

Pleistocene population differentiation in the ant *Myrmica scabrinodis* (Hymenoptera: Formicidae) – a taxonomic borderline case

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Received 16 November 2023 | Accepted 25 January 2024

Published online at www.soil-organisms.de 1 April 2024 | Printed version 15 April 2024

DOI 10.25674/357

Abstract

Three taxa have been supposed in the past to be conspecific with or heterospecific from *Myrmica scabrinodis* Nylander 1846: *M. rugulosoides* Forel 1915, *M. pilosiscapus* Bondroit 1920 and *M. martini* Seifert et al. 2014. The taxonomic relations of these taxa are investigated here based on 146 nest samples with 479 worker individuals collected in the whole Palaearctic range extending from the Pyrenees and England to South Central Siberia (Baikal region). Exploratory and hypothesis-driven data analyses considering 17 morphometric characters confirmed two main clusters: a western cluster distributed from 5°W to about 22°E and an eastern cluster occurring from 6°E to 104°E. The classification error of the exploratory data analyses NC-NMDS.kmeans and PCA relative to the controlling linear discriminant function (LDA) were 1.4% and 0.7% respectively. At the first hand, this seemed to justify considering the two clusters as separate species. The posterior probabilities of the type series when run as wild-card runs in a LDA were 0.9366 in *M. rugulosoides*, 0.9999 in *M. martini*, 0.0284 in *M. scabrinodis* and 0.0081 in *M. pilosiscapus* – allocating the former two to western cluster and the latter two to the eastern cluster. However, strong and highly significant reduction of morphological distance between the western and eastern cluster in the sympatric zone (ranging from 6°E to 22°E) compared to the situation in the allopatric ranges indicates frequent hybridization and introgression of genes. Due to this morphological convergence in sympatry, *M. rugulosoides*, *M. pilosiscapus* and *M. martini* are considered as junior synonyms of *M. scabrinodis*. The placement of the main Pleistocene refuge centers of *M. scabrinodis* concluded from data of this study is congruent to the picture derived from a mtDNA haplotype analysis of Leppänen et al. (2012) in *Myrmica rubra* (Linnaeus, 1758): South France and the Apennine Peninsula for the western group and the Balkans, Middle Asia and SW Siberia for the eastern group. Conclusions on nearly equal glacial retreat and postglacial expansion routes of *M. scabrinodis* and *M. rubra* are also supported by the high overlap of their realized niches found in the broad-based survey of Seifert (2017) and their almost congruent actual geographic ranges.

Keywords Cryptic species | numeric morphology-based alpha-taxonomy | zoogeography | glacial refuge centers | ecological niche

1. Introduction

Species-level taxonomy, or formation of species, is influenced by the strong climate variation in the Pleistocene. One of the most important drivers of species divergence in this context is isolation in separated glacial refuge centers. De Lattin (1949, 1967) distinguished for Europe an Atlanto-Mediterranean center (Iberia and S France), a Tyrrhenian center (Corsica and Sardinia),

an Adriato-Mediterranean center (basically continental Italy), and a Ponto-Mediterranean center (Balkans and W Turkey). Considering only the main routes, postglacial range expansion from the Atlanto-Mediterranean center was largely directed north and northeast, and from the Ponto-Mediterranean center north and northwest. As result, formerly isolated populations came in contact again. For elements of temperate or subboreal faunas, the contact zone was frequently situated in Central Europe. One

outcome of this contact may be unrestrained crossbreeding of the populations in a broad sympatric zone but with the separate glacial refuge centers still genetically traceable – in ants shown by Leppänen et al. (2011, 2012) for *Myrmica rubra* (Linnaeus, 1758). The secondary contact may also result in confrontation at a front line with crossbreeding only along a very narrow zone and establishment of strict parapatry. For ants this was shown in *Temnothorax nylanderi* (Foerster, 1850) and *T. crassispinus* (Karavajev, 1926) (Seifert 1995, Pusch et al. 2006). Another situation, development of broader sympatric ranges of cryptic species with probably no or very little introgression may be concluded from NUMOBAT data in connection with western versus eastern distributional centers. Examples in ants are *Camponotus piceus* (Leach 1825) and *C. atricolor* (Nylander, 1849) (Seifert 2019), *Lasius paralienus* Seifert, 1992 and *L. bombycina* Seifert & Galkowski, 2016 (Seifert 2020) and *Solenopsis fugax* (Latreille, 1798) and *S. juliae* (Arakelian, 1991) (Csösz et al. 2023).

Despite offering plenty of morphological characters, the taxonomy of ants of the genus *Myrmica* Latreille 1805 remains disputed. A then complete compilation of all taxa described from the Old World, with drawings of all 137 extant species proposed to be valid, was provided by Radchenko & Elmes (2010). Details of the taxonomy of cryptic *Myrmica* species under application of Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT) were investigated by Seifert (2000, 2002, 2003, 2005, 2011), Bagherian et al. (2012) and Seifert et al. (2009, 2014). This was followed by a taxonomic study integrating NUMOBAT and nuclear DNA data (Seifert et al. 2018).

The ant *Myrmica scabrinodis* Nylander 1846 has a wide, largely temperate and boreal Eurosiberian range stretching east to at least 104°E in the Baikal region. The range includes Asia Minor, the Caucasus and the mountains of Middle Asia. It goes north to 68°N in Fennoscandia and up to 1960 m in the Alps at 46.6°N. In the southern parts of its range, at more or less 42°N, it is not found below 1300 m and seems to be absent from Iberia south of the Pyrenees where it is replaced by the closely related *Myrmica aloba* Forel 1909. *Myrmica scabrinodis* is hygrophilous, moderately thermophilous and achieves the highest densities in fresh meadows and ecotones with moderate height of grasses as well as in open bogs with much *Sphagnum* moss, with dense populations occurring even in wettest quaking *Sphagnum* stands (Seifert 2017). A condensed synopsis of its life history is given in (Seifert 2018).

There are three taxa supposed to be conspecific or very closely related with *Myrmica scabrinodis*:

M. rugulosoides Forel 1915, *M. pilosiscapus* Bondroit 1920 and *M. martini* Seifert et al. 2014. The former two taxa have been considered as synonyms of *M. scabrinodis*

by Seifert (1984) and Radchenko & Elmes (2010) and the latter taxon has been described as separate cryptic species with a western distributional center. However, a considerable recent extension of sample size with material from western Europe and introduction of new morphological characters suggested that *M. rugulosoides* could possibly represent a separate cryptic species of which *M. martini* is a junior synonym. This shall be investigated in this paper.

2. Material

NUMOBAT data were recorded in a total of 146 nest samples with 479 workers. They originated from Andorra (5 samples), Austria (4), Belgium (2), Belorussia (1), Bosnia-Herzegovina (1), Czechia (1), Finland (1), France (32), Georgia (9), Germany (51), Great Britain (7), Kazakhstan (8), Kyrgyzstan (1), Poland (2), Russia (4), Spain (2), Sweden (1), Switzerland (8) and Turkey (6). With the exception of type specimens (see below), detailed data of this material are not presented in the main text of this paper but listed up in the electronic supplementary information S11. The investigated type series had the following data

Myrmica scabrinodis Nylander, 1846

Lectotype worker (des. Radchenko 2007) plus three paralectotype workers on two pins labelled 'Kuusamo', 'W. Nyland.', 'Mus. Fenn.'; depository Finnish Museum of Natural History, Helsinki / Finland.

Myrmica scabrinodis var. *rugulosoides* Forel, 1915

Lectotype worker plus for paralectotype workers labeled 'Typus', 'LECTOTYPE (bottom specimen) desig. Radchenko & Elmes 2000' [published by Radchenko & Elmes 2010], 'Tourbières de Roche' [Forel's handwriting], 'Myrmica scabrinodis Nyl. v. rugulosoides For. type' [Forel's handwriting], 'ANTWEB CASENT0907654'; depository Muséum d'histoire naturelle de Genève, Genève, Switzerland.

Myrmica pilosiscapus Bondroit, 1920

Lectotype worker labeled 'Mouthe Jura', 'nid dans Sphagnum tres humide', 'M.pilosiscapus type Bondr.' [all

three labels in Bondroits handwriting], 'LECTOTYPE (upper specimen) desig. Radchenko & Elmes 2000' [published by Radchenko & Elmes 2010]; paralectotypes: 1 worker, 2 gynes and 2 males on the same pin with lectotype; 1 paralectotype worker together with 4 males on another pin labeled 'Mouthe Jura', 'Type', 'M. pilosiscapus type Bondr.' [all three labels in Bondroits handwriting], 'PARALECTOTYPE desig. Radchenko & Elmes 2000'; depository Muséum des sciences naturelles de Belgique, Bruxelles / Belgium

***Myrmica martini* Seifert, Bagherian-Yazdi & Schultz, 2014**

Holotype and five paratype worker labeled 'FRA: 44.1002°N, 7.2332°E St.-Martin-Vésudrie-3.8 NNW, 1629 m, Larix-Pinus, clearing, Schultz 2002.05.15 – 126' and 'Holotype *Myrmica martini* Seifert et al.'; depository Senckenberg Museum of Natural History Görlitz, Germany.

3. Methods

3.1 Equipment and measurement procedures

A pin-holding stage, permitting full rotations around X, Y, and Z axes and a Leica M165C high-performance stereomicroscope equipped with a 2.0x planapochromatic objective (resolution 1050 lines/mm) was used for spatial adjustment of specimens at magnifications of 120–360x. A Schott KL 1500 cold-light source equipped with two flexible, focally mounted light-cables, providing 30°–inclined light from variable directions, allowed sufficient illumination over the full magnification range and a clear visualization of silhouette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimal resolution of tiny structures. Simultaneous or alternative use of the cold-light sources depending upon the required illumination regime was quickly provided by regulating voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks ranging over 52% of the visual field was used. To avoid the parallax error, its measuring line was constantly kept vertical within the visual field. Z-stack images of mounted specimens were produced with a KEYENCE VHX-7000 digital microscope.

3.2 The morphometric characters and terminology

Seventeen phenotypic characters were investigated and numerically recorded in worker ants. In bilaterally developed characters, arithmetic means of both body sides were calculated. All measurements were made on mounted and fully dried specimens.

CL – maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of occiput and/or clypeus reduce CL. Longitudinal carinae or rugae on anterior clypeus are included into the measurement - if exactly median, in their full height and, if of doubtful position, in their half height.

ClyEx – depth of excision on anteromedian clypeal margin in a position in which the dorsal and ventral margins of the excision superimpose. This is usually given after tilting the head by $\pm 45^\circ$ from dorsal towards the frontal viewing position (dorsofrontal view). Average valleys and peaks of sculpture.

CS – cephalic size; the arithmetic mean of CL and CW, used as a less variable indicator of body size.

CW – maximum cephalic width; in *Myrmica* always across eyes

EYE – eye-size index: the arithmetic mean of the large (EL) and small diameter (EW) of the elliptic compound eye.

FL – maximum anterior divergence of frontal carinae (= maximum frontal lobe distance, Fig. 1). In specimens with frontal carinae parallele or converging frontad FL is not defined, then FL = FR

FR – minimum distance between frontal carinae (Fig. 1).

MetL – the height of metapleuron including the propodeal lobe measured in lateral view (Fig. 2) perpendicular to the straight section of metapleuro-coxal border (red, horizontal dashed line). The measuring line is positioned in the middle between the level of frontalmost point of subspinal excavation (left vertical dashed line) and the caudalmost point of propodeal lobe (right vertical dashed line).

MetSp – the height of subspinal excavation from upper margin of propodeal lobe to lower spine margin measured along the dorsal continuation of the measuring line for MetL (Fig.2).

PEW – maximum width of petiole

PEH – maximum petiole height measured perpendicular to a reference line. The reference line extends from the center of the petiole-propodeal junction (left red disc) and the center of petiole-postpetiolar junction (right red dot).

PEL – maximum measurable diagonal petiole length from the tip of subpetiolar process to the dorsocaudal

corner of the caudal cylinder (Fig. 2; do not confuse this with the corner of the movable inner sclerite).

PoOc – postocular distance. Use a cross-scaled ocular micrometer and adjust the head to the measuring position of CL. Caudal measuring point: median occipital margin; frontal measuring point: median head at the level of the posterior eye margin. Note that many heads are asymmetric and average the left and right postocular distance (Fig.1).

PPHL – length of longest hair on dorsal postpetiole

PPW – maximum width of postpetiole

SL – maximum straight line scape length. Distal measuring point: the most distal point of the dorsal lamella of the hinge joint capsule. Proximal measuring point: the most proximal point of scape shaft near the neck of articular condyle (Fig. 1). Note that the border region between shaft and condylar neck is usually asymmetric. To measure the real maximum avoid caudal viewing positions and use frontal to dorsal views!

SP – length of propodeal spines as bilateral arithmetic mean measured in dorsofrontal view (Fig. 3). SP is

measured from spine tip to a dashed reference line which is directed perpendicular to the spine axis and crosses the bottom point of the interspinal meniscus. With spine's dorsal edge in measuring plane, the spine tip must be focused at a large magnification with low depth of focus. Then, while keeping this focusing level, the sharpest point at the bottom of interspinal meniscus must be determined. A cross-scaled ocular micrometer should be used in this procedure. This mode of measuring is less ambiguous than other methods but results in some spine length in species with reduced spines.

SVP – Standard viewing positions (SVP) of scape defined by their position relative to the moving plane of the hinge joint between scape and first funiculus segment (Fig. 4). Dorsal view (SVP d) is directed perpendicular to this moving plane (in this position the anterior margins of upper and lower lobe of the distal scape end are congruent and the basal curvature of scape is not or only weakly visible). Frontal view (SVP f) and caudal view (SVP c) are perpendicular to SVP d and perpendicular to the longitudinal scape axis.



Figure 1. Head measurements.

SVP's such as cd and df describe intermediate viewing positions.

SW – maximum distance between caudal margin of basal scape lobe and the anterior scape margin measured perpendicular to the longitudinal scape axis. The scape must be carefully tilted until the maximum distance is situated in the measuring plane. This is given in more or less dorsofrontal viewing position (SVP df). In cases when maximum SW would be measured proximal of basal break point of anterior scape margin, the linear continuation of the anterior scape margin distal of the break is used as anterior measuring point (Fig. 5).

3.3 Removal of allometric variance

In order to make shape components such as CL/CW, SL/CS or SP/CS interspecifically comparable in synoptic tables independent from body size and to increase the discriminative power of principal component analyses,

removal of allometric variance (RAV) followed the procedure described by Seifert (2008). RAV was calculated for the assumption of all individuals having an identical cephalic size of 1.15 mm. Overall genus-specific RAV functions were applied the parameters of which were calculated as the arithmetic mean of the species-specific functions of 36 Palaearctic *Myrmica* species with sufficient sample size. Allometries are weak in *Myrmica* compared to other ant genera but it was to be tested if RAV improved the discriminative power of principal component analysis as it was found in other studies. The RAV functions were as follows

$$\begin{aligned} CL/CW_{1.15} &= CL/CW / (-0.0487*CS+1.0900)*1.0340 \\ SL/CS_{1.15} &= SL/CS / (-0.0802*CS+0.8947)*0.8024 \\ EYE/CS_{1.15} &= EYE/CS / (0.0179*CS+0.1772)*0.1978 \\ FL/CS_{1.15} &= FL/CS / (0.0227*CS+0.4036)*0.4297 \\ FR/CS_{1.15} &= FR/CS / (0.0095*CS+0.3222)*0.3331 \\ FL/FR_{1.15} &= FL/FR / (-0.0572*CS+1.4270)*1.3612 \\ PEW/CS_{1.15} &= PEW/CS / (-0.0201*CS+0.2842)*0.2610 \end{aligned}$$



Figure 5. Measuring the maximum width of scape at lobe level.

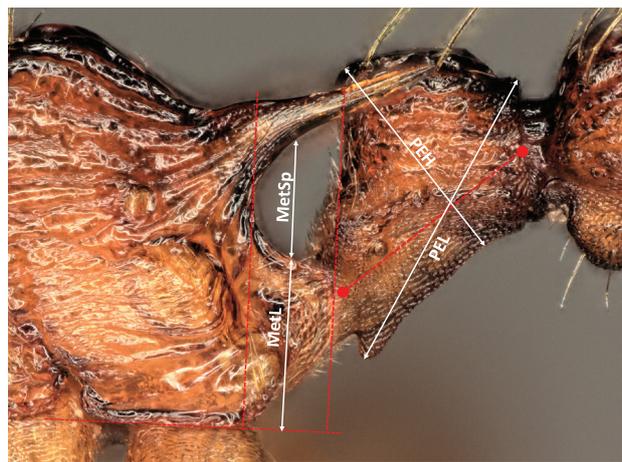


Figure 2. Measurements of caudal mesosoma and petiole.



Figure 3. Measuring length of propodeal spines.

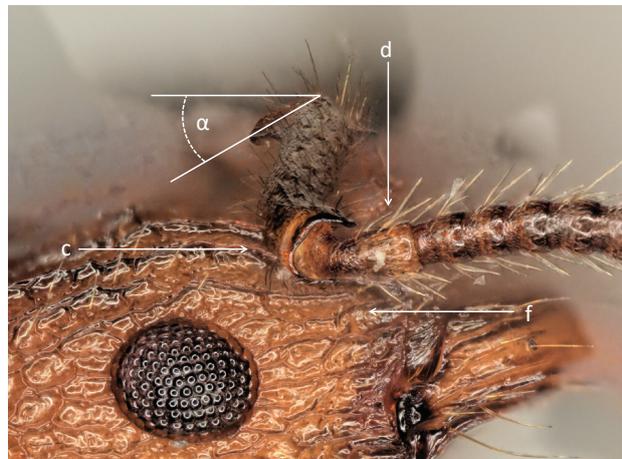


Figure 4. View along the axis of scape with definition of standard viewing positions and the slope angle of basal scape lobe.

$$\begin{aligned} \text{PPW/CS}_{1.15} &= \text{PPW/CS} / (0.0709 \cdot \text{CS} + 0.3208) \cdot 0.4024 \\ \text{PEH/CS}_{1.15} &= \text{PEH/CS} / (0.0051 \cdot \text{CS} + 0.3230) \cdot 0.3288 \\ \text{PEL/CS}_{1.15} &= \text{PEL/CS} / (-0.0259 \cdot \text{CS} + 0.4975) \cdot 0.4677 \\ \text{PPHL/CS}_{1.15} &= \text{PPHL/CS} / (-0.0679 \cdot \text{CS} + 0.2535) \cdot 0.1754 \\ \text{SP/CS}_{1.15} &= \text{SP/CS} / (0.0876 \cdot \text{CS} + 0.2353) \cdot 0.3360 \\ \text{MetL/CS}_{1.15} &= \text{MetL/CS} / (-0.0126 \cdot \text{CS} + 0.2487) \cdot 0.2342 \\ \text{MetSp/CS}_{1.15} &= \text{MetSp/CS} / (-0.0113 \cdot \text{CS} + 0.1880) \cdot 0.1749 \\ \text{PoOc/CL}_{1.15} &= \text{PoOc/CL} / (0.0098 \cdot \text{CS} + 0.4154) \cdot 0.4266 \\ \text{SW/SL}_{1.15} &= \text{SW/SL} / (0.0109 \cdot \text{CS} + 0.1385) \cdot 0.1511 \end{aligned}$$

3.4 Explorative and supervised data analyses and statistical testing

Four forms of exploratory data analysis (EDA) were tested. Three methods used nest centroids (NC) as input data (Seifert et al. 2013). The first one was NC-part.kmeans, an iterative vector-quantization method implemented in partitioning algorithms based on recursive thresholding (Csósz & Fisher 2015). The second and third method were hierarchical NC-Ward clustering and NC-NMDS.kmeans clustering, an analysis combining nonmetric multidimensional scaling with iterative vector-quantization (Seifert et al. 2013). The fourth method of exploratory data analysis was principal component analysis (PCA). Clustering hypotheses proposed by the EDA's were checked by linear discriminant analysis (LDA) in which controversial samples were run as wild-cards (i.e., without imposing an hypothesis). The 'final classification hypothesis' was established by the LDA in an iterative procedure following the rationale described in Seifert et al. (2013) and there remained no undecided cases even if their posterior probabilities were close to 0.5. PCA, LDA and ANOVA tests were run with the SPSS 15.0 software package.

4. Results

NC-part.kmeans did not indicate any cluster separation within the total material of 146 nest samples. This was the case when either all RAV-corrected characters, a selection of RAV-corrected characters, all raw measures or a selection of raw measures was considered. Relative to the final classification determined by the controlling LDA, the PCA also failed to confirm two clusters when all RAV-corrected characters, a selection of seven RAV-corrected characters and all raw measures were considered, showing of errors 13.7%, 13.0% and 7.5% respectively. However, using only the five raw measures FL, FR, PeL, MetSp and SW, the error dropped to 0.7% – meaning misclassification of only a single nest

sample. The errors of these four PCA settings refer to the three-dimensional space as they were determined by running the first three principal components in an LDA. NC-Ward clustering suggested two big clusters but showed an error of > 5% in any character constellation – the best result (error of 5.5%) was achieved when the five raw measures mentioned above were used. By far the best performance over all character setting was achieved by NC-NMDS.kmeans clustering. The error relative to the controlling LDA was 2.7% using all 18 RAV-corrected characters, 1.4% using a selection of seven RAV-corrected characters, 2.0% using all 17 raw measures and 1.4% using the five raw measures mentioned above.

As result, considering all character settings and all four tested exploratory data analyses together, the most parsimonious approach using the five raw measures showed the lowest mean error (Fig. 6). According to this we have a cluster with a western center of geographical distribution bearing the type samples of *Myrmica rugulosoides* Forel 1915 and *M. martini* Seifert et al. 2014 and a cluster of eastern geographical distribution including the type samples of *Myrmica scabrinodis* Nylander 1846 and *M. pilosiscapus* Bondroit 1920. Using the five raw measures FL, FR, PeL, MetSp and SW and running the type samples in a LDA, allocation to the western cluster is given with $p = 0.9366$ in *M. rugulosoides* and $p = 0.9999$ in *M. martini* whereas placement in the eastern cluster is given with $p = 0.9726$ in *M. scabrinodis* and $p = 0.9919$ in *M. pilosiscapus*. There is no indication that *M. martini* could represent another taxonomic entity than *M. rugulosoides*. In the tree of NC-Ward clustering (Fig. 6) both taxa are found in the same sub-cluster of the big western branch. It is obvious that *M. martini* is a junior synonym of *M. rugulosoides*.

The latter statement requires a comment. The description of *M. martini* in 2014, though being based on objective analysis methods, was a mistake caused by a bias in geographic sample distribution. As much as 12 of the 23 nest samples allocated to the *M. martini* cluster came from the type locality and just this population expressed some characters of the western clade in a strong way. The consequence of this extreme bias was that only 11 samples from Western Europe were grouped by the exploratory data analyses together with the *M. martini* types and, most importantly, the type sample of *M. rugulosoides* Forel was not allocated to this cluster. Today, after more samples from France, the Benelux countries and England became available, all 12 samples from the type locality of *M. martini* and each other sample from Western Europe are allocated to the same cluster as the type of *M. rugulosoides*.

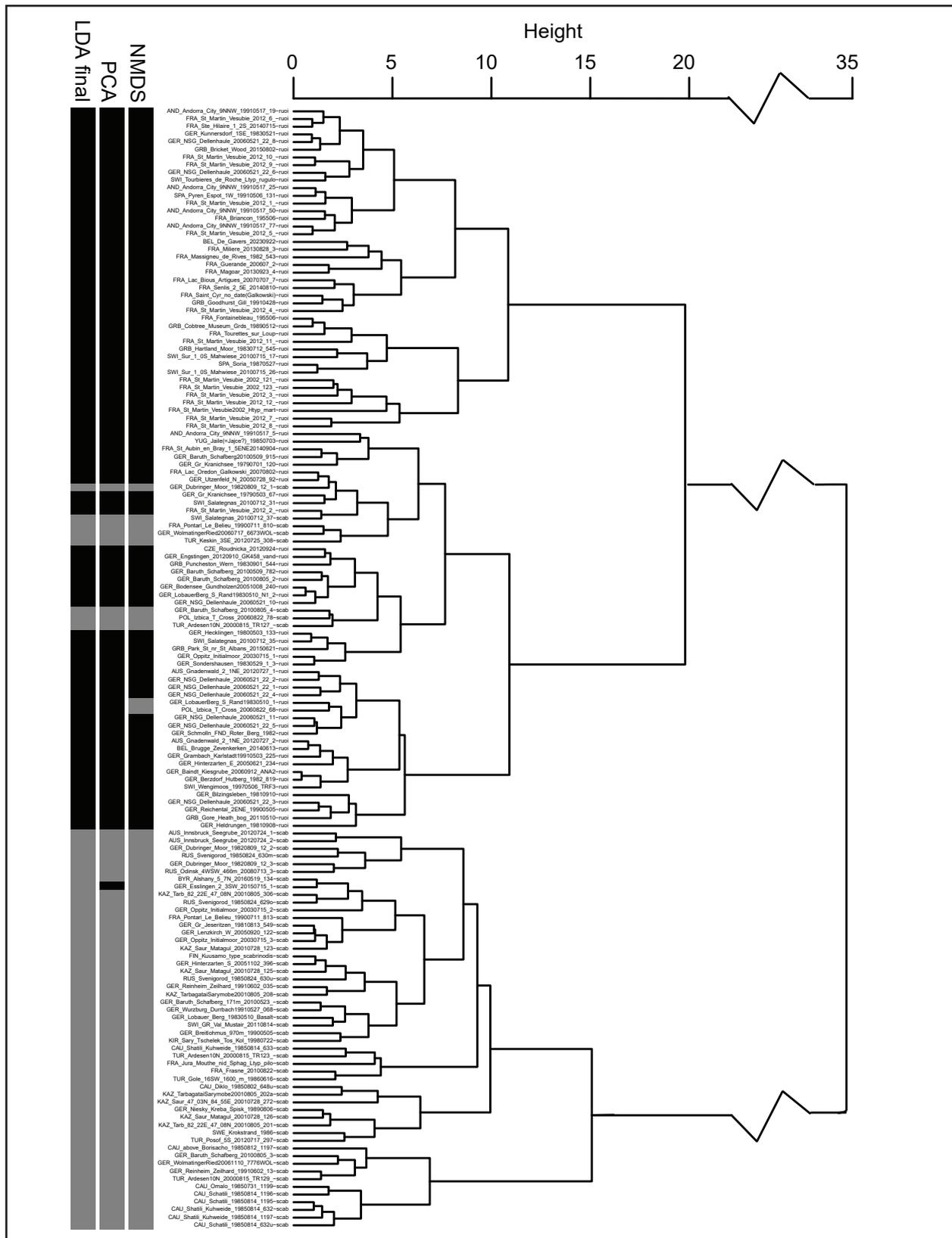


Figure 6. Classification of the western (black bars) and eastern cluster (grey bars) within 146 nest samples of *Myrmica scabrinodis* by the exploratory data analyses NC-Ward (5.5% error), NC-NMDS.kmeans (1.4% error) and a principal component analysis (PCA, 0.7% error) relative to the final classification determined by a linear discriminant analysis (LDA final).

Calling the western population *Myrmica rugulosoides* and the eastern one *M. scabrinodis*, the 146 nest samples of this study can be fully separated by a linear discriminant function. With all measurements in mm, a the nest sample mean of the discriminant

$$32.005*FL-28.086*FR+111.81*SW+12.629*PeL-18.809*MetSp-22.056$$



Figure 7. Dorsal aspect of head in a worker of the western population of *Myrmica scabrinodis*. St. Martin-de-Vesubie /France.

has values < 0 in *M. rugulosoides* and > 0 in *M. scabrinodis*. The error of this LDA was 5.4% in 479 worker individuals. Figs 7–8 illustrate the lower extremes of development of basal scape lobe and of divergence of frontal carinae observed in the *M. rugulosoides* population and Figs. 9–10 the upper extremes in the *M. scabrinodis* population. Tab. 1 shows data of absolute size and of 17 RAV-corrected shape variables. These



Figure 8. Scape of a worker of the western population of *Myrmica scabrinodis* in dorsocaudal view. St. Martin-de-Vesubie /France.

Table 1. Head size and RAV-corrected shape characters of workers of *Myrmica rugulosoides* and *M. scabrinodis*. Sequence of data: arithmetic mean \pm standard deviation [minimum, maximum]. The column between the morphometric data gives F values and statistical significance of a one-way ANOVA. The most separating characters are indicated by F values printed in heavy type.

| | <i>Myrmica rugulosoides</i> (n=289) | ANOVA $F_{1,477}^*$ p | <i>Myrmica scabrinodis</i> (n=190) |
|------------------------------|--|--------------------------|---------------------------------------|
| CS [mm] | 1.071 \pm 0.060 [0.919,1.225] | 12.96, 0.000 | 1.091 \pm 0.060 [0.908,1.285] |
| CL/CW _{1.15} | 1.031 \pm 0.018 [0.973,1.085] | 0.16, n.s. | 1.030 \pm 0.017 [0.984,1.070] |
| SL/CS _{1.15} | 0.781 \pm 0.015 [0.740,0.821] | 38.13, 0.000 | 0.790 \pm 0.016 [0.733,0.840] |
| SW/SL _{1.15} | 0.142 \pm 0.009 [0.108,0.166] | 330.11 , 0.000 | 0.159 \pm 0.011 [0.142,0.189] |
| PoOc/CL _{1.15} | 0.426 \pm 0.010 [0.405,0.452] | 3.41, n.s. | 0.428 \pm 0.010 [0.398,0.460] |
| EYE _{1.15} | 0.202 \pm 0.005 [0.187,0.219] | 1.64, n.s. | 0.201 \pm 0.006 [0.185,0.215] |
| FL/CS _{1.15} | 0.437 \pm 0.013 [0.407,0.468] | 209.66 , 0.000 | 0.454 \pm 0.013 [0.422,0.501] |
| FR/CS _{1.15} | 0.325 \pm 0.013 [0.292,0.361] | 72.08 , 0.000 | 0.314 \pm 0.015 [0.279,0.367] |
| FL/FR _{1.15} | 1.339 \pm 0.059 [1.168,1.517] | 295.13 , 0.000 | 1.443 \pm 0.072 [1.259,1.671] |
| PEW/CS _{1.15} | 0.271 \pm 0.012 [0.226,0.312] | 41.64, 0.000 | 0.278 \pm 0.012 [0.249,0.314] |
| PPW/CS _{1.15} | 0.401 \pm 0.015 [0.354,0.453] | 29.42, 0.000 | 0.409 \pm 0.015 [0.374,0.467] |
| PEH/CS _{1.15} | 0.329 \pm 0.010 [0.297,0.364] | 39.44, 0.000 | 0.336 \pm 0.012 [0.296,0.370] |
| PEL/CS _{1.15} | 0.469 \pm 0.014 [0.430,0.506] | 58.00 , 0.000 | 0.479 \pm 0.014 [0.447,0.514] |
| PPHL/CS _{1.15} | 0.168 \pm 0.011 [0.139,0.195] | 16.56, 0.000 | 0.163 \pm 0.011 [0.135,0.193] |
| MetL/CS _{1.15} | 0.237 \pm 0.009 [0.212,0.260] | 31.21, 0.000 | 0.242 \pm 0.009 [0.215,0.262] |
| MetSp/CS _{1.15} | 0.164 \pm 0.012 [0.134,0.216] | 0.20, n.s. | 0.164 \pm 0.011 [0.130,0.195] |
| SP/CS _{1.15} | 0.385 \pm 0.024 [0.315,0.454] | 11.41, 0.001 | 0.393 \pm 0.028 [0.318,0.453] |
| ClyEx/CS _{1.15} [%] | 0.72 \pm 0.28 [0.00,1.45] | 2.96, n.sp. | 0.77 \pm 0.27 [0.00,1.47] |



Figure 9. Dorsal aspect of head in a worker of the eastern population of *Myrmica scabrinodis*. Odinsk / South Central Siberia.



Figure 10. Scape of a worker of the eastern population of *Myrmica scabrinodis* in dorsocaudal view. Odinsk / South Central Siberia.

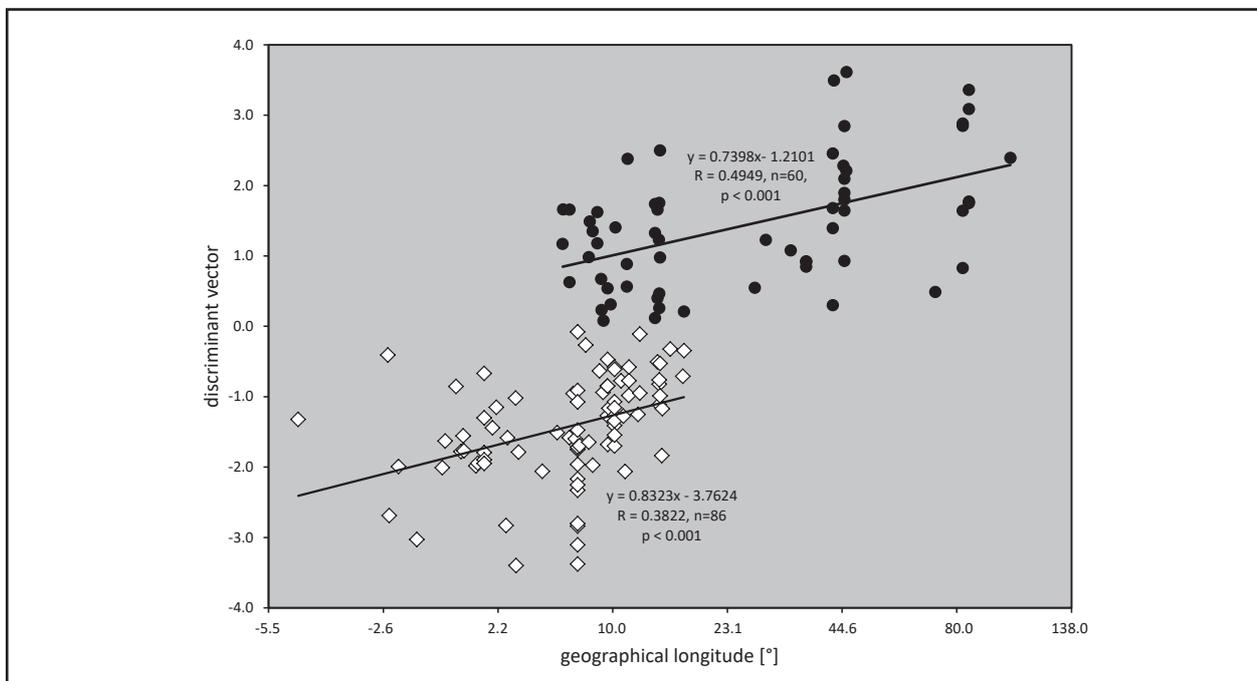


Figure 11. Discriminant values separating the western (white rhombs) and eastern population (black dots) of *Myrmica scabrinodis* showing a strong morphological convergence in the allopatric range. Geographic longitude in logarithmic scale.

Table 2. Distribution of discriminant values of *Myrmica rugulosoides* and *M. scabrinodis* within the allopatric and sympatric range. Sequence of data: arithmetic mean \pm standard deviation [minimum, maximum]. The line between the allopatric and sympatric data sets gives F values and statistical significance of a one-way ANOVA.

| nest sample means of discriminant | |
|--|-------------------------------------|
| <i>M. rugulosoides</i> in allopatry west of 6°E (n=27) | -1.755 \pm 0.681 [-3.399, -0.408] |
| ANOVA $F_{1,84}$ 8.093, p = 0.006 | |
| <i>M. rugulosoides</i> in sympatry(n=59) | -1.284 \pm 0.726 [-3.379, -0.078] |
| <i>M. scabrinodis</i> in allopatry east of 22°E (n=30) | 1.840 \pm 0.940 [0.299, 3.611] |
| ANOVA $F_{1,58}$ 14.223, p = 0.000 | |
| <i>M. scabrinodis</i> in sympatry (n=30) | 1.048 \pm 0.664 [0.079, 2.499] |

data show that the biggest contributions to separation of the two taxa are provided by the width of basal scape lobe and the divergence of frontal carinae.

5. Conclusion

The good separation of *Myrmica rugulosoides* and *M. scabrinodis* by the LDA and only 1.4% misclassification by NC-NMDS.kmeans could indicate heterospecificity but a look on the geographic variation of morphology throws doubt on this hypothesis. According to the classification provided by the LDA, the sympatric range of both taxa is within a zone in Europe extending from 6°E and about 22°E. This means *M. rugulosoides* is in allopatry west of 6°E whereas *M. scabrinodis* is in allopatry east of 22°E. A statistics of the discriminant values provided by Tab. 2 clearly indicates morphological convergence and bilateral introgression within the sympatric zone. The same conclusion results from linear regressions of discriminant values against geographical longitude (Fig. 11).

The two morphological clusters distinguished here diverged in separate Pleistocene refuges which were situated in *Myrmica rugulosoides* in southern France north of the Pyrenees and in *M. scabrinodis* in the Balkans and farther east. After postglacial spreading they formed a sympatric range in Europe between 6°E and probably about 22° E. Significant morphological convergence within this area of contact clearly indicates hybridization and introgression of genes. The degree of morphological convergence appears too large to accept the heterospecificity suggested by the exploratory data analyses NC-NMDS.kmeans and PCA. Accordingly, the taxon *Myrmica rugulosoides* is considered here as junior synonym of *M. scabrinodis* as it is the case with *M. pilosiscapus* and *M. martini*.

The situation indicated by morphology and zoogeography is similar to the phylogeographic picture found by Leppänen et al. (2012) in *Myrmica rubra* (Linnaeus 1758). Analyzing mtDNA haplogroups, Leppänen et al. concluded on Pleistocene refuges in south France and the Apennine Peninsula for the western group and in the Balkans, Middle Asia and SW Siberia for the eastern group. The placement of the Pleistocene refuge of the western population of *M. scabrinodis* north of the Pyrenees is most probable because it is absent from Iberia south of the Pyrenees where it is replaced by the closely related *M. aloba* Forel 1909. Retreat of *Myrmica scabrinodis* and *M. rubra* into similar (or equal) Pleistocene refuge centers appears also very likely by ecological data gathered in the broad-based survey of

Seifert (2017). The realized niche overlap of $O_R = 0.1955$, which indicates the probability of both species to occur in the same habitat spot, is at rank 6 within the overlap values calculated for 78 pairs of 12 Central European *Myrmica* species (see SI3). Arithmetic mean \pm standard deviation [minimum, maximum], and sample size of this data set are 0.0469 ± 0.0671 [0.0000, 0.2689], $n = 78$. Very similar glacial retreat and postglacial expansion routes of these distantly related species are also indicated by their almost congruent actual geographic ranges (see distribution maps in Radchenko & Elmes 2010, supplemented by data of this study).

6. Acknowledgements

I wish to thank Igor Antonov, Phill Attewell, Christophe Galkowski, Wolfgang Münch and Roland Schultz for providing valuable samples from Siberia, Middle Asia and Western Europe. This research was co-financed by tax money on the basis of the state budget passed by the Sächsischer Landtag according to the Antragsnummer 100590787 of the Sächsische Aufbaubank issued 3 August 2021.

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