social parasite (*Formicoxenus nitidulus* (Nylander, 1846) (also considered Vulnerable by the IUCN, Social Insect Specialists Group, 1996) was found in about 980 samples containing its host species (a queen was found in the same locality of *M. myrmicoxena* in a trap retrieved on 16.VII.2018). This collection number represents a lower rate compared to the parasite *Myrmica* spp. despite literature data suggesting this species is frequent in the Italian Alps (Baroni Urbani, 1971). Inquiline social parasites are difficult to detect during standardized field surveys with a generic focus on ants (e.g., Glaser, 2004; Glaser et al., 2012;

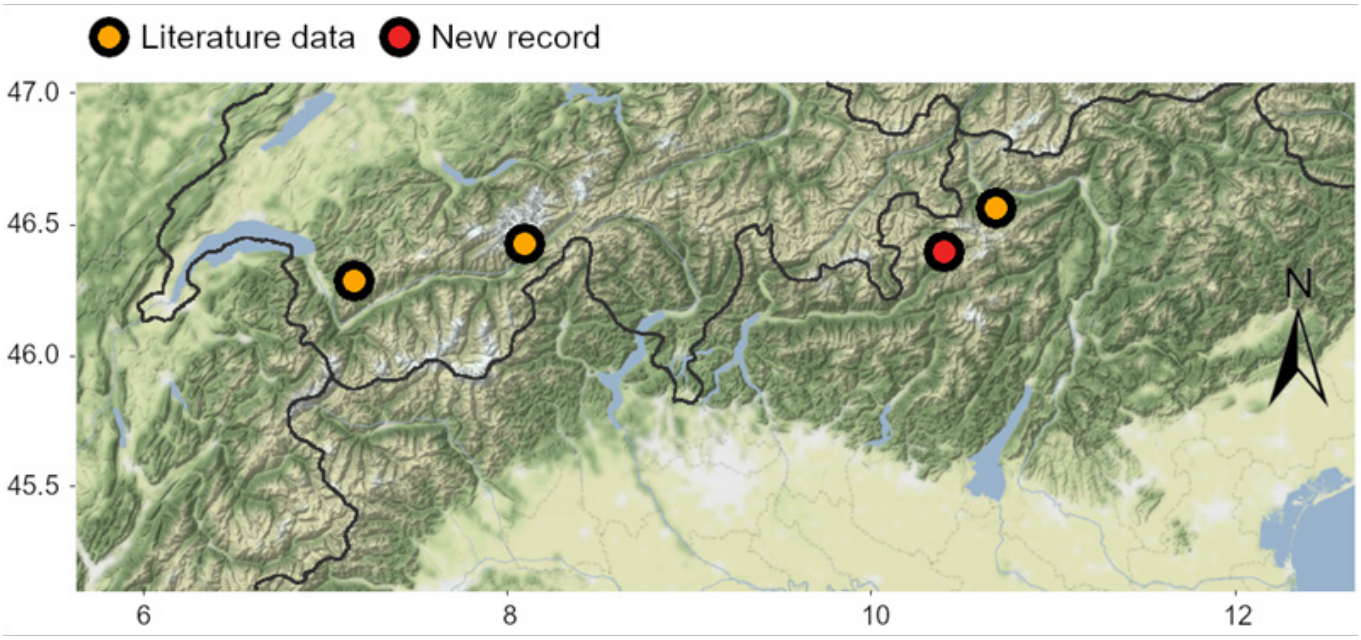

Fig 2. The four Alpine sites where *Myrmica myrmicoxena* has been found in Italy and Switzerland.

Spotti et al., 2015), and pitfall trapping is not a proper sampling method useful to detect inquiline parasites. Yet, our findings demonstrate that even on these ants, important information can still be recovered thanks to the extensive use of this sampling tool in studies with a broader ecological aim. In both our findings, the parasites were found in the same sample along with several individuals of their respective host species, which suggests they may have been caught during colony relocation. Finally, insect decline and its severe potential outcomes on ecosystems functioning has recently attained much attention showing the need for a deeper commitment in field survey, the development of effective monitoring systems, and biodiversity database implementation in the face of global changes and contexts with different anthropic impact (Campanaro et al., 2011; Gibb et al., 2017; Leather, 2017; Homburg et al., 2019).

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Authors' Contributions

ES: conceptualization, methodology, investigation, data curation, writing-original draft, writing-review & editing, visualization.
CC: conceptualization, methodology, validation, resources, data curation, writing-review & editing, supervision, project administration.
LP: methodology, validation, resources, data curation, supervision, project administration, funding acquisition.
MGo: methodology, validation, resources, data curation, writing-review & editing, supervision, project administration, writing-review & editing.
VL: validation, resources, data curation, supervision, project administration, funding acquisition.
FAS: validation, resources, writing-review & editing.
AM: resources, writing-review & editing, supervision, project administration, funding acquisition.
DG: visualization, writing-review & editing.
DAG: resources, writing-review & editing, supervision, project administration, funding acquisition.
MGh: resources, writing-review & editing.
MG: funding acquisition.

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