

RESEARCH ARTICLE

New analyses in the species triple *Myrmica sabuleti* Meinert 1861, *lonae* Finzi 1926 and *spinosior* Santschi 1931 (Hymenoptera: Formicidae)

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Abstract

New multivariate analyses considering new differential characters and based on hypothesis formation by NC-clustering are presented for three closely related species of the Westpalaeartic *Myrmica sabuleti* species complex. Investigation of 345 nest samples with 1080 workers resulted in the confirmation of *Myrmica sabuleti* Meinert 1861, *M. lonae* Finzi 1926 and *M. spinosior* Santschi 1931 as valid species. The analysis provided suggestions for local hybridization between *M. sabuleti* and *M. lonae* but the overall morphological, zoogeographic and ecological evidence favors a treatment as different species.

Keywords Cryptic species | numeric morphology-based alpha-taxonomy | hybridization | ecological niche

1 Introduction

Radchenko & Elmes (2010) defined a Westpalaeartic *Myrmica sabuleti* species complex that included the species *Myrmica sabuleti* Meinert 1861, *M. lonae* Finzi 1926, *M. spinosior* Santschi 1931, *M. vandeli* Bondroit 1920, *M. bibikoffi* Kutter 1963 and *M. hirsuta* Elmes 1978. The males of the first three species are very similar and there are no thorough investigations so far to identify diagnostic characters. Based on multivariate analysis of phenotypic characters in large series of workers, Seifert (2005) provided proof that these three entities form morphologically separable clusters. This concept was accepted by Radchenko & Elmes (2010). Commenting on regional populations with less clear separation between *Myrmica sabuleti* and *M. lonae* and suggesting occasional

hybridization, Seifert (2018) argued that a number of arguments in favor of species separation outweigh a possible counter-argument based on hybridization. Ebsen et al. (2019), using alone mitochondrial Cyt-B and COI genes as indicators, supposed conspecificity of the two taxa without formally stating a synonymy. First of all, it is surprising that a method shown for long as misleading by numerous papers (see, e.g., the impressive meta-analyses of Funk & Omland 2003 and Ross 2014) was used in the year 2019 to draw taxonomic conclusions. Referring to ants in particular, there is an average classification error by mtDNA barcoding per sample of 17% over 10 genera with 66 species with the extremes ranging from 0 to 32% (Seifert 2024). Notably, the error in Tibetan *Myrmica* species was near the upper range with 24% (Seifert et al. 2018).



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A recent investigation by Lavanchy et al. (2025) of 918 *Myrmica* samples from Switzerland, which combined mtDNA barcoding (based on COI) and RAD sequencing, could not confirm a separate *Myrmica lonae* lineage. However, alone the ratio of 138 morphologically identified *Myrmica sabuleti* samples against only two morphologically identified *M. lonae* samples suggests that samples of latter species were misclassified as *M. sabuleti*. Notably, the average abundance ratio of *M. sabuleti* and *M. lonae* in Central Europe and south Fennoscandia based on extensive sampling was estimated by Seifert (2000, 2018) as 8 : 1 and within 69 *Myrmica* samples from Switzerland investigated by the author five were classified by multivariate analyses as *Myrmica lonae*. Furthermore, the two supposed *M. lonae* samples in the study of Lavanchy et al. (2024) were finally identified by the “integrated evidence” of morphology and genetics as *M. lobicornis* (!) and the other one as *M. sabuleti*.

As there was no published investigation of the three focal species since Seifert (2005) and in particular no published response to the implied synonymization by Ebsen et al. (2019), it appeared necessary to check the hypotheses with new methods of Numeric Morphology-Based Alpha-taxonomy (NUMOBAT) under consideration of new differential characters in *Myrmica*. On this occasion, the cluster allocation of the type series of *Myrmica spinosior* and of *Myrmica rolandi reticulata* Stärcke 1942, both described from the Pyrenees and belonging to the *Myrmica scabrinodis* species complex, was re-analysed and the results of these studies are presented here. Though not belonging to the *Myrmica sabuleti* species complex, the western population of *Myrmica scabrinodis* Nylander 1846 – *Myrmica scabrinodis rugulosoides* Forel 1915 – was included in the analysis because it occurs in the Pyrenees and because the workers show some similarities to the afore-mentioned types. *Myrmica rugulosoides* was synonymized by Seifert (2024) with *M. scabrinodis* Nylander 1846 but using the name in subspecific rank makes sense within the context considered here.

2 Material

NUMOBAT data were recorded in a total of 345 nest samples with 1080 workers. These figures were 86 and 289 in *Myrmica scabrinodis rugulosoides*, 126 and 382 in *M. sabuleti*, 36 and 100 in *M. spinosior* and 97 and 309 in *M. lonae*. The material originated from Albania (1 sample) Andorra (5), Austria (20), Belgium (2), Bosnia-Herzegovina (1), Bulgaria (7), Czechia (7), Denmark (1), Finland (9), France (35), Germany

(152), Great Britain (17), Greece (9), Hungary (1), Italy (12), Netherlands (1), Norway (1), Poland (1), Russia (1), Serbia (1), Spain (2), Sweden (1), Switzerland (8), Turkey (6). With the exception of type specimens (see detailed report below), data of this material are not presented in the main text of this paper but listed up in the electronic supplementary information SI1 and SI2. Note that the number of worker individuals used in the exploratory and hypothesis-driven analyses is often lower than the numbers given above because of missing data in some characters.

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

Sixteen 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.

ASCB – the tangens of the slope angle α at scape base relative to the plane defined by standard viewing positions SVPc and SVP f (Fig. 4).

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.

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 parallel 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 metapleurocoxal 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 dot) and the center of petiole-postpetiolar junction (right red dot).

PEL – maximum measurable diagonal petiole length



Figure 1. Head measurements.

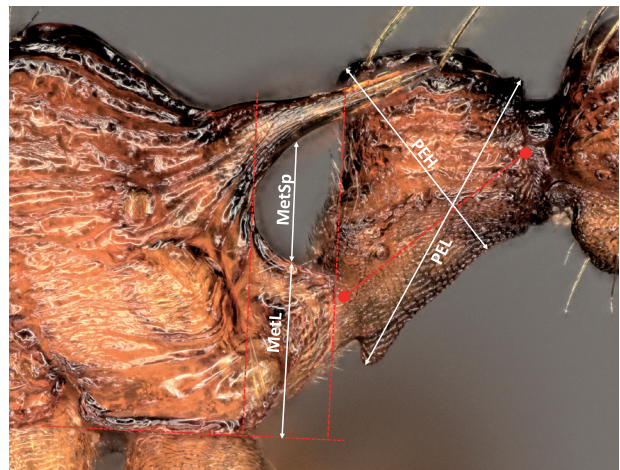


Figure 2. Measurements of caudal mesosoma and petiole.



Figure 3. Measuring length of propodeal spines.

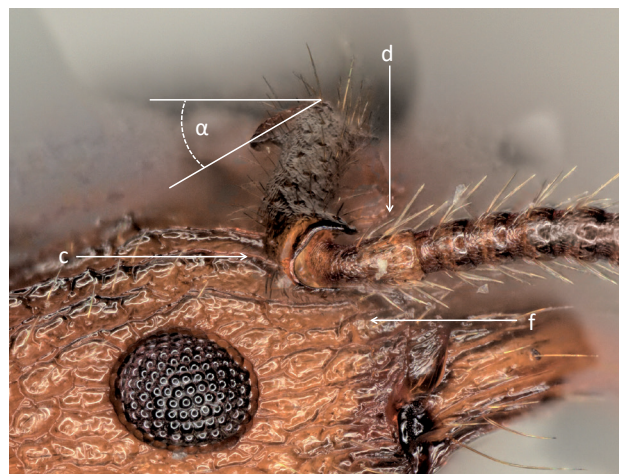


Figure 4. View along the axis of scape with definition of standard viewing positions and the slope angle of basal scape lobe.

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



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

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. 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

$$\begin{aligned} CL/CW_{1.15} &= CL/CW / (-0.0487*CS+1.0900)*1.0340 \\ SL/CS_{1.15} &= SL/CS / (-0.0802*CS+0.8947)*0.8024 \\ EYE/CS_{1.15} &= EYE/CS / (0.0179*CS+0.1772)*0.1978 \\ FL/CS_{1.15} &= FL/CS / (0.0227*CS+0.4036)*0.4297 \\ FR/CS_{1.15} &= FR/CS / (0.0095*CS+0.3222)*0.3331 \\ FL/FR_{1.15} &= FR/CS / (-0.0572*CS+1.4270)*1.3612 \\ PEW/CS_{1.15} &= PEW/CS / (-0.0201*CS+0.2842)*0.2610 \\ PPW/CS_{1.15} &= PPW/CS / (0.0709*CS+0.3208)*0.4024 \\ PEH/CS_{1.15} &= PEH/CS / (0.0051*CS+0.3230)*0.3288 \\ PEL/CS_{1.15} &= PEL/CS / (-0.0259*CS+0.4975)*0.4677 \\ PPHL/CS_{1.15} &= PPHL/CS / (-0.0679*CS+0.2535)*0.1754 \end{aligned}$$

$$\begin{aligned} SP/CS_{1.15} &= SP/CS / (0.0876*CS+0.2353)*0.3360 \\ MetL/CS_{1.15} &= MetL/CS / (-0.0126*CS+0.2487)*0.2342 \\ MetSp/CS_{1.15} &= MetSp/CS / (-0.0113*CS+0.1880)*0.1749 \\ PoOc/CL_{1.15} &= PoOc/CL / (0.0098*CS+0.4154)*0.4266 \\ SW/SL_{1.15} &= SW/SL / (0.0109*CS+0.1385)*0.1511 \end{aligned}$$

3.5 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 [Csösz & 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 species 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 and discussion

4.1 Investigation of type specimens

Myrmica sabuleti Meinert 1861

Investigated was the lectotype worker labelled “9/8 1858 Buur Krat Vestjyll, Meinert” and “LECTOTYPE desig. Radchenko & Elmes 2002”, deposited in Zoological Museum of the University of Copenhagen. The specimen shows over all characters an average *sabuleti* morphology with exception of the petiole profile which is more similar to the *scabrinodis* condition. The subpetiolar process of the specimen was concealed in glue and no manipulations were done to remove the glue. Accordingly, the substitution data for the missing PeL value was calculated by an intraspecific correlation analysis against CS, PeW and PeH.

The lectotype was allocated in a wild-card run of a 2-class LDA considering CS and 13 RAV-corrected values and *Myrmica sabuleti* and *lonae* as alternatives (for details see below under cluster analyses) with $p=0.9835$ to the *sabuleti* cluster.

Myrmica lonae Finzi 1926

This taxon was described as *Myrmica sabuleti* subsp. *lonae* Finzi 1926. Investigated were the lectotype worker (published in Seifert 2000) from Karislojo, Finland, leg. Forsius; 4 worker paralectotypes (labelled as cotypes) from Monte Baldo, Trentino/Italy, leg. Lona July 1923; 3 worker paralectotypes (labelled as cotypes) from Monte Nanos (NE of Trieste), leg. Finzi May 1923; 1 worker and 1 gyne paralectotype (labelled as cotypes) from Ratzes / Tirol [= Razzes / Trentino]. All material is stored in MCZ Cambridge. The lectotype was allocated in a wild-card run of a 2-class LDA considering CS and 13 RAV-corrected values and *Myrmica sabuleti* and *lonae* as alternatives (for details see below under cluster analyses) with $p=0.9996$ to the *lonae* cluster. The paralectotype samples were allocated by the same analysis to the *lonae* cluster with $p=0.9998$ (Ratzes), $p=0.9663$ (Monte Baldo) and $p=0.9995$ (Monte Nanos).

Myrmica rolandi var. *reticulata* Stärrcke 1942

Myrmica scabrinodis rolandi var. *reticulata* Santschi 1931

This taxon was described from the Central Pyrenees. Santschi (1931) reported as collecting sites: “Pyrénées centrales, Cirque d’Espingno, entre 1850 et 2000 m., août 1929 w,g,m (types) - Val Astos, Commune d’ Oô, entre 1100 et 1200 m., août - Val de la Frèche, fond de la vallée de la Pique, entre 1550 et 1650m. Haute-Garonne, sous des pierres (H. GADEAU DE KERVILLE leg.).“ For explanation of geographic names: the “Val de Oueil” is situated at about 42.83°N and 0.54°E, the “Commune d’ Oô” at 42.796°N, 0.506°E and 966 m, and the “Cirque d’Espingno” or “Espingo” at 42.728°N, 0.499°E, 1920 m.

We have the problem that each label of Santschi in the collection of Naturhistorisches Museum Basel shows disagreements with his statements in the original description. Furthermore Santschi's series contain two species. I selected a lectotype in a worker series showing the least disagreement with Santschi's statements: worker lectotype (by present designation) and 4 paralectotype workers on the same pin labelled “Mt. Espigno. Val d’ Oueil Ht. Garon. 1200 m Kerville. 20 VIII 29” [Santschi's handwriting], “M. scabrinodis rolandi

Bond. *reticulata* San. “[Santschi’s handwriting], “Lectotype + paralectotypes *Myrmica scabrinodis rolandi reticulata* Santschi, des. and remounted by B. Seifert 2012”, “SYNTYPE desig. Radchenko & Elmes 2002”. Four additional paralectotype workers on another pin, but remaining in the original mode of mounting, were labelled with laser printed label copies “label copy by B. Seifert, read as: Mt. Espigno. Val d’ Oueil Ht. Garon. 1200 m Kerville. 20 VIII 29”, “Paralectotypes *Myrmica scabrinodis rolandi reticulata* Santschi, des. B. Seifert 2012”. These 8 workers were originally placed by Santschi on a single pin. Dividing the sample and remounting was necessary to enable a proper investigation. The lectotype series was allocated with $p=0.9992$ to the *sabuleti* cluster in a wild-card run of a 2-class LDA considering CS and 13 RAV-corrected values and the species *Myrmica sabuleti* and *lonae* (for details see below under cluster analyses). Furthermore, it was allocated to the *sabuleti* cluster with $p=0.9554$ in a wild-card run of a 3-class LDA considering CS and 17 RAV-corrected characters and the species *Myrmica sabuleti*, *M. scabrinodis rugulosoides* and *M. spinosior* (for details see below under cluster analyses).

Note: Without placing a lectotype label on a pin within the NHM Basel collection, Radchenko & Elmes (2010) published a lectotype fixation with the following text: “lectotype of *M. rolandi* var. *reticulata* (designated here), male (upper specimen on the pin with 3 males and 1 queen), labelled “*M. scabrinodis rolandi* Bondr. v. *reticulata* Sant.”, “[very hardly readable text, most probably: M° Espigno, Val d’Oueu, H^a Garon, 1200 m, Kerville, 20.VIII.29]”. During a search in 2012 I could not identify a pin in the NHM Basel collection which is in agreement with these data. A recent scrutiny search by the curator Seraina Klopstein 28 August 2026 could also not detect such specimens within the series of *Myrmica rolandi reticulata*, *Myrmica scabrinodis* and *M. scabrinodis rolandi*. Present in NHM Basel are 3 males, 1 gyne on the same pin, labelled “Val de la Frèche fond val de la Pique Hte Gar. 1600 m G.Kerville 23 IX 30” [Santschi’s handwriting], “Vol nuptial mixte” [Santschi’s handwriting], “SYNTYPE desig. Radchenko & Elmes 2002” and a second pin, also with 3 males, 1 gyne, labelled only “Vol nuptial mixte” [Santschi’s handwriting] and “*M. scabrinodis rolandi*” [Santschi’s handwriting]. The males and gynes in these two series belong to *Myrmica scabrinodis rugulosoides*. As it can be seen from the data above, a pin with three males and a gyne matching the information published

by Radchenko & Elmes (2010) does not exist in NHM Basel. Accordingly, the lectotype designation by these authors is considered invalid under the provisions of article 74.7.2 of ICZN.

***Myrmica spinosior* Santschi 1931**

Myrmica sabuleti var. *spinosior* Santschi 1931

Investigated was the lectotype worker labelled “Typus”, “*M.sabuleti* v. *spinosior* Sant”, “Pyren. occ. Irun 25-26 3. 1926 Lindberg”, “LECTOTYPE desig. Radchenko & Elmes 2002”, NHM Basel and 2 paralectotype workers on one pin, labelled “Pyren. occ. Irun 25-26 3. 1926 Lindberg”, “*M.sabuleti* v. *spinosior* Sant”, “Paralectotype *M. spinosior* Santschi det. Seifert 2004”, NHM Basel. These specimens are in morphology and locality label consistent with the lectotype.

The lectotype series was allocated to the *spinosior* cluster with $p=0.9881$ in a wild-card run of a 3-class LDA considering CS and 17 RAV-corrected characters and the species *Myrmica sabuleti*, *M. scabrinodis rugulosoides* and *M. spinosior* (for details see below under cluster analyses).

4.2 Cluster analyses

The first cluster analysis included material belonging to the taxa *Myrmica sabuleti*, *M. spinosior*, *M. scabrinodis rugulosoides*. Material with very strong development of scape lobes, presumably representing *Myrmica lonae* and with certainty not belonging to *spinosior* or *rugulosoides*, was excluded from this analysis and checked in a second analysis against those ants classified by the first analysis as *sabuleti*.

The first analysis included only specimens in which data of the full character set were available. This included cephalic size CS plus the 17 RAV-corrected characters CL/CW_{1.15}, PoOc/CL_{1.15}, EYE/CS_{1.15}, SL/CS_{1.15}, SW/SL_{1.15}, ASCB_{1.15}, FL/CS_{1.15}, FR/CS_{1.15}, FL/FR_{1.15}, MetL/CS_{1.15}, MetSp/CS_{1.15}, SP/CS_{1.15}, PeW/CS_{1.15}, PeH/CS_{1.15}, PeL/CS_{1.15}, PpW/CS_{1.15}, and PpHL/CS_{1.15}. The exploratory data analyses NC-Ward, NC-part.kmeans, and NC-NMDS.kmeans suggested three main clusters within 173 considered nest samples (Fig. 6). The controlling Linear Discriminant Analysis (LDA) used the hypothesis provided by NC-part.kmeans. The classification error of the exploratory data analyses relative to the final species hypothesis was 0% in NC-Ward and NC-part.hclust and 0.6% in NC-part.kmeans and NC-NMDS.kmeans. The LDA indicated 1.0% misclassified specimens in 512 worker individuals with any specimen of *M. scabrinodis rugulosoides* correctly classified. When run as wild-cards in the

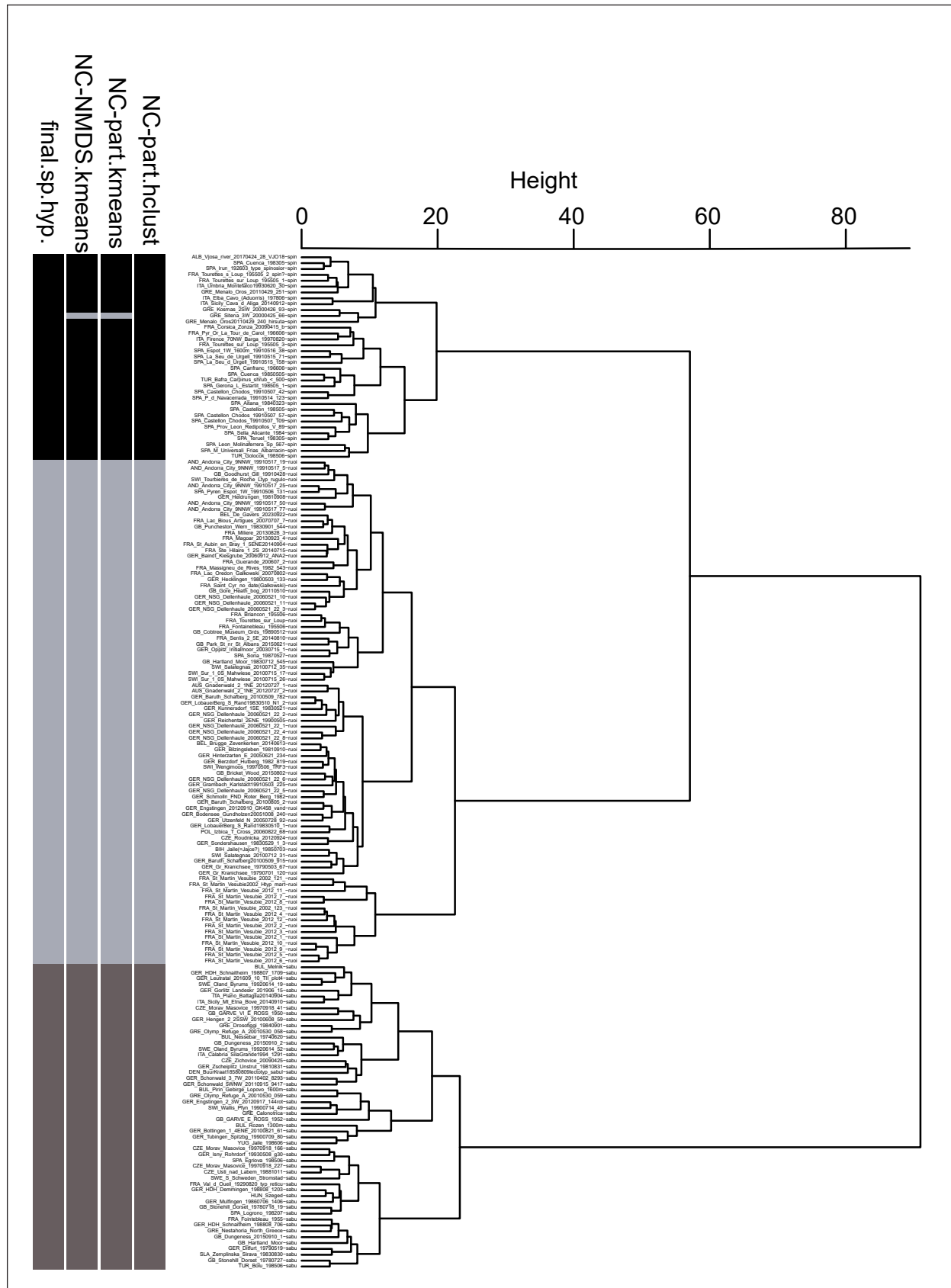


Figure 6. Separation of 173 worker nest samples of *Myrmica spinosior* (black bars), *Myrmica scabrinodis rugulosoides* (light grey bars) and *Myrmica sabuleti* (dark grey bars) by four variants of NC-clustering considering 18 morphometric characters. The mean error of all analyses is 0.3 %.

LDA, the type series of *M. spinosior* was allocated to the *spinosior* cluster with $p=0.9881$ whereas the type series of *M. reticulata* and the lectotype of *M. sabuleti* were allocated to the *sabuleti* cluster with $p=0.9554$ and $p=1.000$ respectively. The type series of *M. scabrinodis rugulosoides* and *M. martini* Seifert et al. were placed in the *rugulosoides* cluster with $p=0.9999$ and $p=1.000$. As result, *M. reticulata* is considered a junior synonym of *M. sabuleti*, *M. martini* as junior synonym of *M. scabrinodis rugulosoides* whereas *M. spinosior* is exposed as bona species (see also Fig. 7).

The second analysis considered samples classified by the first analysis as *Myrmica sabuleti* and samples supposed to be *Myrmica lonae*. This included a total of 220 nest samples and considered cephalic size CS plus the 13 RAV-corrected characters CL/CW_{1.15}, PoOc/CL_{1.15}, EYE/CS_{1.15}, SL/CS_{1.15}, SW/SL_{1.15}, FL/CS_{1.15}, FR/CS_{1.15}, FL/FR_{1.15}, SP/CS_{1.15}, PeW/CS_{1.15}, PeH/CS_{1.15}, PeL/CS_{1.15} and PpW/CS_{1.15}. Ignoring NC-part.hclust which split the samples into eight clusters, the exploratory data analyses NC-Ward, NC-part.kmeans and NC-NMDS.kmeans proposed two main clusters (Fig. 8). NC-part.kmeans subdivided the *lonae* cluster into two subsamples which was caused by oversampling of the morphologically extreme *M. lonae* population from

Harprechtsmoos – a turf bog in Baden-Württemberg. This subdivision was ignored and the hypotheses were set with $K=2$. According to the controlling LDA, the classification error on nest-sample level was 2.3 % in NC-Ward, 4.1 % in NC-part.kmeans and 1.4 % in NC-NMDS.kmeans and on individual level 3.6 % in 589 workers. Reducing the considered character to SL/CS_{1.15}, SW/SL_{1.15}, FL/CS_{1.15}, PeH/C_{1.15} and PEL/CS_{1.15} the error of the exploratory data analyses became lower: 0.9 % in NC-Ward, 0.4 % in NC-part.kmeans and 1.4 % in NC-NMDS.kmeans. These data and the strong differences in habitat selection and geographic distribution (detailed description in Seifert 2000, summarized in Seifert 2018) as well as a different response to several ecological niche variables such as soil temperature and moisture (Seifert 2017) provide good arguments to consider *M. lonae* as a separate species.

However, there are also observations suggesting hybridization. A PCA considering the reduced ('refined') character set shows that intermediate samples are not rare (Fig. 9) and, using the principal components 1 and 3 as characters in a LDA, results in 2.7 % misclassified samples. A direct suggestion for local hybridization comes from comparing the mean values of discriminant vectors from 133 sites with only one species present

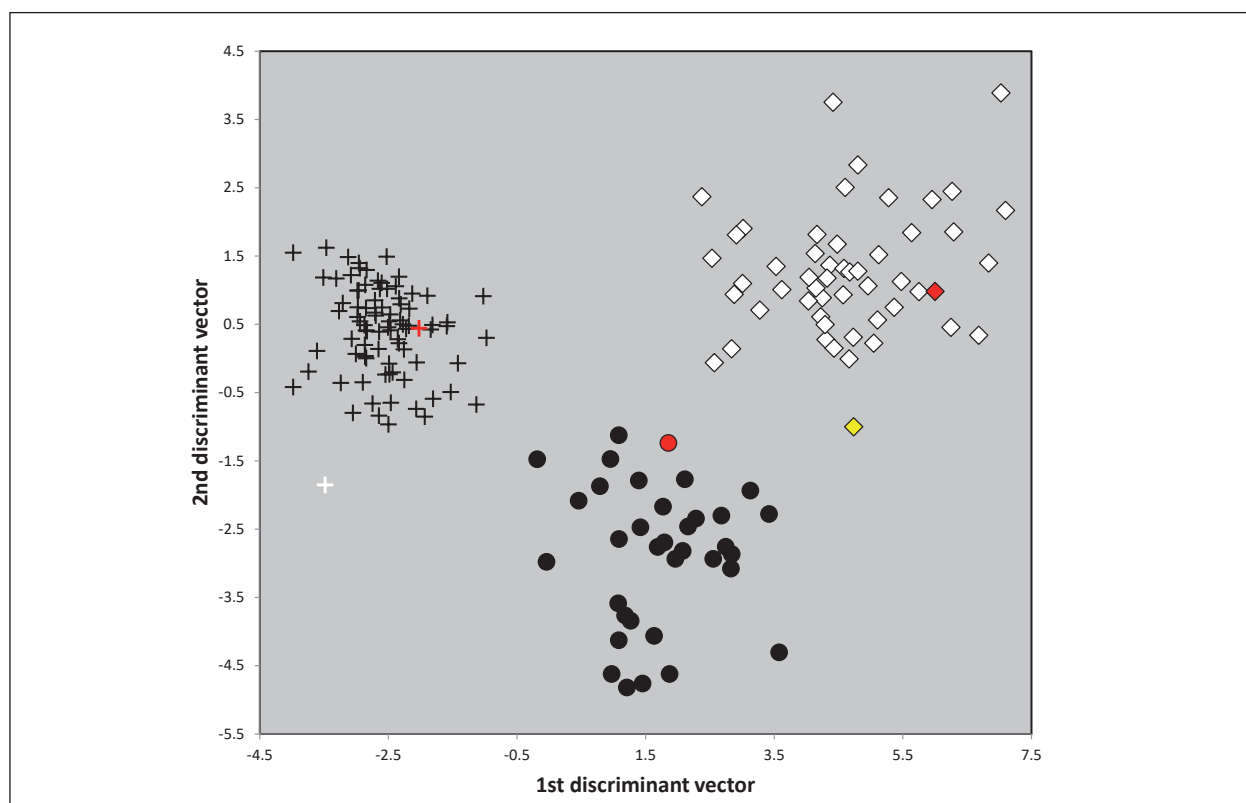


Figure 7. Separation of 173 worker nest samples of *Myrmica spinosior* (dots), *M. scabrinodis rugulosoides* (crosses) and *M. sabuleti* (rhombs) by a linear discriminant analysis considering 18 morphometric characters. Type series are marked as follows: *M. sabuleti* (red rhomb), *M. reticulata* (yellow rhomb), *M. spinosior* (red dot), *M. scabrinodis rugulosoides* (red cross), *M. martini* (white cross).

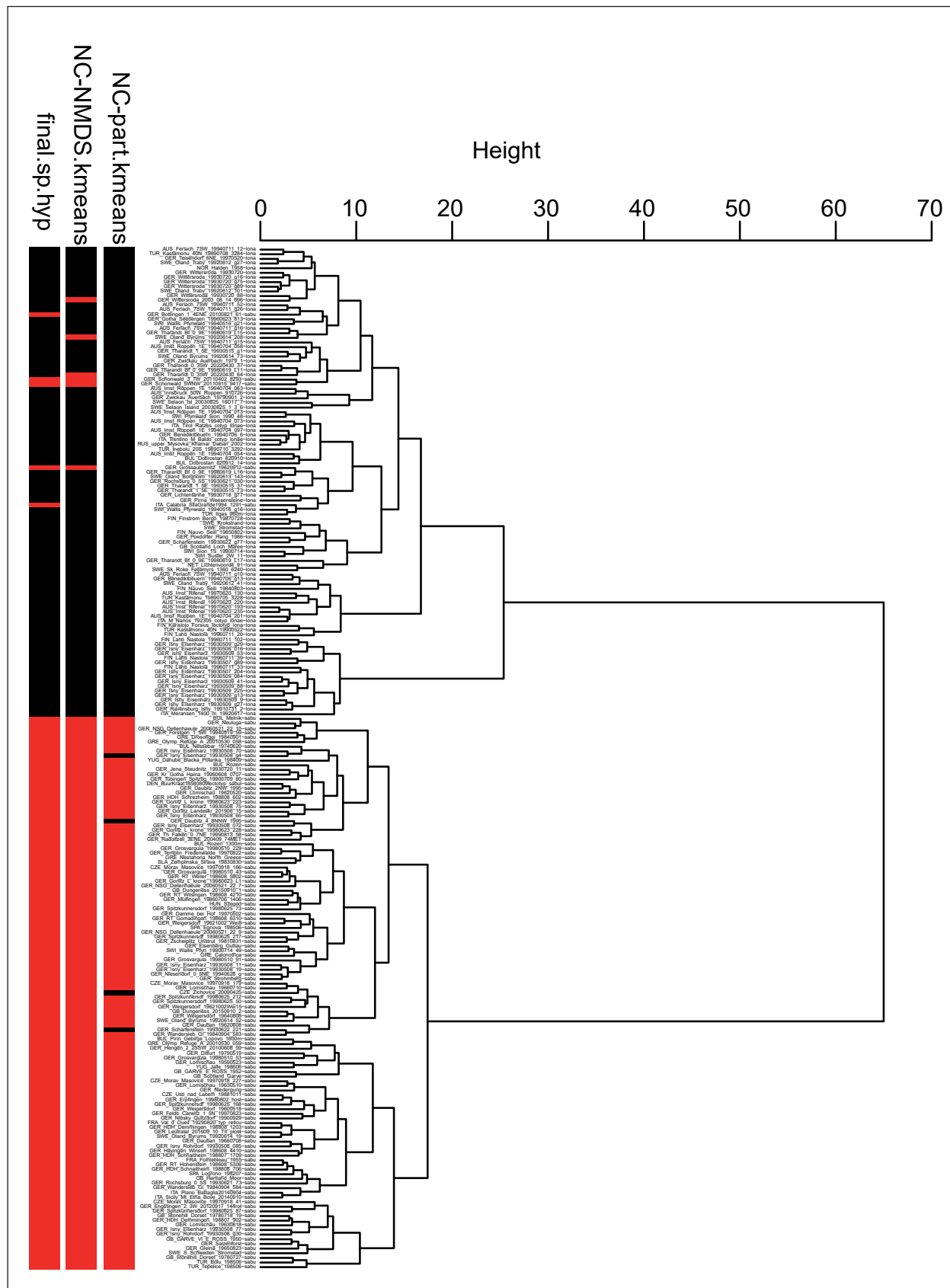


Figure 8. Separation of 220 worker nest samples of *Myrmica lonae* (black bars) and *Myrmica sabuleti* (red bars) by three variants of NC-clustering considering 14 morphometric characters. The mean error of all analyses relative to the final species hypothesis is 2.6 %.

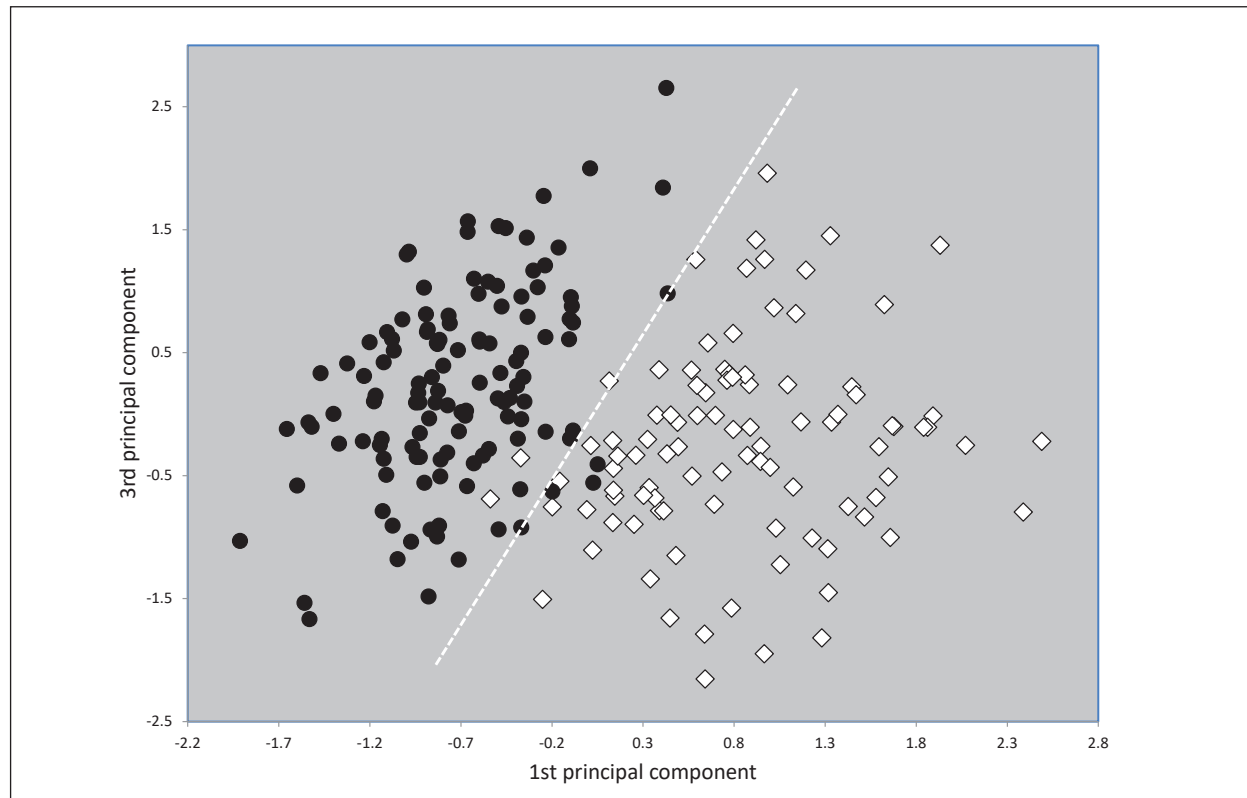


Figure 9. Separation of 220 worker nest samples of *Myrmica lonae* (white rhombs) and *M. sabuleti* (black dots) by a principal component analysis considering five RAV-corrected characters. The dashed line marks the separation of the species by an LDA using the principal components 1 and 3 as characters. The vector of the LDA is directed perpendicular to the dashed line.

against those from the 6 sites with syntopic occurrence of both species. We observe in both species a reduction of the absolute values of the discriminant scores at sites with syntopic occurrence (Tab. 1). This trend to morphological convergence is significant in case of *M. sabuleti* ($p=0.014$) and not significant in *M. lonae* ($p=0.067$) and might indicate hybridization in the rare cases of direct contact. Testing the case with a linear mixed-effects model could yield other results but this is outside the scope of this paper. I consider the strong ecological separation as one major factor to keep the populations of the two species separate in most parts of their geographical range.

Tab. 2 shows RAV-corrected data of all investigated characters with the most similar species placed in neighboring columns. *Myrmica scabrinodis rugulosoides* is separable from *M. spinosior* by smaller absolute size, shorter scape, the much steeper slope of the plane at scape base, and a larger ratio MetL / MetSp. With all measurements in mm, a discriminant $26.30 \cdot SL + 78.86 \cdot SW - 37.48 \cdot EYE - 16.60 \cdot MetL + 26.78 \cdot MetSp - 1.661 \cdot \tan(ASCB) - 24.87$ offers a perfect separation with all 289 individuals of *M. sc. rugulosoides* having values < 0 and all 92 individuals of *M. spinosior* having values > 0 .

Table 1. Comparison of discriminant values of worker individuals of *Myrmica sabuleti* and *M. lonae* from sites with syntopic occurrence of both species and from sites where only one species was found. Values of a one-tailed ANOVA test are placed between the lines of compared samples.

	discriminant for 14 characters
<i>M. sabuleti</i> from 67 sites with only <i>sabuleti</i>	-1.833 ± 0.769 (n=171)
one tailed ANOVA	$F_{1,166} \ 6.174, p=0.014$
<i>M. sabuleti</i> from 6 sites with both species	-1.354 ± 0.617 (n=17)
<i>M. lonae</i> from 6 sites with both species	1.119 ± 0.920 (n=27)
one tailed ANOVA	$F_{1,222} \ 3.381, p=0.067$
<i>M. lonae</i> from 66 sites with only <i>lonae</i>	1.554 ± 1.181 (n=197)

Table 2. RAV-corrected data of workers of *Myrmica scabrinodis rugulosoides*, *M. spinosior*, *M. sabuleti*, and *M. lonae*. The most similar species are placed in neighboring columns divided by a column with values of one-tailed ANOVA test. No ANOVA was calculated for characters in which only 6 specimens were measured in *M. lonae*.

	<i>scabrinodis rugulosoides</i> (n=289)	ANOVA F, p	<i>spinosior</i> (n=100)	ANOVA F, p	<i>sabuleti</i> (n=382)	ANOVA F, p	<i>lonae</i> (n=309)
CS [μ m]	1071 $\pm$ 60 [919,1225]	288.7 0.000	1195 $\pm$ 71 [1003,1346]	24.36 0.000	1159 $\pm$ 64 [942,1322]	43.76 0.000	1190 $\pm$ 61 [998,1386]
CL/CW _{1.15}	1.031 $\pm$ 0.018 [0.973,1.085]	2.28 n.s.	1.034 $\pm$ 0.018 [0.996,1.089]	5.12 0.024	1.030 $\pm$ 0.016 [0.988,1.074]	29.69 0.000	1.023 $\pm$ 0.017 [0.979,1.070]
SL/CS _{1.15}	0.781 $\pm$ 0.015 [0.740,0.821]	636.6 0.000	0.829 $\pm$ 0.020 [0.790,0.866]	183.3 0.000	0.802 $\pm$ 0.017 [0.761,0.851]	18.98 0.000	0.807 $\pm$ 0.017 [0.764,0.861]
SW/SL _{1.15}	0.142 $\pm$ 0.009 [0.108,0.166]	78.91 0.000	0.152 $\pm$ 0.011 [0.131,0.176]	656.9 0.000	0.199 $\pm$ 0.018 [0.159,0.245]	1693.1 0.000	0.263 $\pm$ 0.023 [0.208,0.328]
tan(ASCB)	0.879 $\pm$ 0.149 [0.49,1.28]	363.2 0.000	0.507 $\pm$ 0.199 [0.03,1.04]	49.90 0.000	0.364 $\pm$ 0.128 [0.10,0.67]		0.335 $\pm$ 0.120 [0.25,0.42]
FL/CS _{1.15}	0.437 $\pm$ 0.013 [0.407,0.468]	3.67 n.s.	0.440 $\pm$ 0.013 [0.410,0.466]	163.9 n.s.	0.458 $\pm$ 0.012 [0.421,0.489]	709.9 0.000	0.485 $\pm$ 0.015 [0.444,0.529]
FR/CS _{1.15}	0.325 $\pm$ 0.013 [0.292,0.361]	0.85 n.s.	0.326 $\pm$ 0.013 [0.297,0.354]	270.4 0.000	0.300 $\pm$ 0.015 [0.262,0.356]	26.89 0.000	0.294 $\pm$ 0.015 [0.258,0.335]
FL/FR _{1.15}	1.339 $\pm$ 0.059 [1.168,1.517]	4.06 0.045	1.353 $\pm$ 0.064 [1.231,1.485]	424.6 0.000	1.530 $\pm$ 0.079 [1.306,1.739]	372.9 0.000	1.658 $\pm$ 0.095 [1.400,1.914]
PoOc/ CL _{1.15}	0.426 $\pm$ 0.010 [0.405,0.452]	27.54 0.000	0.432 $\pm$ 0.010 [0.408,0.452]	0.24 n.s.	0.432 $\pm$ 0.009 [0.408,0.456]	3.59 n.s.	0.430 $\pm$ 0.009 [0.411,0.455]
EYE/CS _{1.15}	0.202 $\pm$ 0.005 [0.187,0.219]	191.2 0.000	0.192 $\pm$ 0.006 [0.179,0.212]	13.64 0.000	0.195 $\pm$ 0.006 [0.181,0.220]	3.31 n.s.	0.194 $\pm$ 0.006 [0.183,0.214]
MetL/ CS _{1.15}	0.237 $\pm$ 0.009 [0.212,0.260]	55.80 0.000	0.228 $\pm$ 0.013 [0.194,0.258]	4.68 0.031	0.231 $\pm$ 0.011 [0.194,0.267]		0.229 $\pm$ 0.013 [0.202,0.248]
MetSp/ CS _{1.15}	0.164 $\pm$ 0.012 [0.134,0.216]	257.1 0.000	0.188 $\pm$ 0.014 [0.149,0.221]	12.55 0.000	0.181 $\pm$ 0.016 [0.147,0.250]2		0.177 $\pm$ 0.009 [0.166,0.191]
SP/CS _{1.15}	0.385 $\pm$ 0.024 [0.315,0.454]	0.19 n.s.	0.384 $\pm$ 0.030 [0.328,0.453]	26.16 0.000	0.398 $\pm$ 0.023 [0.303,0.455]	13.22 0.000	0.392 $\pm$ 0.020 [0.343,0.480]
PeW/ CS _{1.15}	0.270 $\pm$ 0.012 [0.226,0.312]	20.99 0.000	0.278 $\pm$ 0.015 [0.244,0.315]	4.64 0.032	0.281 $\pm$ 0.012 [0.252,0.318]	0.82 n.s.	0.280 $\pm$ 0.014 [0.240,0.325]
PPW/ CS _{1.15}	0.401 $\pm$ 0.015 [0.354,0.453]	9.86 0.002	0.396 $\pm$ 0.015 [0.350,0.429]	2.59 n.s.	0.399 $\pm$ 0.016 [0.351,0.453]	2.77 n.s.	0.397 $\pm$ 0.016 [0.358,0.452]
PeH/ CS _{1.15}	0.329 $\pm$ 0.010 [0.297,0.364]	0.88 n.s.	0.331 $\pm$ 0.013 [0.305,0.372]	9.03 0.003	0.335 $\pm$ 0.012 [0.307,0.373]	1.40 n.s.	0.336 $\pm$ 0.011 [0.310,0.371]
PeL/CS _{1.15}	0.469 $\pm$ 0.014 [0.430,0.506]	148.8 0.000	0.490 $\pm$ 0.015 [0.444,0.532]	39.90 0.000	0.479 $\pm$ 0.014 [0.441,0.523]	1.42 n.s.	0.480 $\pm$ 0.013 [0.448,0.525]
PpHL/ CS _{1.15}	0.168 $\pm$ 0.011 [0.139,0.195]	27.07 0.000	0.174 $\pm$ 0.011 [0.151,0.215]	0.02 n.s.	0.173 $\pm$ 0.013 [0.115,0.205]		0.165 $\pm$ 0.013 [0.143,0.177]

The most important characters for separation of *Myrmica spinosior* and *M. sabuleti* are scape lobe distance FL, the frontal index FL / FR and scape length. With all measurements in mm, a discriminant $16.05*CW-18.33*SL+47.40*SW+9.55*FL/FR-29.26*PeL+16.97*PpW-12.83$

offers a perfect separation with 94 individuals of *M. spinosior* having values < 0 and 131 individuals of *M. sabuleti* values > 0 .

The separation of *Myrmica sabuleti* and *M. lonae* is more difficult and is largely based on the width of the scape lobe, frontal lobe distance and absolute size. With all measurements in mm, a discriminant $53.98*SW-6.056*SL+15.461*FL-12.53*PeL+9.985*PeH-9.701$ separates the two species sufficiently with *M. sabuleti* having negative and *M. lonae* having positive values. Using CS plus the RAV-corrected characters $CL/CW_{1.15}$, $PoOc/CL_{1.15}$, $EYE/CS_{1.15}$, $SL/CS_{1.15}$, $SW/SL_{1.15}$, $FL/CS_{1.15}$, $FR/CS_{1.15}$, $SP/CS_{1.15}$, $PeW/CS_{1.15}$, $PeH/CS_{1.15}$, $PeL/CS_{1.15}$ and $PpW/CS_{1.15}$, the classification error in *M. sabuleti* is 1.3 % in 317 worker individuals and 0 % in 124 nest sample means whereas 5.1 % of 272 individuals and 0 % of 96 nest samples are misclassified in *M. lonae*.

5 Conclusions

The inclusion of new characters and new evaluation methods confirmed the taxonomic status the following taxa as it was earlier proposed (Seifert 2000, 2005, Radchenko & Elmes 2010): *Myrmica sabuleti* Meinert 1861, *Myrmica lonae* Finzi 1926 and *Myrmica spinosior* Santschi 1931 were clustered as separate species. There are suggestions for local hybridization between *Myrmica sabuleti* and *M. lonae*. Under which conditions and to which degree gene flow between these species occurs is a matter future investigations which inevitably have to use informative systems of nuDNA. *Myrmica rolandi* var. *reticulata* Strcke 1942, which had been supposed by Seifert (1988) as junior synonym of *M. scabrinodis*, is considered now by investigation of the worker lectotype as junior synonym of *M. sabuleti*. The published data for a lectotype fixation in a male (Radchenko & Elmes 2010) did not allow to identify this specimen in the type collection of F. Santschi at NHM Basel. If this specimen should really exist, *Myrmica r. reticulata* would fall under junior synonymy of *Myrmica scabrinodis*.

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