

Distribution, host specificity, and the potential for cryptic speciation in hoverfly *Microdon myrmicae* (Diptera: Syrphidae), a social parasite of *Myrmica* ants

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Abstract. 1. In 2002 *Microdon myrmicae*, a social parasite of *Myrmica* ants, was taxonomically separated from *Microdon mutabilis*. The original study in the U.K. found *Microdon myrmicae* to be specific to one ant species, *Myrmica scabrinodis*, yet it became apparent that the range of *Microdon myrmicae* includes at least the western Palaearctic.

2. Current knowledge of the European distributions of both *Microdon myrmicae* and *Microdon mutabilis* in Europe is reviewed. Also, in detailed studies of two Polish populations, *Microdon myrmicae* was found to survive equally well with two *Myrmica* ant species. We examine, however, the possibility that this reflects the presence of two separate *Microdon* species, each connected to one species of *Myrmica*.

3. Forty populations of *Microdon myrmicae* and 37 populations of *Microdon mutabilis* are currently known in Europe. All the populations in central and southern Europe that were visited after the separation of the two species were identified as *Microdon myrmicae*, while *Microdon mutabilis*' recognised range is now restricted to the British Isles and Scandinavia. *Myrmica scabrinodis* was found to host *Microdon myrmicae* in 26 out of 31 populations investigated. Four other *Myrmica* species were identified to the host *Microdon myrmicae*: *Myrmica gallienii* (eight populations), *Myrmica rubra* (four), *Myrmica vandeli* (one), and *Myrmica sabuleti* (one). *Microdon myrmicae* occurs in waterlogged grassland habitats, mainly of the '*Molinietum*' type, resulting in a patchy distribution relative to its host ants.

4. In two populations *Myrmica scabrinodis* and *Myrmica gallienii* are both abundant and rear *Microdon myrmicae* in equal proportions. *Microdon myrmicae* pupae from *Myrmica gallienii* nests were heavier and the anterior respiratory organs were of significantly different shape. In contrast, the comparisons of *Microdon myrmicae* pupae among all other populations showed no significant differences, suggesting only one species throughout the European range.

Key words. Cryptic lineages, distributions, habitat preferences, *Microdon*, myrmecophily.

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Introduction

Non-ant social parasites in ant colonies, so-called myrmecophiles, have long been seen as enigmatic species in conservation, partly because they have been shown to be very sensitive to environmental change, but also because a large proportion of species is recorded as rare and endangered (Settele & Kühn, 2009; Thomas *et al.*, 2009). Yet, the conservation of these species depends largely on gaining a good understanding of their host ant specificity, as well as on how consistent such specificity is at a larger geographical scale (Thomas *et al.*, 2005, 2009). This is particularly the case for *Microdon myrmicae*, which was separated from *Microdon mutabilis* on patterns of its host ant use in the British Isles (Schönrogge *et al.*, 2002; Thomas *et al.*, 2005). Resolving questions on its patterns of host ant specificity on a broader geographical scale and on how such variation might arise will be important for ensuring its long-term survival.

Obligate social parasites of ants is a species-rich group with an estimated 10 000 species worldwide, many of which are listed in national and international red data books (Thomas *et al.*, 2005). Parasites of social insects and other parasitic organisms are thought to be particularly prone to evolve cryptic lineages. The ability to circumvent host defences provides strong selection, although often on physiological rather than morphological traits. One example is the use of chemical mimicry to infiltrate host ant colonies (Akino *et al.*, 1999; Elmes *et al.*, 1999; Schönrogge *et al.*, 2004). The intricacy of the subterfuge for social parasites interacting with ants has been demonstrated in detailed studies of chemical mimicry in *Maculinea* butterflies (Akino *et al.*, 1999; Thomas *et al.*, 2002; Schönrogge *et al.*, 2004; Thomas & Settele, 2004; Nash *et al.*, 2008). In one host–parasite pair, *Myrmica rubra* (Linnaeus) and *Maculinea alcon* (Denis and Shiffermüller), it has been demonstrated that the combination of recognition compounds varies in both species at population level such that mosaic type coevolution is the most likely explanation (Nash *et al.*, 2008).

Another less well-studied group of social parasites are hoverflies of the sub-family Microdontinae where 405 species are currently recognised worldwide. Most of the species are tropical, but all appear to have associations with ants (Cheng & Thompson, 2008). A number of them are obligate myrmecophilous social parasites where they act as predators of ant brood in the host colonies (Elmes *et al.*, 1999; Schönrogge *et al.*, 2006), whereas the adult stage lives outside the nests. Five species of the genus *Microdon* are known from Europe: *Microdon analis* (Macquart), *Microdon miki* (Doczkal & Schmid), *Microdon devius* (Linnaeus), *Microdon mutabilis* (Linnaeus), and *Microdon myrmicae* (Schönrogge *et al.*) (see Doczkal & Schmid, 1999; Stankiewicz, 2003; Gammelmo & Aarvik, 2007). *Microdon myrmicae* and *Microdon mutabilis* larvae have been described as predators of ant brood (Schönrogge *et al.*, 2002, 2006; Thomas *et al.*, 2005), while the other three species associate with ants the nature of the relationship is as yet largely unknown (Andries, 1912; Doczkal & Schmid, 1999). The adult stages of both *Microdon myrmicae* and *Microdon mutabilis* are short lived and very similar. The main characters distinguishing *Microdon myrmicae*

from *Microdon mutabilis* are in morphological traits of the pupae and – according to the original study carried out in the British Isles – in their host use, with *Microdon mutabilis* being specific to colonies of *Formica lemni* (Bondroit) and *Microdon myrmicae* to those of *Myrmica scabrinodis* (Nylander) (Schönrogge *et al.*, 2002).

Since the description of *Microdon myrmicae*, it has been recorded from Ireland, Sweden, Norway, the Netherlands, Germany, Switzerland, Hungary, and Poland (Speight, 2002, 2003; Stankiewicz, 2003; Beuker, 2004; Schmid, 2004; Bonelli *et al.*, 2005; Gammelmo & Aarvik, 2007; Bartsch, 2009). Little is known, however, about the consistency of host use by *Microdon myrmicae* at a European scale. Polish *Microdon myrmicae* have been recorded from *Myrmica gallienii* (Bondroit), a species absent from the U.K. and *Myrmica rubra* (Stankiewicz, 2003), yet the extent to which these species are used by *Microdon myrmicae* is unclear. Also, beyond information on the presence of the species in various countries of Europe, very little is known about potential differences in life-cycles or host use between populations of *Microdon myrmicae* across Europe as they have been described for other social parasites such as *Maculinea* butterflies (Als *et al.*, 2002; Thomas *et al.*, 2005; Nash *et al.*, 2008). This may be an important issue since Schönrogge *et al.* (2002) suggest that *Microdon* hoverflies, as in other myrmecophilous social parasites, may be prone to cryptic speciation. Selection on traits important for the ant–parasite interaction is likely to be strong, yet the traits themselves are likely to be physiological rather than morphological (Akino *et al.*, 1999; Nash *et al.*, 2008). The existence of cryptic species has been demonstrated in many different taxonomic groups, yet where cryptic lineages comprise a species complex of rare and endangered species with different ecological requirements this poses a particular problem to conservationists (Blaxter & Floyd, 2003; Stuart *et al.*, 2006).

We assess the host range and host use of *Microdon myrmicae* across a large part of its European range, and ask whether they vary at scales of regions or populations. We also examine the larval and pupal morphologies for indications of further cryptic lineages within the species *Microdon myrmicae*.

Materials and methods

Surveys for this study were carried out between 2002 and 2009 in seven European countries: Italy, Hungary, Great Britain, Poland, Slovakia, Romania, and Ukraine. Thirty-one populations of *Microdon myrmicae* were investigated and specimens from one *Microdon mutabilis* population on the Burren in Ireland were used as a reference (Fig. 1).

Myrmica ant nests of all species present were opened and searched for parasites from the end of April until July when fully grown larvae, pupae, or fresh exuviae can be found in the ant nests. These later developmental stages of *Microdon myrmicae* move to the top of the nests where they are easier to detect. In this way host association of *Microdon myrmicae* with *Myrmica* species was recorded in 31 populations (Table S1). Furthermore, the relative abundance of the ant species and



Fig. 1. Map with distribution of populations of *Microdon myrmicae* in Europe (Italy: 1, Caselette; Hungary: 2, Aggtelek; 3, Drahos-ret; 4, Fülesd; 5, Gyilkos – rét; 6, Ipolytarnóc; 7, Kercaszomor; 8, Kétvölgy; 9, Lófő-tisztás; 10, Meszes; 11, Nógrádszakál; 12, Vörös – rét; Romania: 13, Rascruci; 14, Sardu; Slovakia: 15, Hačava; Poland: 16, Aleksandrówka; 17, Wojciechów; 18, Brzeźno; 19, Ciesacin; 20, Augustówka; 21, Kosyń; 22, Makowa; 23, Przemyśl; 24, Kraków; 25, Orosz; 26, Wiesiołka; 27, Kapice; 28, Ławki; Ukraine: 29, Leliaky; England: 30, Rimsmoor Pond; 31, Winfrith; Ireland: 32, Burren (*Microdon mutabilis*); 33, Co. Offaly (Speight; 2003); the Netherlands (Beuker; 2004): 34, Leemputten; 35, Smitsveen; Norway (Gammelmo & Aarvik; 2007): 36, Adalstjernet; Germany (Schmid; 2004): 37, Jeseritzen; 38, Breitlohmiß; Switzerland (Schmid; 2004): 39, Les Grandes/Pontarlier; Sweden (Bartsch; 2009): 40, Gardshults naturreservat 1; 41, Gardshults naturreservat 2 (square, populations presented in this paper; cross, populations of published data).

the proportions infested with *Microdon myrmicae* at seven of the 31 populations were established (Table 1). To ensure the correct identification of the *Myrmica* ants, between 10 and 20 workers from each colony were collected and used the identification keys by Seifert (1988), Czechowski *et al.* (2002) and Radchenko *et al.* (2003).

Morphometric analysis

Morphometric measurements were taken from specimens collected at seven populations at Caselette, Italy, IT (51 pupae), Meszes, Hungary, HU_1 (36 pupae), Aggtelek, Hungary, HU_2 (31 pupae), Rimsmoor Pond, U.K., UK_OW (nine pupae), Winfrith, U.K., UK_WI (26 pupae), Brzeźno, Poland, PL_1 (39 pupae), and Ciesacin, Poland, PL_2 (69 pupae). All 261 pupae were processed by SB in Turin. They were weighed to the nearest 0.1 mg using a Precisa® digital balance and measured under a Leica Optical Microscope equipped with a graticule eyepiece. The following measurements were recorded to the nearest 0.01 mm for each pupa (Fig. 2): the width of the pupae, later referred to as PWIDTH, and taken at mid-length; the basal diameter of the anterior respiratory organ (AROBAS); the length of the anterior respiratory organ (AROEI); the basal diameter of the posterior respiratory organ (PROBAS); and the length of the posterior respiratory organ (PROHEI). The same measurements were taken from five pupae of *Microdon mutabilis* from the Burren in Ireland.

Statistical analysis

Morphometric data were analysed using univariate ANOVA, discriminant analysis and principal component analysis (PCA). To minimise distortion caused by allometric relationships, all body measurements were log transformed and non parametric one-sample Kolmogorov–Smirnov tests were used to assess their distribution. The multivariate analyses were based on a correlation matrix where the first component extracted by PCA was used as the multivariate expression of body size. To determine the level of discrimination obtained by discriminant analysis, we used jackknifing to calculate the percentage of individuals correctly assigned to each population (Manly, 1986). Five pupae of *Microdon mutabilis* were used to assess the interspecific and intraspecific range of morphometric variability within this group.

Using non-parametric partial Mantel test (Manly, 1991), spatial correlations of morphotypes with distance between sampling sites was investigated. The dependent matrix was of overall morphometric distances. It considered both size and shape effects and was calculated from the significant discriminant functions of log-transformed morphometric characters. The independent matrix was the matrix of geographic distances. Finally, data for discriminant analyses using country names to conveniently represent different biogeographical regions were pooled: Italy (IT); Hungary (HU); Poland (PL) and the United Kingdom (U.K.). All data were analysed using SPSS (SPSS Inc.).

Table 1. The relative abundance of *Myrmica* ants at seven sites and the infestation rates by *Microdon myrmicae*.

Localities and species	Nests opened (% of all nests)	Nests with <i>M. myrmicae</i> (% nests infested)	No. <i>M. myrmicae</i> (% of the detected)	Max. no of <i>Microdon</i> per nest
Brzeźno				
<i>M. scabrinodis</i>	92 (73.6%)	24 (26%)	54 (63.5%)	5
<i>M. rubra</i>	2 (1.6%)	0	0	—
<i>M. gallienii</i>	31 (24.8%)	8 (26%)	31 (36.5%)	7
Ciesacin				
<i>M. scabrinodis</i>	30 (25.9%)	6 (20%)	20 (27.8%)	10
<i>M. rubra</i>	4 (3.4%)	0	0	—
<i>M. gallienii</i>	82 (70.7%)	15 (18%)	52 (72.2%)	11
Kosyń				
<i>M. scabrinodis</i>	28 (22.2%)	7 (25%)	12 (22.2%)	3
<i>M. rubra</i>	36 (28.6%)	3 (8%)	9 (16.7%)	5
<i>M. ruginodis</i>	1 (0.8%)	0	0	—
<i>M. gallienii</i>	61 (48.4%)	16 (26%)	33 (61.1%)	7
Augustówka				
<i>M. scabrinodis</i>	45 (77.6%)	7 (16%)	17 (54.8%)	4
<i>M. rubra</i>	12 (20.7%)	3 (25%)	14 (24.2%)	12
<i>M. ruginodis</i>	1 (1.7%)	0	0	—
Wojciechów				
<i>M. scabrinodis</i>	122 (86.5%)	7 (6%)	25	11
<i>M. rubra</i>	6 (4.3%)	0	0	—
<i>M. ruginodis</i>	2 (1.4%)	0	0	—
<i>M. gallienii</i>	11 (7.7%)	0	0	—
Aleksandrówka				
<i>M. scabrinodis</i>	142 (81.6%)	6 (4%)	16	9
<i>M. rubra</i>	19 (10.9%)	0	0	—
<i>M. ruginodis</i>	1 (0.6%)	0	0	—
<i>M. gallienii</i>	12 (6.9%)	0	0	—
Caselette				
<i>M. scabrinodis</i>	215	35 (16%)	75	7

Please note the meaning of the per cent values: 'Nests opened' – per cent gives the relative abundance of that *Myrmica* species on the site; and 'Nests with *Microdon myrmicae*' – per cent gives the proportion of that *Myrmica* species containing one or more *Microdon*; 'No. *Microdon myrmicae*' – per cent is the proportion of *Microdon* individuals detected in the colonies of the respective *Myrmica* species. None of the differences in infestation rates are significant (Fisher's exact test $P > 0.5$).

Results

The European distribution of Microdon myrmicae

The identity of *Microdon myrmicae* has been positively confirmed at 40 populations in 13 countries, which include the 31 populations we studied and nine recently published by other authors (Fig. 1).

Pupae in Myrmica scabrinodis and Myrmica gallienii nests: morphology and infestation rates

Microdon myrmicae infested *Myrmica scabrinodis* colonies in 26 of the 31 populations where pupae and larvae were recorded (Table S1). In 13 populations, *Microdon myrmicae* was also found in nests of other *Myrmica* species, namely of *Myrmica gallienii* (eight populations), *Myrmica rubra* (five), *Myrmica vandeli* (Bondroit) (a temporary social parasite of *Myrmica scabrinodis*) (one), and *Myrmica sabuleti* (Meinert) (one) (Table S1).

In the seven populations where ant relative abundance and parasite infestation rates were established, we found combinations of *Myrmica scabrinodis* with *Myrmica gallienii*, *Myrmica rubra* and *Myrmica ruginodis* (Nylander), but not with *Myrmica sabuleti* and *Myrmica vandeli*. We did not detect *Microdon myrmicae* in nests of *Myrmica ruginodis* at any of the sites (Table 1). At three Polish localities (Brzeźno, Ciesacin, and Kosyń), both *Myrmica scabrinodis* and *Myrmica gallienii* were abundant, yet the relative abundances for either ranged from 22% to 74% of all nests inspected. Despite the differences in relative abundance between the two host ants, the range in *Microdon* infestation rates were surprisingly similar in both, with 20–26% for *Myrmica scabrinodis* and 18–26% for *Myrmica gallienii* (Table 1). At Brzeźno the majority of nests belonged to *Myrmica scabrinodis* [Fisher's exact test for proportions (FETp) $P < 0.001$], while at Ciesacin and Kosyń *Myrmica gallienii* was clearly more abundant (FETp $P < 0.001$ for both; Table 1). However, no significant difference in infestation rates between the nests of the two ant species was found at any of the sites (FETp $P > 0.5$ for all).

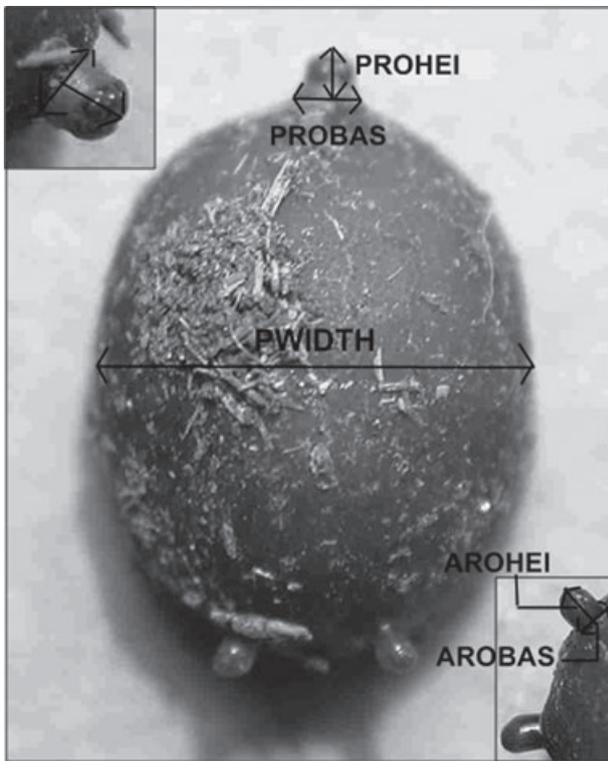


Fig. 2. *Microdon myrmicae* pupa indicating the five morphological measurements taken for the multivariate analysis.

Studying the *Microdon myrmicae* larvae reared by the two *Myrmica* species, it was found that pupae reared by *Myrmica gallienii* colonies were consistently heavier (GLM with normal errors: $F_{1,108} = 13.508$, $P < 0.001$, while controlling for site differences $F_{1,108} = 9.364$, $P = 0.003$) at Brzeźno and Ciesacin. Differences in the ratio of width to length of the anterior respiratory organ, a character that distinguishes between *Microdon myrmicae* and *Microdon mutabilis*, were close to significance ($F_{1,106} = 3.56$, $P = 0.062$), where anterior respiratory organs of pupae collected from *Myrmica gallienii* nests were relatively longer (mean = 1.07 ± 0.116 mm) than on pupae from *Myrmica scabrinodis* nests (mean = 1.02 ± 0.122 mm).

Phenotypic differences among populations

Microdon mutabilis vs. *Microdon myrmicae* – pupal morphometric analysis. To confirm the distinctness of *Microdon myrmicae* pupae from seven populations throughout its European range from *Microdon mutabilis* pupae we compared their morphometric measurements with those from five pupae of *Microdon mutabilis* collected on the Burren in Ireland. We used a multivariate discriminant analysis on the parameters given in Table 2. The differences in the width to height ratio of the ARO are visible to the bare eye: 0.68 ± 0.02 for *Microdon myrmicae* and 0.15 ± 0.04 for *Microdon mutabilis*, and all pupae were assigned correctly in the discriminant analysis (jackknifed: Wilks' $\Lambda = 0.016$; $N = 258$; d.f. = 5, 7, 250, $F =$

49.84; $P < 0.001$). Five canonical functions were extracted and four of them showed significant differences (ANOVA; Score 1: $F = 1031.55$; d.f. = 7; $P < 0.001$, Score 2: $F = 25.15$; d.f. = 7; $P < 0.001$, Score 3: $F = 6.27$; d.f. = 7; $P < 0.001$, Score 4: $F = 2.08$; d.f. = 7; $P = 0.046$) (Fig. 3a). The first PCA component (size) explained more than 90% of the total variance.

Microdon myrmicae pupal morphometrics between populations. Discriminant analyses performed separated pupae from the seven *Microdon myrmicae* populations significantly, but only 36% of the samples were correctly assigned (jackknifed: Wilks' $\Lambda = 0.395$; $N = 253$; d.f. = 5, 6, 246 (30, 970); $F = 8.44$; $P < 0.001$) (Fig. 3b). Four canonical functions were extracted and three of them showed significant differences among populations (ANOVA; Score 1: $F = 29.86$; d.f. = 6, 246; $P < 0.001$, Score 2: $F = 9.46$; d.f. = 6, 246; $P < 0.001$, Score 3: $F = 5.44$; d.f. = 6, 246; $P < 0.001$). The first PCA component (size), now without *Microdon mutabilis*, explained 34.4% of the variance, while the second component explained 28.1% of the total variance. The second component strongly correlated with both the height and length of the anterior respiratory organs reflecting its shape ($F = 18.29$, $P < 0.001$, see Fig. 2; canonical loadings of the four principal components in Table S2). Using a Mantel test correlating geographic distance with Euclidean distance calculated over all morphometric measures had no significant result, suggesting there are no geographical gradients in shape among our populations.

When data were pooled by biogeographic region, discriminant analysis separated the pupae significantly and 53% of the samples were correctly assigned (jackknifed: Wilks' $\Lambda = 0.49$; $N = 253$; d.f. = 15, 676; $F = 12.965$; $P < 0.001$). The respective percentage of correctly assigned samples was 44% for Italy, 70% for Hungary, 52% for Poland and 54% for the U.K. (Fig. 3c). The three canonical functions extracted showed significant differences among geographical areas (ANOVA; Score 1: $F = 48.53$; d.f. = 3, 249; $P < 0.001$, Score 2: $F = 14.56$; d.f. = 3, 249; $P < 0.001$, Score 3: $F = 6.55$; d.f. = 3, 249; $P < 0.001$).

Discussion

Since 2002, when *Microdon myrmicae* was described as a separate species from *Microdon mutabilis* based on studies in the British Isles, new records have substantially changed the understanding of the current distribution of both species (Speight, 2003; Stankiewicz, 2003; Beuker, 2004; Gammelmo & Aarvik, 2007; Bartsch, 2009). Using pupae collected from seven populations, a much broader geographical range than in previous studies supports the distinction between *Microdon myrmicae* and *Microdon mutabilis*. Here we record *Microdon myrmicae* from 31 populations throughout Europe, our investigation and further records published elsewhere show that *Microdon myrmicae* is present in Italy, Poland, Hungary, Romania, Slovakia, Ukraine, Germany, Switzerland, England, the Netherlands, Norway, Sweden, and Ireland (Speight, 2003; Stankiewicz,

Table 2. Morphometric measurements taken from *Microdon myrmicae* pupae collected in four geographical regions.

Population	<i>n</i>	Weight	AROBAS	AROHEI	PROBAS	PROHEI	Pupal width
Caselette, IT	50	0.11 ± 0.03	0.75 ± 0.15	1.10 ± 0.15	1.45 ± 0.15	9.74 ± 1.17	9.74 ± 0.90
Meszses, HU	36	0.14 ± 0.04	0.81 ± 0.14	1.10 ± 0.13	1.41 ± 0.12	1.21 ± 0.13	10.96 ± 0.85
Aggtelek, HU	31	0.16 ± 0.03	0.81 ± 0.11	1.06 ± 0.13	1.50 ± 0.14	1.13 ± 0.15	11.00 ± 0.60
Brzeźno, PL	39	0.09 ± 0.02	0.70 ± 0.08	1.10 ± 0.10	1.15 ± 0.09	1.13 ± 0.10	9.70 ± 0.80
Ciesacin, PL	69	0.09 ± 0.01	0.70 ± 0.13	1.11 ± 0.12	1.16 ± 0.10	1.14 ± 0.10	10.50 ± 0.90
Rimsmoor Pond, UK	9	0.15 ± 0.02	0.81 ± 0.09	1.17 ± 0.10	1.50 ± 0.09	1.28 ± 0.10	11.18 ± 0.63
Winfrith, UK	19	0.14 ± 0.04	0.73 ± 0.13	1.18 ± 0.16	1.50 ± 0.08	1.34 ± 0.11	10.24 ± 1.06
<i>M. mutabilis</i>	5	0.15 ± 0.04	1.17 ± 0.118	1.50 ± 0.091	1.50 ± 0.10	1.37 ± 0.173	11.83 ± 0.76

IT, Italy; HU, Hungary; PL, Poland; UK, England. Weights are measured in grams and length in millimetres and all measurements are given as mean ± 1 SD.

AROBAS, basal diameter of the anterior respiratory organ; AROHEI, the length of the anterior respiratory organ; PROBAS, the basal diameter of the posterior respiratory organ; PROHEI, the length of the posterior respiratory organ.

2003; Beuker, 2004; Gammelmo & Aarvik, 2007; Bartsch, 2009). Including the new records from Hungary, Romania, Slovakia, Ukraine, and Italy presented here, the species distribution might be very patchy and tied to specific habitat requirements, but *Microdon myrmicae* occurs throughout central and western Europe (Fig. 1), while eastern range boundaries are yet unknown. Establishing the current range of *Microdon mutabilis* seems more difficult. We are aware of five populations in the British Isles and recently published records from Sweden indicate 32 populations (Bartsch, 2009). All recently checked populations in central and southern Europe, however, were reclassified as *Microdon myrmicae*, suggesting a distinctly western and northern range for *Microdon mutabilis*.

Microdon mutabilis has been described as being an extreme specialist (Elmes *et al.*, 1999). In Scottish and Irish populations 1.1% and 0% of hundreds of non-host colonies contained pupae, while 23.7% and 31.6% of 1678 *F. lemni* colonies were infested (Schönrogge *et al.*, 2002, 2008). None of the 327 colonies of *Myrmica scabrinodis* that were inspected in these surveys showed any evidence of infestation by *Microdon mutabilis*. Even more extreme, *Microdon mutabilis* has been reported as being specific to local ant populations, and individual super-colonies of their host species (Elmes *et al.*, 1999; Schönrogge *et al.*, 2002, 2006). In contrast, *Microdon myrmicae* seems to be less host-specific than *Microdon mutabilis*, since it is associated with three distinct host species such as *Myrmica scabrinodis* and *Myrmica gallienii* and to a less degree with *Myrmica rubra* (Table 1). The individual record for *Myrmica sabuleti* is likely to be the result of the parasite's attack causing the real host colony (*Myrmica scabrinodis*) to leave the nest site so that it was replaced by *Myrmica sabuleti*, which became a host (Thomas *et al.*, 2005). *Myrmica vandeli* is a temporary parasite of *Myrmica scabrinodis* and it is possible that the recorded colony was in transition and the workforce mixed (Radchenko & Elmes, 2003). *Myrmica scabrinodis* was clearly the most common host species present at 26 of the 31 sites although locally not always dominant. *Myrmica gallienii* was recorded at eight sites, and at Ciesacin, Brzeźno and Kosyń, where more detailed studies were conducted, infestation rates were as high as those found in *Myrmica scabrinodis* colonies (16–26%).

In three Polish populations pupae reared in nests of *Myrmica gallienii* were heavier than those found in colonies of *Myrmica scabrinodis*. While this might reflect higher resource provision, which could have fitness implications (Czechowski *et al.*, 2002), we also found that the shape of the anterior respiratory organ differed between '*Myrmica scabrinodis*' and '*Myrmica gallienii* types'; a character of pupal morphology that also distinguishes *Microdon myrmicae* from *Microdon mutabilis* (Schönrogge *et al.*, 2002). With 6.2% the significance level for that difference was just outside the traditional probability level of 5%, and while it could be an indication of further cryptic lineages, in the absence of molecular data current evidence is rather weak. To our knowledge *Microdon myrmicae* and *Microdon mutabilis* never live sympatrically and have very different habitat requirements. In contrast, '*Myrmica scabrinodis*' and '*Myrmica gallienii* types' were found at the same location and it is unclear what would separate them reproductively. One possibility, however, could be a difference in phenology to separate them in time. In species with a very short-lived adult stage, different flight periods could easily lead to isolation (Kawecki & Ebert, 2004; Schönrogge *et al.*, 2006).

Studies of *Microdon mutabilis* suggest the use of chemical mimicry to infiltrate host ant colonies, although the exact mode of the interactions is yet unknown (Elmes *et al.*, 1999). With a detailed understanding of the interactions between *Microdon myrmicae* and its host ants, it should become apparent whether *Myrmica scabrinodis* and *Myrmica gallienii* are particularly similar in any relevant aspect and how different levels of specificity arise. Based on the data available, we would regard *Microdon myrmicae* as a generalist parasite of *Myrmica* ants.

Species differentiation and cryptic speciation has been linked to host use in the inquiline ant *Acromyrmex insinuator* Schultz, Bekkevold and Boomsma for instance (Schultz *et al.*, 1998), and Schönrogge *et al.* (2002, 2006, 2008) suggest that in social parasites divergent selection results from the need to penetrate efficiently the nestmate recognition systems of their host colonies. There might be selection pressure for parasites to penetrate the more resource-rich brood chambers of their host colonies but, as these are typically more forcefully defended (Hölldobler & Wilson, 1990; Thomas *et al.*, 2005), such parasites may only succeed if they evolve more specific and more sophisticated procedures to overcome such defences.

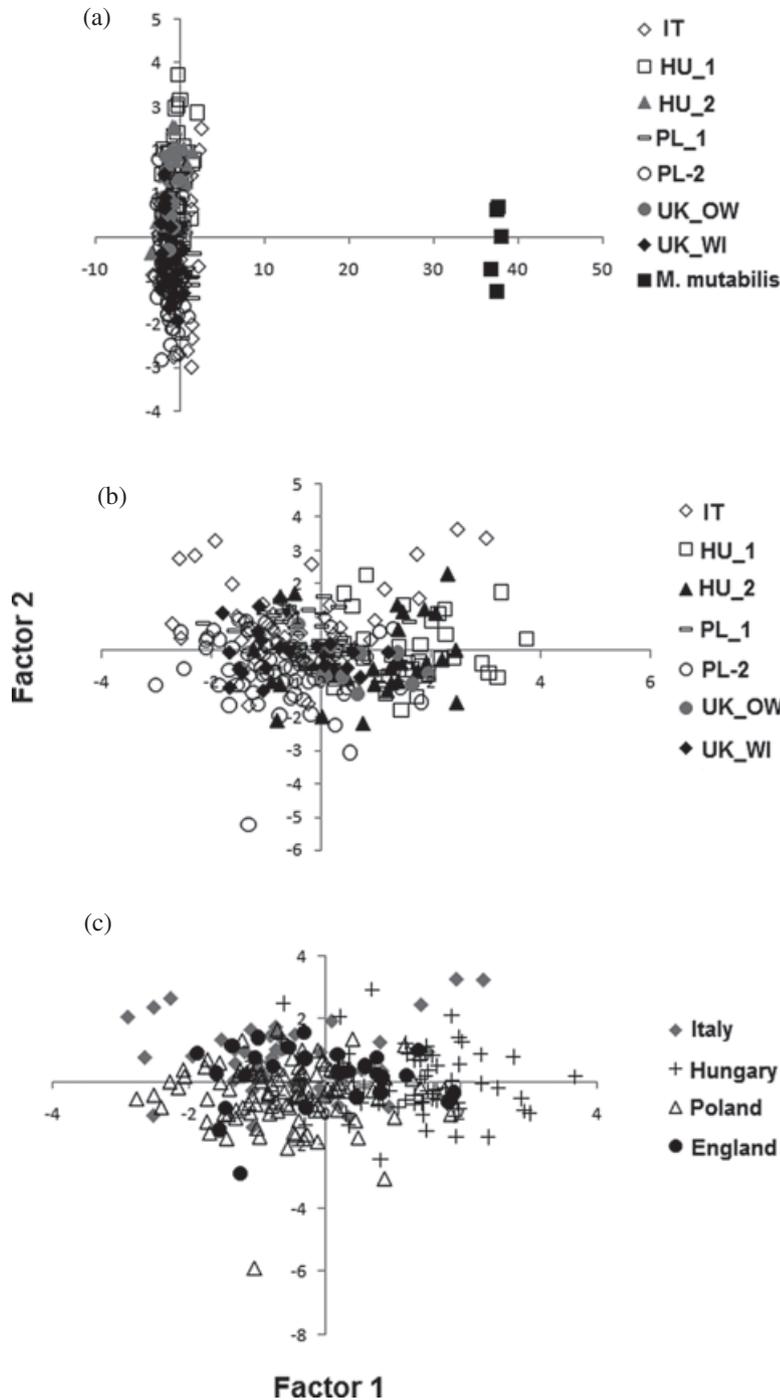


Fig. 3. Plot with the first and second component of the analysis of morphometric traits of: (a) pupae of *Microdon myrmicae* from seven European populations and *Microdon mutabilis* from the Burren in Ireland; (b) pupae of *Microdon myrmicae* from seven European populations (one population from Italy, two from Hungary, two from Poland and two from England); (c) pupae from four geographical regions (IT, Italy; HU, Hungary; PL, Poland; UK, England).

Selection might act on physiological traits such as cuticular hydrocarbons (Schönrogge *et al.*, 2002; Nash *et al.*, 2008), yet the shape of an anterior respiratory organ might be adaptive in relation to differences in the frequency of biting as part of the host workers aggressive or inquisitive behaviour. Different kind of adaptations against aggressive behaviour of ant workers were found in other myrmecophilous insects like lycaenid larvae (Pierce *et al.*, 2002) or in coccinellids larvae (Schwartzberg *et al.*, 2010).

Records of *Microdon mutabilis* from before 2002 still need re-evaluation. The different habitat requirements of the two species could be useful in this. Without information on pupal morphology or host ant use for material in entomological collections, information on the habitat type, where available, can provide a useful guide to species identity. *Microdon mutabilis* occurs in relatively dry habitats such as some well-drained, frequently grazed grasslands, while *Microdon myrmicae* was recorded from wet and often temporarily

waterlogged places (Napper, 2004). The Polish, Italian, and the most of the Hungarian sites in this study were wet meadows dominated by *Molinia* with an abundance of *Cladium*, *Carex*, and *Phragmites*. At the Hungarian Lófő-tisztás, the Slovakian Hačava, and at the British sites, *Microdon myrmicae* was found around a sinkhole or doline formation surrounded partly by deciduous forest and wet heathland, while the herbaceous vegetation layer was usually dominated by *Sphagnum*, *Juncus*, and *Molinia coerulea* [(L.) Moench]. The 'Molinietum' type of meadow is very rare in Europe ('Habitats Directive' Annex 1) and *Microdon myrmicae* is strongly associated with it as a character species.

The most common host ant of *Microdon myrmicae*, *Myrmica scabrinodis*, is almost ubiquitous in grasslands of the western Palaearctic (Czechowski *et al.*, 2002), *Microdon myrmicae* is rare in relation to the range of its host, which is a poorly understood feature common to many myrmecophilous species (Thomas *et al.*, 2005). *Formica lemani*, the host ant of *Microdon mutabilis*, is a boreo-montane species, abundant in the north of Europe, but only locally present in central and southern Europe, where it is restricted to subalpine conditions and inhabits both dry and wet open mountain meadows (Czechowski *et al.*, 2002). One would expect that, like *Microdon myrmicae*, *Microdon mutabilis*'s distribution would be patchy within the range of the host ant, but if more of the older *Microdon mutabilis* records are found to be *Microdon myrmicae* instead, *Microdon mutabilis* could rapidly become one of the rarest hoverfly species in Europe.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference:

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Table S1. *Myrmica* species found in *Microdon myrmicae* populations ('H' means that species was found as the host, 'X' means that the presence of species was detected but it was not found as host, '-' means that the presence of species was not detected on the site).

Table S2. Component loadings of a PCA on pupal morphometric characters from seven European populations.

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