

## *Myrmica martini* sp.n. – a cryptic species of the *Myrmica scabrinodis* species complex (Hymenoptera: Formicidae) revealed by geometric morphometrics and nest-centroid clustering

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### Abstract

Palaeartic populations of *Myrmica* ants so far known under the name *M. scabrinodis* NYLANDER, 1846 were studied by combining geometric morphometrics (GM) with nest-centroid (NC) clustering and hypothesis-driven data analysis. A new cryptic species, *Myrmica martini* sp.n., showing a rather limited geographical range extending over largely the montane to subalpine zones of the Pyrenees and French Alps, was identified. 41 landmarks and 252 semilandmarks were fixed in the clypeus, head capsule, mesosoma and petiole aspects of 359 ant workers belonging to 106 nest samples. Extracting the 14 most diagnostic shape components from a set of 316 relative warps and running these data in NC-clustering, resulted in a complete species separation despite minute interspecific differences and large overlap in any character. The species identification provided by NC-Ward, NC-K-means and NMDS-K-Means clustering and by the controlling linear discriminant analysis agreed in each nest sample. There was no classification in disagreement with zoogeographic data. The lectotype samples of the five most similar and possibly synonymous taxa had near-to-zero probabilities of belonging to the *M. martini* sp.n. cluster: *M. scabrinodis* ( $p = 0.00015$ ), *M. scabrinodis* var. *rugulosoides* FOREL, 1915 ( $p = 0.0037$ ), *M. pilosiscapus* BONDROIT, 1920 ( $p = 0.0008$ ), *M. sabuleti* var. *spinosior* SANTSCHI, 1931 ( $p = 0.0006$ ), and *M. rolandi* var. *reticulata* STÄRCKE, 1942 ( $p = 0.00001$ ). *Myrmica rolandi* var. *reticulata*, of which a lectotype was designated here, is established as junior synonym of *M. spinosior* whereas *M. scabrinodis* var. *rugulosoides* and *M. pilosiscapus* are confirmed as junior synonyms of *M. scabrinodis*. We provide a rather simple system to discriminate *M. martini* from *M. scabrinodis* requiring 8 - 10 minutes of investigation time per specimen and resulting in an error of 3.6% on the nest sample level.

**Key words:** Taxonomy, cryptic species, species delimitation, numeric morphology-based alpha-taxonomy, lectotype designation.

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### Introduction

Ants of the genus *Myrmica* LATREILLE, 1804 are widely distributed across the Holarctic and have important functions in temperate ecosystems. The current estimate of the Palaeartic fauna approaches 150 species (RADCHENKO & ELMES 2010). The species delimitation within *Myrmica* is poorly understood due to the overwhelming dominance of subjective eye-inspection taxonomy instead of using explorative and hypothesis-driven analyses of reproducibly recorded data. The crucial importance of numeric morphology-based alpha-taxonomy (NUMOBAT) in the identification of cryptic species was emphasized by SEIFERT (2009) who defined cryptic species as "two or more species which are not safely separable by primary visual or acoustic perception of an expert". This reflects the immediate sense of the word and restricts the terminus to the truly cryptic cases – i.e., to species not safely separable by training of innate pathways of the human cognitive system.

In personal contacts with colleagues, the first author frequently experienced criticism that such complex NUMOBAT approaches are unacceptable because of being too time-consuming. Those critics confuse origin and end. First, we have to do our best in credibly delimiting species on the basis of testable data sets under the best currently available investigation standards. Then, and always after this, we have to do the service to the public and to ourselves: finding the least time consuming and most simple way to identify the recognized entities with an acceptable error rate.

The identification of cryptic species in *Myrmica* is in its early beginning. NUMOBAT approaches delimited cryptic species in the *M. sabuleti*, *M. lobicornis* and *M. speciosoides* species complexes resulting in four rank elevations (SEIFERT 2000, 2005, SEIFERT & al. 2009) but also in four synonymizations (SEIFERT 2011). This means a zero balance of accepted species numbers and rebuts the wide-spread

opinion NUMOBAT could result in an oversplitting. Considering the continued philosophy of most taxonomists of prolifically publishing one new name after the other on the basis of untested subjective impressions, it is not really surprising that these detailed NUMOBAT analyses could not identify a single undescribed taxon so far.

The methodology of NUMOBAT in ants experienced a fast evolution during the last years. In *Myrmica*, it started with hypothesis-driven analyses of conventional linear morphometrics (SEIFERT 2000, 2005). Later, explorative analyses of more complex data sets (SEIFERT & al. 2009, SEIFERT 2011) and geometric morphometrics (BAGHERIAN YAZDI & al. 2012) were added to the toolbox. The most recent methodological improvement is NC-clustering (SEIFERT & al. 2014). NC clustering (nest-centroid clustering) is a high-resolution explorative data analysis applicable to any group of eusocial organisms or to any cohesive biological system providing repeats of definitely conspecific elements.

Here, we provide the first case for *Myrmica* in which advanced NUMOBAT resulted in the description of a new cryptic species. We combined geometric morphometrics with NC-clustering and hypothesis-driven data analysis for studying so far inseparable populations of the *M. scabrinodis* species complex and identified a new species. This new species, *Myrmica martini* sp.n., has a rather limited geographical range extending over largely the montane to subalpine zones of the Pyrenees and French Alps. It is closely related to *Myrmica scabrinodis* NYLANDER, 1846. We firstly present the formal description of the new species. Then we present our argumentation why *Myrmica martini* sp.n. is no junior synonym of *Myrmica scabrinodis* var. *rugulosoides* FOREL, 1915, *Myrmica pilosiscapus* BONDROIT, 1920, *Myrmica sabuleti* var. *spiniosior* SANTSCHI, 1931, or *Myrmica rolandi* var. *reticulata* STÄRCKE, 1942. These taxa show a similar morphology and real or potential geographic overlap. The type localities of *M. spiniosior* and *M. reticulata* are within the range of *M. martini* sp.n. and those of *M. scabrinodis* var. *rugulosoides* and *M. pilosiscapus* are 150 km north-northeast and 200 km north of the next known site of *M. martini* sp.n.

## Material

Collecting dates are given in alphanumeric format yyyy.mm.dd. Sample numbers refer to the primary numbers in the field books.

### *Myrmica martini* sp.n.

A total of 23 nest samples with 84 workers was investigated both by geometric morphometrics (GM) and conventional linear morphometrics (CLM): **A n d o r r a**: Andorra City-9 km NNW, 1991.05.17, samples No 5, 19, 25, 50, 77 [42.680° N, 1.470° E, 1850 m]. – **F r a n c e**: Briançon, 1955.06 [44.90° N, 6.63° E, 1300 m]; Fontainebleau, 1955.06 [43.33° N, 5.537° E, 500 m]; Saint-Martin-Vésubie, 2002.05.15, samples No 121, 123, 126 [44.101° N, 7.235° E, 1611 m]; Saint-Martin-Vésubie, 2012.08.15, samples No 1 - 4 [44.104° N, 7.230° E, 1766 m]; Saint-Martin-Vésubie, 2012.08.15, samples No 5 - 12 [44.103° N, 7.233° E, 1686 m]. – **S p a i n**: Espot-1 km W, 1991.05.06, sample No 131 [42.588° N, 1.071° E, 1400 m].

### *Myrmica scabrinodis* NYLANDER, 1846

89 nest samples with 275 workers were investigated by CLM only and 83 nest samples with 253 workers by both

CLM and GM: **A u s t r i a**: Gnadenwald-2.1 km NE, 2012.07.27, samples 1, 2 [47.337° N, 11.571° E, 1632 m]; Innsbruck: Seegrube, 2012.07.24, samples 1, 2 [47.305° N, 11.377° E, 1925 m]. – **C z e c h R e p u b l i c**: Roudnicka, 2012.09.24 [50.174° N, 15.829° E, 236 m]. – **F r a n c e**: Frasné, 2010.08.22 [46.831° N, 6.154° E, 840 m]; Jura: Mouthe, lectotype of *M. pilosiscapus* [48.710° N, 6.190° E, 940 m]; Tourettes-sur-Loup, 1955.06 [43.720° N, 7.060° E, 400 m]; Tourettes-s-Loup, 1955.05 - 2 [43.720° N, 7.060° E, 400 m]. – **G e o r g i a**: Borisacho, 1985.08.12, sample No 1197 [42.533° N, 44.933° E, 1500 m]; Diklo, 1985.08.02, sample No 648u [42.402° N, 45.685° E, 1800 m]; Omalo, 1985.07.31, sample No 1199 [42.380° N, 45.630° E, 1500 m]; Schatili, 1985.08.14, samples No 1195, 1196, 632u [42.658° N, 45.159° E, 1450 m]; Shatili-Kuhweide, 1985.08.14, samples No 632, 633, 1197 [42.658° N, 45.159° E, 1450 m]. – **G e r m a n y**: Baidt: Kiesgrube, 2006.09.12, sample No ANA2 [46.836° N, 9.664° E, 495 m]; Baruth, 1965.08.27 [51.230° N, 14.590° E, 150 m]; Baruth: Schafberg, 2010.08.05, samples No 2, 3, 4 [51.232° N, 14.601° E, 165 m]; Baruth: Schafberg, 2010.05.09, samples No 782, 915 [51.232° N, 14.601° E, 165 m]; Baruth: Schafberg, 2010.05.23, [51.231° N, 14.601° E, 171 m]; Berzdorf: Hutberg, 1982, sample No 819 [51.055° N, 14.886° E, 280 m]; Bodensee: Gundholzen, 2005.10.08, sample No 240 [47.699° N, 8.963° E, 443 m]; Breitlohms, 1990.05.05 [48.695° N, 8.419° E, 970 m]; Dubringer Moor, 1982.08.09 / 12, sample No 1 [51.398° N, 14.163° E, 135m]; Engstingen, 2012.09.10, sample No GK458 [48.393° N, 9.258° E, 782 m]; Grambach: Karlstadt, 1991.05.03, sample No 225 [50.006° N, 9.774° E, 280 m]; Görlitz: Königshain-1 km N, 1989.08.01 [51.198° N, 14.851° E, 300 m]; Heldrungen, 1981.09.08 [51.300° N, 11.190° E, 130 m]; Hinterzarten-E, 2005.06.21 - 234 [47.908° N, 8.110° E, 880 m]; Hinterzarten-S, 2005.11.02 sample No 396 [47.983° N, 8.105° E, 940 m]; Kunnersdorf-1 km SE, 1983.05.21 [51.197° N, 14.947° E, 258 m]; Lenzkirch-W, 2005.09.20 - 122 [47.865° N, 8.170° E, 844 m]; Löbauer Berg, 1983.05.10-Basalt [51.090° N, 14.693° E, 420 m]; LöbauerBerg: S-Rand, 1983.05.10, sample No N1 + 2 [51.088° N, 14.699° E, 360 m]; Kreba: Spisk, 1989.08.06 [51.361° N, 14.714° E, 144 m]; NSG Dellenhäule, 2006.05.21 / 22, samples No 1 - 6, 8 [48.816° N, 10.241° E, 582 m]; Oppitz: Initialmoor, 2003.07.15, samples No 1 - 3 [51.296° N, 14.423° E, 131 m]; Reinheim-Zeilhard, 1991.06.02 - 035 [49.850° N, 8.780° E, 230 m]; Reichental-2 km ENE, 1990.05.05 [48.735° N, 8.415° E, 660 m]; Schmölln: FND Roter Berg, 1982 [50.930° N, 12.450° E, 230 m]; Sondershausen, 1983.05.29 [51.374° N, 10.834° E, 356 m]; Utzenfeld-N, 2005.07.28 - 92 [47.841° N, 7.863° E, 1100 m]; Wolmatinger Ried, 2006.07.17, sample No 6673WOL [47.674° N, 9.143° E, 393 m]; Wolmatinger Ried, 2006.11.10, sample No 7776WOL [47.703° N, 9.103° E, 395 m]; Würzburg: Dürrbach, 1991.05.27 - 068 [49.816° N, 9.915° E, 250 m]. – **K a z a k h s t a n**: Saur Mountains, 2001.07.28, sample No 272 [47.055° N, 84.924° E, 2000 m]; Saur Mountains: Matagul, 2001.07.28, samples No 123, 125, 126 [47.049° N, 84.914° E, 1655 m]; Tarbagatai Mts., 2001.08.05, samples No 306, 201 [47.059° N, 82.368° E, 2000 m]; Tarbagatai Mts.: Sarymobe, 2001.08.05, sample No 202a [47.130° N, 82.372° E, 1887 m]; Tarbagatai Mts.: Sarymobe, 2001.08.05, sample No 208 [47.132° N, 82.377° E, 1886 m]. – **K y r g y z s t a n**: Sary-Tschelek: Tos-Kol, 1998.07.22 [41.867° N, 71.983° E, 1600 m]. – **P o**

land: Izbica: T-Cross, 2006.08.22, samples No 78, 68 [54.658° N, 17.419° E, 2 m]. – Russia: Odinsk-4 km WSW, 2008.07.13, sample No 3 [52.400°N, 103.700°E, 466 m]. – Spain: Soria, 1987.05.27 [41.760° N, 2.460° W, 1100 m]. – Sweden: Krokstrand, 1986 [59.000° N, 11.433° E, 60 m]. – Switzerland: GR: Val Müstair, 2011.08.14 [46.633° N, 10.335°E, 1890 m]; Roche bogs, lectotype of *M. scabrinodis* var. *rugulosoides* [46.370° N, 6.920° E, 380 m]; Sur-1 km S: Mähwiese, 2010.07.15, samples No 17, 26 [46.514° N, 9.631° E, 1635 m]; Salategnas, 2010.07.12, samples No 31, 35, 37 [46.516° N, 9.651° E, 1960 m]. – Turkey: Ardesen, 2000.08.15, samples No TR123, TR132, TR127, TR129 [41.230° N, 42.440° E, 1800 m]; Göle-16SW, 1986.06.16 [40.698° N, 42.464° E, 1600 m]; Posof-5 kmS, 2012.07.17 - 297 [41.473° N, 42.741° E, 1900 m]; Keskin-3 km SE, 2012.07.25-308 [39.642° N, 33.624° E, 1120 m]. – Serbia: Jaile (=Jajce?), 1985.07.03 [putatively 44.330° N, 17.270° E, 1100 m].

### ***Myrmica spinosior* SANTSCHI, 1931**

30 nest samples with 83 workers were studied by CLM: France: Briancon, 1955.05 [44.90° N, 6.63° E, 1300 m]; Pyr.Or.: La Tour de Carol, 1966.06 [42.470° N, 1.880° E, 1400 m]; Val d'Oueil, 1929.08.20, lectotype of *M. reticulata* [42.804° N, 0.503° E, 1200 m]; Tourettes-sur-Loup, 1955.05, samples No 1, 3 [43.720° N, 7.060° E, 400 m]; Corsica: Zonza, 2009.04.15-b [41.733° N, 9.167° E, 780 m]. – Italy: Elba: Cavo (Aduorris), 1978.06 [42.860° N, 10.430° E, 6 m]; Firenze-70 km NW: Barga, 1997.08.20 [44.070° N, 10.480° E, 700 m]; Umbria: Montefalco 1993.06.20 / 30 [42.880° N, 12.650° E, 440 m]. – Spain: Aitana, 1984.03.23 [38.650° N, 0.270° W, 440 m]; Canfranc, 1966.06 [42.720° N, 0.530° W, 1100 m]; Castellon, 1978 [40.0° N, 0.0° E, 40 m]; Castellon, 1985.05 [40.0° N, 0.0° E, 40 m]; Castellon: Chodos, 1991.05.07, samples No 57, 42, 109 [40.253° N, 0.299° W, 1250 m]; Cuenca, 1983.05 [40.000° N, 2.000° W, 1000 m]; Cuenca, 1985.05.05 [40.0° N, 2.0° W, 1000 m]; Espot-1 km W, 1991.05.16, sample No 38 [42.588° N, 1.071° E, 1700 m]; Gerona, L'Estartit-1985.05, sample No 1 [42.530° N, 3.194° E, 9 m]; Irun-1926.03, lectotype of *M. spinosior* [43.330° N, 1.790° W, 250 m]; La Seu de Urgell, 1991.05.15, sample No 71 [42.379° N, 1.231° E, 1900 m]; La Seu de Urgell, 1991.05.15, sample No 158 [42.366° N, 1.262° E, 1400 m]; Leon: Molinaferrera Sp 567 [42.400° N, 6.370° W, 1100 m]; Leon: Redipollos, 1989.05 [43.000° N, 5.260° E, 1150 m]; M. Universali: Frias: Albarracin [40.340° N, 1.620° W, 1400 m]; Puerto de Navacerrada, 1991.05.14, sample No 123 [40.853° N, 4.027° W, 1250 m]; Sella Alicante, 1984 [38.610° N, 0.270° W, 400 m]; Teruel, 1983.05 [40.350° N, 1.100° W, 1000 m].

### **Type material investigated**

***Myrmica scabrinodis* var. *rugulosoides* FOREL, 1915:** Lectotype worker together with a paralectotype worker on the same pin labeled "Typus", "LECTOTYPE (bottom specimen) desig. Radchenko & Elmes 2000" [published by RADCHENKO & ELMES 2010], "Tourbières de Roche" [Forels handwriting], "*Myrmica scabrinodis* Nyl. v. *rugulosoides* For. type" [Forels handwriting], "ANTWEB CAS-ENT0907654"; 3 paralectotype workers on a single pin labeled "Typus, PARALECTOTYPE desig. Radchenko & Elmes 2000, Tourbières de Roche [Forels handwriting],

*Myrmica scabrinodis* Nyl. v. *rugulosoides* For. type" [Forels handwriting], all MHN Genève.

***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"; IRSNB Bruxelles.

***Myrmica sabuleti* var. *spinosior* SANTSCHI, 1931:** Lectotype worker labeled "Typus, *M. sabuleti* v. *spinosior* Sant, Pyren. occ. Irun 25 - 26 3. 1926 Lindberg, LECTOTYPE desig. Radchenko & Elmes 2002" [published by SEIFERT 2005], NHM Basel. 2 paralectotype workers on one pin, labeled "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.

***Myrmica rolandi* var. *reticulata* STÄRCKE, 1942:** The lectotype sample of 8 workers has been mounted by Santschi on a single pin in a way not allowing a reasonable investigation. Accordingly, the 4 best conserved specimens were washed off, cleaned and remounted on a single pin labeled "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" [lectotype by present designation: top specimen with CW = 1.237 mm and FL / FR 1.377 ], "SYNTYPE desig. Radchenko & Elmes 2002"; 4 workers, placed on another pin, remained in Santschi's original mounting and got laser-printed labels "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"; 3 males, 1 gyne, labeled "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"; 3 males, 1 gyne, labeled only "Vol nuptial mixte" [Santschi's handwriting] and "*M. scabrinodis rolandi*" [Santschi's handwriting]; all material in NHM Basel.

Comments: In his original description, Santschi gave the following information on type localities: "Pyrénées centrales, Cirque d'Espigno, 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.)". To explain geography, Haute Garonne is a region around Toulouse including the Central Pyrenees, Val de Oueil (Val Astos) is a valley in the Pyrenees approximately at 42.83° N, 0.54° E, Commune d'Oô is a village in the Pyrenees at 42.796° N, 0.506° E, 966 m and Cirque d'Espigno (or "Espigno") is situated at 42.728° N, 0.499° E and 1920 m.

RADCHENKO & ELMES (2010) published the following lectotype designation: "male (upper specimen on the pin with 3 males and 1 queen), *M. scabrinodis rolandi* Bondr.

*var. reticulata* Sant [very hardly readable text, most probably as follows] M<sup>o</sup> Espigno, Val d'Oueu, Ha Garon., 1200 m, Kerville 20.VIII.29."

This lectotype could not be identified by an existing label. Santschi's original labeling cited by Radchenko and Elmes is exactly that of the 8 workers designated by us as lectotype and paralectotypes. Apparently, Radchenko and Elmes recorded the labels correctly but confused which castes were associated with which labels. The statements in Santschi's original description also disagree with the situation found in the collection and the text on the lectotype label is a disagreement in itself: Mt. Espigno is at 1920 and not at 1200 m. The smallest mismatch between Santschi's statements and the labeling is found in the worker series in which at least the name of location, elevation and month of collection agree. Accordingly, we selected and labeled as lectotype a worker from the nest sample from Val d'Oueil collected at an elevation of 1200 m. Furthermore, selecting a male as primary type specimen in *Myrmica* would be a wrong decision because the delimitation of closely related species is usually most difficult and in many cases unsolved just in this caste.

## Methods

An average of three mounted workers per sample was investigated by two-dimensional geometric morphometrics and conventional linear morphometrics. The number of investigated workers was increased to six in problematic samples.

**Geometric morphometrics:** The methodology of geometric morphometrics, including optical equipment, digitizing, symmetrizing, Generalized Procrustes Analysis, calculating deformation grids and the used software packages are described elsewhere (BAGHERIAN YAZDI & al. 2012). We fixed 41 landmarks and 252 semilandmarks in the clypeus, head capsule, mesosoma and petiole aspects and extracted a total of 316 relative warps. MorphoJ software version 1.05a (KLINGENBERG 2011) was used to calculate and to display the shape changes related to the LDA scores as wireframe graphs. The basic requirements for geometric analysis were fulfilled in the investigated material:

a) Variation of the specimens in shape space was perfectly correlated with tangent space for all anatomical aspects.

b) Centroid sizes of both species were normally distributed in all anatomical aspects (Asymp. Sig. > 0.05) and means of centroid sizes were significantly different between the species in all anatomical aspects ( $p < 0.001$ ).

c) No directional asymmetry was demonstrable in the investigated ants with any test system applied.

**Conventional linear morphometrics:** The optical equipment used, the character recording methods and estimation of measuring errors are given in SEIFERT (2011). The definitions for 19 characters are as follows.

CL Maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of posterior head margin 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 dorsal and ventral margins

of excision superimpose. This is usually given after tilting the head by  $\pm 45^\circ$  from dorsal towards the frontal viewing position (dorsofrontal view). The assumed surface is a compromise between valleys and peaks of sculpture.

- CS Cephalic size; arithmetic mean of CL and CW, used as a less variable indicator of body size.
- CW Maximum cephalic width; in *Myrmica* this is always across the eyes.
- EYE Eye-size: arithmetic mean of large (EL) and small diameter (EW) of the elliptic compound eye.
- FL Maximum anterior divergence of frontal carinae (= maximum frontal lobe width). In specimens with frontal carinae parallel or converging frontad FL is not defined, then FL = FR.
- FR Minimum distance between frontal carinae. In specimens with parallel frontal carinae or ones converging frontad FR is not defined; FR then is measured at the level of the centre of frontal triangle.
- MetL Height of metapleuron including propodeal lobe measured in lateral view perpendicular to straight section of metapleuro-coxal border. The lower endpoint of measuring line is the metapleuro-coxal border and the upper one the upper margin of propodeal lobe. The level of the measuring line is positioned in the middle between the frontalmost point of subspinal excavation and the caudalmost point of propodeal lobe (see SEIFERT & al. 2009: fig. 1).
- MetSp Height of subspinal excavation from upper margin of propodeal lobe to lower spine margin measured along dorsal continuation of measuring line for MetL.
- PEH Maximum petiole height measured perpendicular to a reference line defined as follows: The frontal endpoint of the reference line is marked by the centre of the petiole-propodeal junction and the caudal endpoint by the centre of petiole-postpetiolar junction (see SEIFERT & al. 2009: fig. 2).
- PEL Maximum measurable diagonal petiole length from tip of subpetiolar process to dorsocaudal corner of caudal cylinder. (Do not confuse this with the corner of the movable inner sclerite.)
- PEW Maximum width of petiole.
- 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 (see SEIFERT & al. 2009: fig. 3).
- PPHL Length of longest hair on dorsal postpetiole.
- PPW Maximum width of postpetiole.
- SL Maximum straight line scape length. Distal measuring point: most distal point of dorsal lamella of hinge joint capsula. Proximal measuring point: most proximal point of scape shaft near neck of articular condyle. Note that the border region between shaft and condylar neck is usually asymmetric. To measure the real maximum, avoid positions near to SVP C; instead use positions between the SVPs F and D! In species with basal scape lobes or dents (e.g., *M. schencki*, *M. scabrinodis* and *M. lobicornis* group) the lobes are excluded from measurement!

Tab. 1: Worker nest sample means of RAV-corrected data of Conventional Linear Morphometrics given as arithmetic mean  $\pm$  standard deviation [lower extreme, upper extreme]. F values and significance levels p are from an univariate ANOVA; the F values of the most separating characters are given in heavy type.

	<i>M. martini</i> (n = 23)	ANOVA F; p	<i>M. scabrinodis</i> (n = 89)	ANOVA F; p	<i>M. spinosior</i> (n = 30)
CL / CW (1150)	1.020 $\pm$ 0.017 [0.986, 1.055]	14.81 0.0001	1.032 $\pm$ 0.014 [0.983, 1.064]	n.s.	1.035 $\pm$ 0.019 [0.999, 1.082]
ClyEx / CS [%] (1150)	0.68 $\pm$ 0.20 [0.18, 1.17]	n.s.	0.76 $\pm$ 0.19 [0.12, 1.20]	7.76 0.006	0.62 $\pm$ 0.36 [0.00, 1.27]
CS [ $\mu$ m]	1089 $\pm$ 45 [1027, 1175]	n.s.	1079 $\pm$ 57 [953, 1198]	<b>95.44</b> 0.0001	1200 $\pm$ 64 [1083, 1342]
EYE (1150)	0.202 $\pm$ 0.003 [0.196, 0.208]	n.s.	0.201 $\pm$ 0.005 [0.189, 0.213]	81.72 0.0001	0.192 $\pm$ 0.005 [0.184, 0.207]
FL / CS (1150)	0.427 $\pm$ 0.008 [0.412, 0.444]	<b>69.84</b> 0.0001	0.449 $\pm$ 0.012 [0.425, 0.493]	21.83 0.0001	0.438 $\pm$ 0.011 [0.413, 0.459]
FL / FR (1150)	1.287 $\pm$ 0.040 [1.189, 1.372]	<b>83.37</b> 0.0001	1.415 $\pm$ 0.064 [1.317, 1.589]	30.48 0.0001	1.340 $\pm$ 0.067 [1.239, 1.469]
FR / CS (1150)	0.332 $\pm$ 0.009 [0.312, 0.351]	30.80 0.0001	0.317 $\pm$ 0.012 [0.286, 0.352]	16.742 0.0001	0.328 $\pm$ 0.013 [0.307, 0.351]
MetL / CS (1150)	0.237 $\pm$ 0.006 [0.222, 0.248]	4.32 0.04	0.240 $\pm$ 0.007 [0.225, 0.255]	59.12 0.0001	0.228 $\pm$ 0.009 [0.202, 0.246]
MetSp / CS (1150)	0.159 $\pm$ 0.005 [0.150, 0.170]	7.49 0.007	0.163 $\pm$ 0.008 [0.143, 0.187]	<b>226.91</b> 0.0001	0.190 $\pm$ 0.010 [0.174, 0.207]
MetSp / MetL (1150)	0.670 $\pm$ 0.027 [0.622, 0.723]	n.s.	0.681 $\pm$ 0.031 [0.579, 0.756]	<b>272.3</b> 0.0001	0.835 $\pm$ 0.062 [0.711, 1.004]
PEH / CS (1150)	0.332 $\pm$ 0.008 [0.309, 0.347]	n.s.	0.333 $\pm$ 0.009 [0.313, 0.358]	n.s.	0.329 $\pm$ 0.011 [0.314, 0.363]
PEL / CS (1150)	0.476 $\pm$ 0.008 [0.463, 0.494]	n.s.	0.473 $\pm$ 0.013 [0.444, 0.511]	36.52 0.0001	0.489 $\pm$ 0.013 [0.452, 0.523]
PEW / CS (1150)	0.271 $\pm$ 0.009 [0.258, 0.292]	n.s.	0.276 $\pm$ 0.010 [0.254, 0.296]	n.s.	0.276 $\pm$ 0.013 [0.250, 0.314]
PoOc / CL (1150)	0.421 $\pm$ 0.007 [0.408, 0.435]	17.06 0.0001	0.428 $\pm$ 0.008 [0.413, 0.449]	10.13 0.02	0.433 $\pm$ 0.007 [0.419, 0.448]
PPHL / CS (1150)	0.175 $\pm$ 0.006 [0.158, 0.186]	<b>39.18</b> 0.0001	0.164 $\pm$ 0.008 [0.146, 0.180]	46.60 0.0001	0.176 $\pm$ 0.009 [0.160, 0.196]
PPW / CS (1150)	0.405 $\pm$ 0.012 [0.379, 0.431]	n.s.	0.405 $\pm$ 0.013 [0.378, 0.438]	16.88 0.0001	0.394 $\pm$ 0.013 [0.367, 0.427]
SL / CS (1150)	0.776 $\pm$ 0.010 [0.755, 0.799]	16.08 0.0001	0.787 $\pm$ 0.012 [0.763, 0.814]	<b>197.06</b> 0.0001	0.828 $\pm$ 0.017 [0.800, 0.861]
SP / CS (1150)	0.378 $\pm$ 0.021 [0.339, 0.419]	10.08 0.002	0.395 $\pm$ 0.022 [0.335, 0.443]	15.17 0.001	0.376 $\pm$ 0.024 [0.337, 0.426]
SW / SL (1150)	0.141 $\pm$ 0.009 [0.116, 0.154]	26.22 0.0001	0.153 $\pm$ 0.010 [0.132, 0.177]	n.s.	0.154 $\pm$ 0.012 [0.136, 0.187]
tan $\alpha$ (1150)	0.949 $\pm$ 0.119 [0.73, 1.09]	6.33 0.013	0.865 $\pm$ 0.142 [0.44, 1.14]	<b>175.02</b> 0.0001	0.463 $\pm$ 0.152 [0.13, 0.91]

SP Maximum length of propodeal spines as bilateral arithmetic mean. Measured in dorsofrontal view from spine tip to a point at bottom of interspinal meniscus (see SEIFERT & al. 2009: fig. 4). With the spines' dorsal edge in measuring plane, the spine tip must be focused at a magnification with low depth of focus. Then, while keeping this focusing, the sharpest point at the bottom of interspinal meniscus is the basal measuring point. 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 of scape defined by their position relative to the moving plane of the hinge joint between scape and first funiculus seg-

ment (see SEIFERT & al. 2009: fig. 6). 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 within the moving plane and perpendicular to the longitudinal scape axis – i.e., when the scape is imagined to be directed strictly laterad from head, SVP F is the frontal and SVP C the caudal aspect of scape. SVPs such as CD and DF describe intermediate viewing positions. SVP L is the view along the longitudinal axis of scape from its distal to proximal end.

**SW** Worker and gyne: maximum distance between caudal margin of basal scape lobe and anterior scape margin measured perpendicular to longitudinal scape axis. The scape must be carefully tilted until the maximum distance is situated in the measuring plane. This is given in approximately SVP DF. In cases when maximum SW would be measured proximal of basal break point of anterior scape margin, a point on an imagined linear continuation of the anterior scape margin distal of the break is used as anterior measuring point.

**tan  $\alpha$**  The tangens of the angle alpha under which the basal scape lobe slopes caudad relative to the plane in which SVP L or SVP C are running. The angle is measured in SVP L in the following way. Focusing the distal scape end, the horizontal axis of the cross-scaled ocular micrometer (x-axis) is aligned with the plane of SVP L and C. Then, after focusing down to the level of basal scape lobe without changing the former adjustment, the y- and x-values (or opposite and adjacent legs) of tangens  $\alpha$  can be read in the cross-scale ocular micrometer. This is done in both scapes and needs some training. See also figures in SEIFERT (2007) and SEIFERT & al. (2009).

**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 (Tab. 1), a removal of allometric variance (RAV) was performed with 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. It can be seen from the functions below that allometries are rather weak and usually less than 5% per 400  $\mu$ m CS change (this is the average intraspecific size difference between the smallest and largest worker). However, SP / CS grows by 10.4% and ClyEx / CS by 27% from smallest to largest workers. The RAV functions were as follows

$$\begin{aligned} \text{CL} / \text{CW}_{1.15} &= \text{CL} / \text{CW} / (-0.0487 * \text{CS} + 1.0900) * 1.0340 \\ \text{SL} / \text{CS}_{1.15} &= \text{SL} / \text{CS} / (-0.0802 * \text{CS} + 0.8947) * 0.8024 \\ \text{EYE} / \text{CS}_{1.15} &= \text{EYE} / \text{CS} / (0.0179 * \text{CS} + 0.1772) * 0.1978 \\ \text{FL} / \text{CS}_{1.15} &= \text{FL} / \text{CS} / (0.0227 * \text{CS} + 0.4036) * 0.4297 \\ \text{FR} / \text{CS}_{1.15} &= \text{FR} / \text{CS} / (0.0095 * \text{CS} + 0.3222) * 0.3331 \\ \text{PEW} / \text{CS}_{1.15} &= \text{PEW} / \text{CS} / (-0.0201 * \text{CS} + 0.2842) * 0.2610 \\ \text{PPW} / \text{CS}_{1.15} &= \text{PPW} / \text{CS} / (0.0709 * \text{CS} + 0.3208) * 0.4024 \\ \text{PEH} / \text{CS}_{1.15} &= \text{PEH} / \text{CS} / (0.0051 * \text{CS} + 0.3230) * 0.3288 \\ \text{PEL} / \text{CS}_{1.15} &= \text{PEL} / \text{CS} / (-0.0259 * \text{CS} + 0.4975) * 0.4677 \\ \text{PPHL} / \text{CS}_{1.15} &= \text{PPHL} / \text{CS} / (-0.0679 * \text{CS} + 0.2535) * 0.1754 \\ \text{SP} / \text{CS}_{1.15} &= \text{SP} / \text{CS} / (0.0876 * \text{CS} + 0.2353) * 0.3360 \\ \text{MetL} / \text{CS}_{1.15} &= \text{MetL} / \text{CS} / (-0.0126 * \text{CS} + 0.2487) * 0.2342 \\ \text{MetSp} / \text{CS}_{1.15} &= \text{MetSp} / \text{CS} / (-0.0113 * \text{CS} + 0.1880) * 0.1749 \\ \text{PoOc} / \text{CL}_{1.15} &= \text{PoOc} / \text{CL} / (0.0098 * \text{CS} + 0.4154) * 0.4266 \\ \text{SW} / \text{SL}_{1.15} &= \text{SW} / \text{SL} / (0.0109 * \text{CS} + 0.1385) * 0.1511 \\ \tan \alpha_{1.15} &= \tan \alpha / (0.391 * \text{CS} + 0.199) * 0.649 \\ \text{ClyEx} / \text{CS}_{1.15} &= \text{ClyEx} / \text{CS} / (-0.460 * \text{CS} + 1.193) * 0.664 \\ \text{FL} / \text{FR}_{1.15} &= \text{FL} / \text{FR} / (-0.0572 * \text{CS} + 1.4270) * 1.3612 \end{aligned}$$

All linear discriminant analyses were run with the SPSS 16.0 software package.

**The applied species concept:** We applied the species concept of DE QUEIROZ (2007). As delimitation criterion we used phenotypic distinctness. The decision threshold in favour of heterospecificity was that the discriminant analysis controlling the explorative data analysis must not place more than 2% of the classified samples in the inter-specific overlap range.

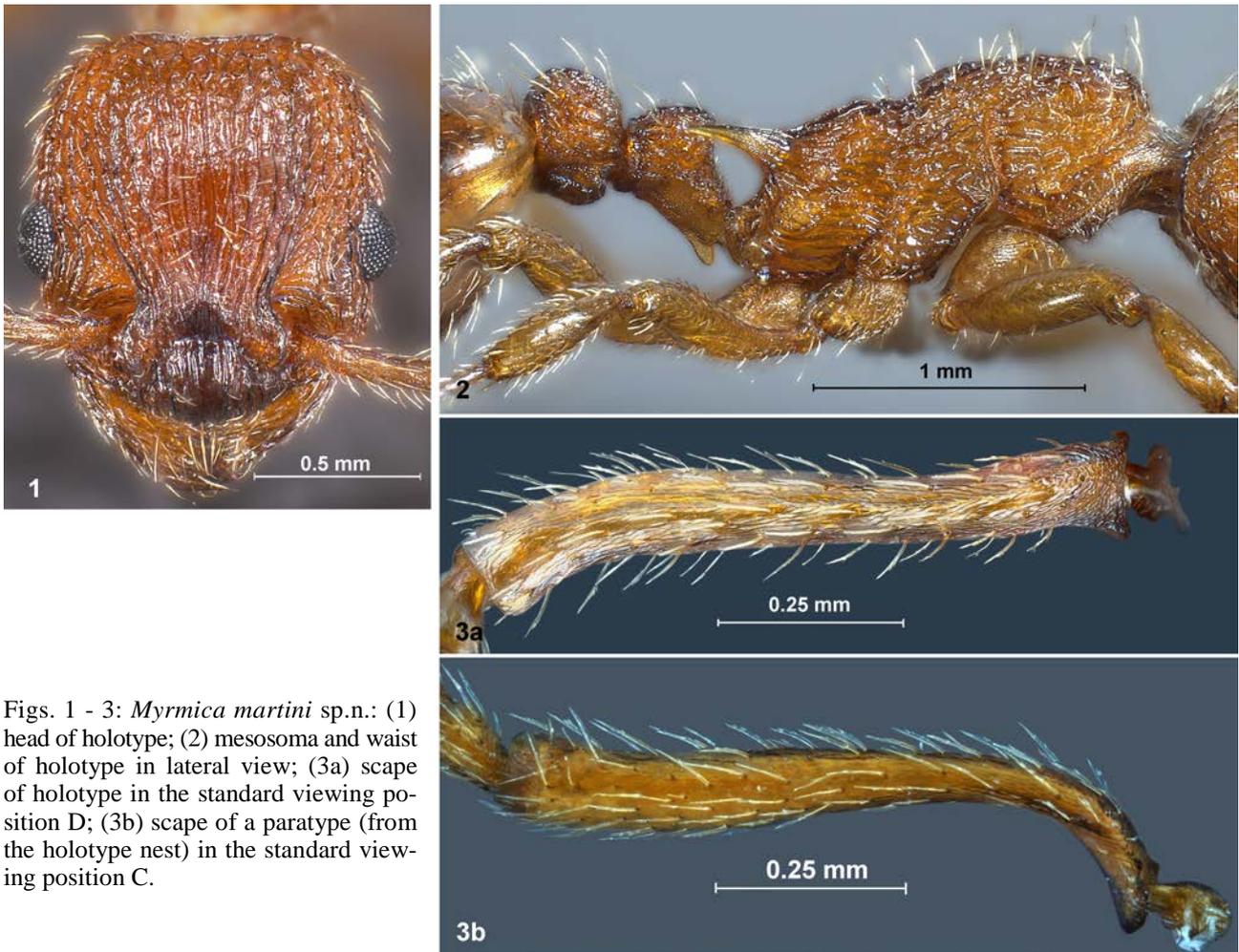
## Results and Discussion

### *Myrmica martini* sp.n.

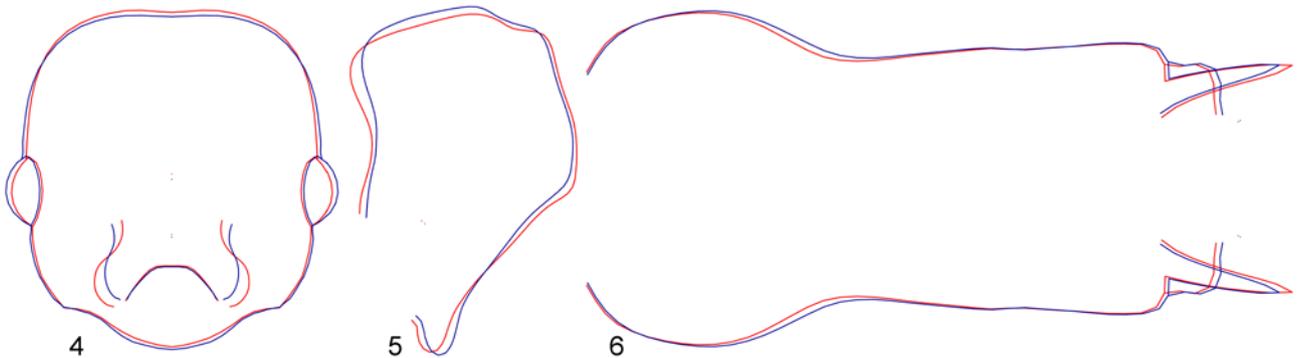
**Etymology:** Named after the titular Saint of the locality Saint-Martin-Vésubie situated close to the locus typicus.

**Type material:** Holotype worker labeled "FRA: 44.1002° N, 7.2332° E St.-Martin-Vésubie-3.8NNW 1629 m, Larix-Pinus, clearing, Schultz 2002.05.15 – 126" and "Holotype *Myrmica martini* Seifert & al."; 8 worker paratypes on three other pins and 120 worker paratypes in ethanol with identical locality labels and "Paratype *Myrmica martini* Seifert & al."; all material stored in Senckenberg Museum of Natural History Görlitz.

**Description:** Worker (Figs. 1 - 6, Tab. 1, all morphometric ratios given in the following verbal description are arithmetic nest sample means of primary data – i.e., without removal of allometric variance): *Myrmica martini* sp.n. seems to lack any exposed morphological character and is most similar to *M. scabrinodis*. Medium-sized (CS 1089  $\mu$ m). Head with a straight posterior margin and strongly convex sides and not elongated (CL / CW 1.023), postocular distance rather low (PoOc / CL 0.420). Frontal lobes moderately diverging (FL / CS 0.426) and frontal width comparably large (FR / CS 0.331) in terms of related species, frontal carinae reaching caudad only to level of eyes. Eyes with few microsetae and medium-sized (EYE / CS 0.200). Anterior clypeal margin in dorsofrontal view not or only feebly emarginated (ClyEX / CS 0.71%). Scape moderately long (SL / CS 0.781), with a clearly developed dorsal and caudal carina at base – the plane demarcated by these carinae form a caudoventral slope by an angle of more or less 44° (tan  $\alpha$  0.949) and is not very wide (SW / SL 0.140), scape base in caudal view varying from curved (Fig. 3a) to almost angular. The convexity of dorsal mesosomal profile is interrupted by a rather deep metanotal depression. Propodeal spines acute, moderately long (SP / CS 0.372), their axes in dorsal view only diverging by 30 - 34°, in lateral view weakly erected, deviating from longitudinal mesosomal axis by 25 - 30°. Central height of propodeal lobe clearly larger than equal-level height of subspinal excavation (MetL / CS 0.237, MetSp 0.159). Petiole in lateral view with almost straight dorsal profile that slopes caudad with only a suggested step, dorsal and frontal profile of petiole node form an angle of 90°. Petiole in dorsal view with weakly convex sides, its width about 67% of postpetiolar width. Setae are present on all dorsal parts of body, those on dorsum of postpetiole are moderately long (PPHL / CS 0.180). Head including clypeus and mesosoma with rather strong longitudinal rugosity, about 12 - 14 rather linear rugae are found between the most approximated parts of frontal carinae. Whole body usually rather uniformly medium brown with a weak yellowish component and sometimes with a lighter mesosoma. Ecology and distribution: found in submontane to subalpine grassland



Figs. 1 - 3: *Myrmica martini* sp.n.: (1) head of holotype; (2) mesosoma and waist of holotype in lateral view; (3a) scape of holotype in the standard viewing position D; (3b) scape of a paratype (from the holotype nest) in the standard viewing position C.



Figs. 4 - 6: Wireframe graphs of *Myrmica martini* sp.n. (blue lines) and of *M. scabrinodis* (red lines) with interspecific differences exaggerated by the factor three. (4) Head shape; (5) petiole shape; (6) mesosoma shape.

or grassy woodland clearings of the Pyrenees and French Alps (Fig. 7). Nests are under stones or in grass tussocks.

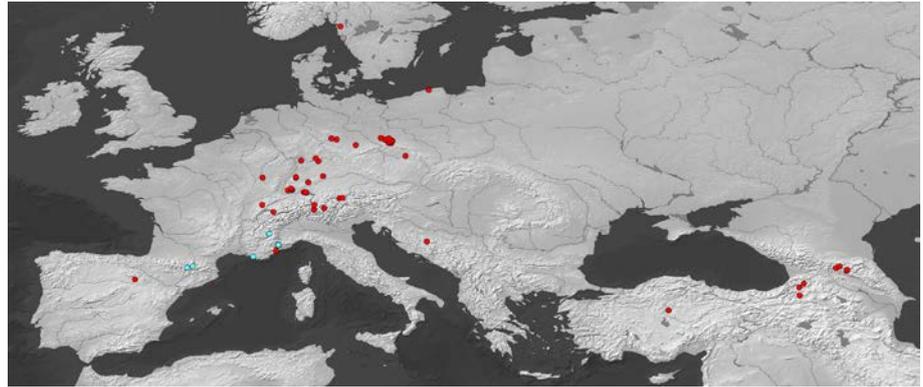
**Biology:** unknown.

**Differential diagnosis derived from geometric and conventional morphometrics**

Both geometric morphometrics (GM) and conventional linear morphometrics (CLM) showed that *Myrmica scabrinodis* and *M. martini* sp.n. are extremely similar. The wire frame graphs exaggerated by a factor of three (Figs. 4 - 6) revealed only very few and often minute interspecific dif-

ferences: *Myrmica martini* sp.n. has a significantly smaller frontal lobe distance, a relatively wider head, a larger minimum frons width, a shorter postocular distance and shorter propodeal spines. All these differences are also indicated by CLM (Tab. 1). In lateral aspect of petiole, *M. martini* sp.n. shows a shorter petiole node relative to petiole height, the angularity between the dorsal and anterior profile is less distinct, the dorsal plane of petiole is shorter and slopes down to caudal cylinder without a distinct step. Furthermore, *M. martini* sp.n. has significantly longer setae on dorsal postpetiole (Tab. 1). The shape of basal scape in

Fig. 7: Sampling sites of *Myrmica martini* sp.n. (blue dots) and of *M. scabrinodis* (red dots) in the Euro-caucasian range.



Tab. 2: Three diagnostic characters to distinguish taxa of the *Myrmica scabrinodis* and *M. sabuleti* species complex given as mean of the holo/lectotype samples – the profile of petiole, the sloping angle  $\alpha$  of basal scape lobe and the ratio between height of subspinal excavation and height of metapleural lobe.

Holo/lectotype sample	Dorsum of petiole node	$\tan \alpha$	MetSp/MetL
<i>M. martini</i> sp.n.	truncate	large: 0.955	small: 0.670
<i>M. s. var. rugulosoides</i>	truncate	large: 0.668	small: 0.699
<i>M. pilosiscapus</i>	truncate	large: 0.787	small: 0.689
<i>M. spinosior</i>	slightly convex	small: 0.427	large: 0.855
<i>M. r. var. reticulata</i>	slightly convex	small: 0.387	large: 0.953

SVP C is no reliable discriminator from *M. scabrinodis* because this character varies in *M. martini* from nearly curved (Fig. 3a) to almost angular (comparable to the situation in *M. scabrinodis*). In the sections below we present conclusive data-based evidence that *M. martini* sp.n. is no synonym of five morphologically similar taxa.

#### Demonstration of distinctness of *Myrmica spinosior* from members of the *M. scabrinodis* complex

*Myrmica spinosior* SANTSCHI, 1931 and its junior synonym *M. rolandi* var. *reticulata* STÄRCKE, 1942 (first available use of *M. scabrinodis rolandi* var. *reticulata* SANTSCHI, 1931) have been described from the Pyrenees and we show in the following that both are not synonyms of *M. martini* sp.n. *Myrmica spinosior*, *M. sabuleti* MEINERT, 1861 and *M. lonae* FINZI, 1926 together form the *M. sabuleti* species complex (SEIFERT 2005). In the normal situation, the workers of the *M. sabuleti* complex can be subjectively separated from *M. martini* sp.n. and *M. scabrinodis* by a higher and not truncate petiolar node, a smaller sloping angle of basal scape lobe  $\alpha$  and a higher subspinal excavation (MetSp) relative to the height of metapleural lobe (MetL). These differences are also expressed in the lectotype samples of *M. spinosior*, *M. rolandi* var. *reticulata*, *M. scabrinodis* var. *rugulosoides* and *M. pilosiscapus* (Tab. 2) but many, even experienced, observers have difficulties to recognize these useful traits. Accordingly, there is a need to apply more complex numeric approaches to demonstrate the differences in the workers convincingly.

We had no data of geometric morphometrics at hand for *Myrmica spinosior* but there are enough samples with data of conventional linear morphometrics. A three-class LDA considering all 19 characters provided a full separation of the *M. spinosior* cluster from the *M. martini* and *M. scabrinodis* clusters (Fig. 8). All *M. spinosior* samples had

a posterior probability of belonging to this cluster of  $p > 0.970$  with the lectotype samples of *M. spinosior* and *M. reticulata* showing  $p = 0.9994$  and  $p = 1.0000$  respectively. All *M. martini* and *M. scabrinodis* samples had a posterior probability of belonging to the *M. spinosior* cluster of  $p < 0.032$  with the lectotype samples of *M. scabrinodis* var. *rugulosoides* and *M. pilosiscapus* showing  $p = 0.0000$  and  $p = 0.0316$ . Figure 8 shows some 2% of doubtful allocations between *M. martini* and *M. scabrinodis* with this character system and type of analysis. This problem is treated in the next section by more advanced geometric analyses. A full separation of *M. spinosior* from *M. martini* and *M. scabrinodis* is also provided by a plot of the 1<sup>st</sup> and 2<sup>nd</sup> components of a PCA (not shown). Accordingly, the separate identity of *M. spinosior* is clearly confirmed by both

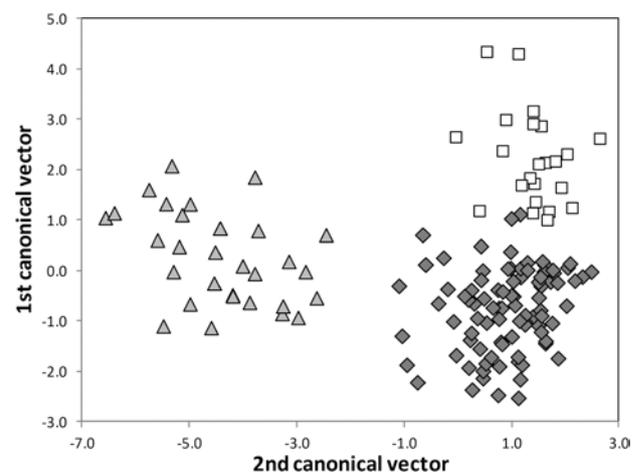


Fig. 8: Nest sample means of a canonical variance analysis of *Myrmica spinosior* (grey triangles), *M. scabrinodis* (grey rhombs) and *M. martini* sp.n. workers (white squares).

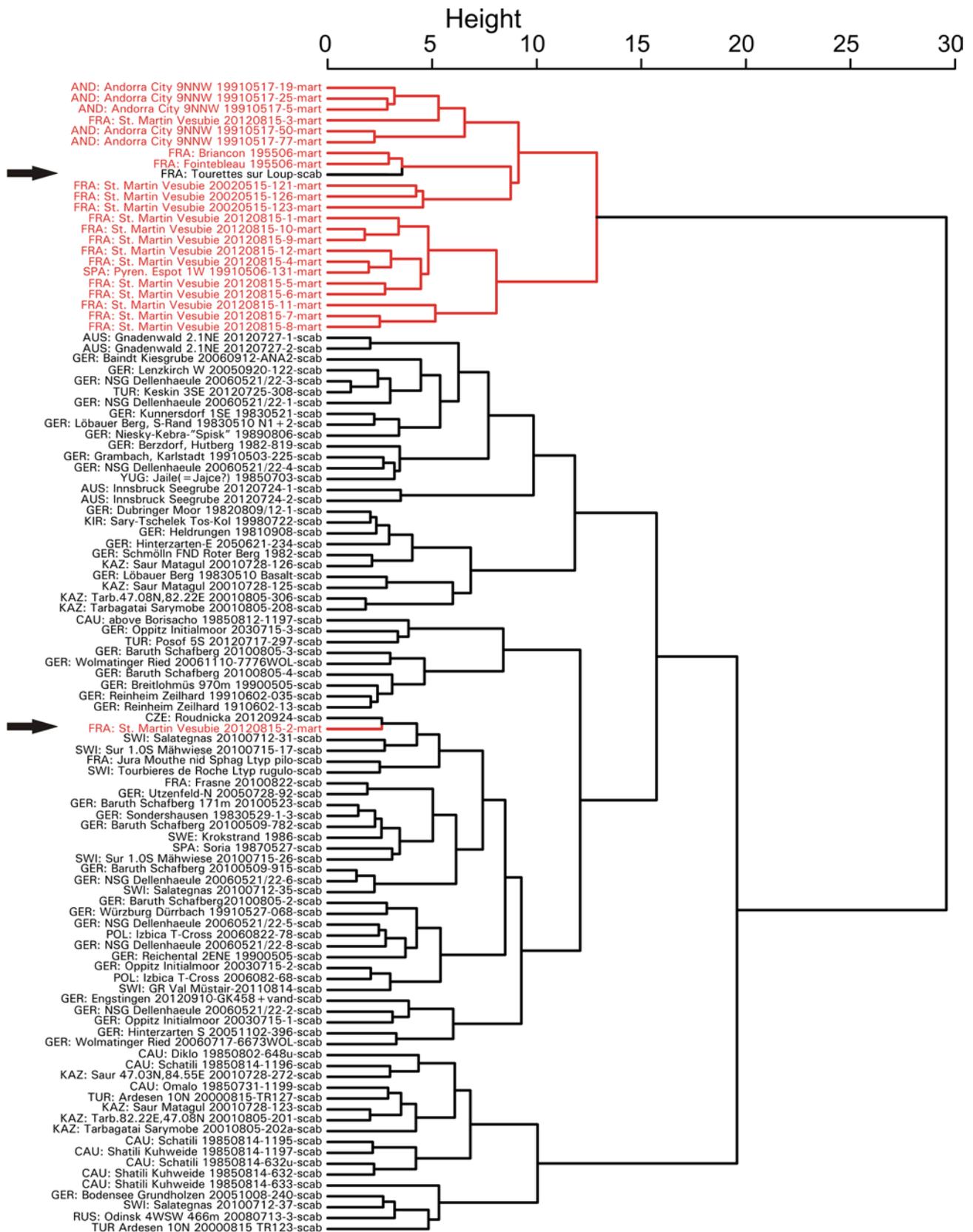


Fig. 9: NC-Ward clustering of the first ten relative warps of *Myrmica martini* sp.n. (red) and *M. scabrinodis* (black). Arrows point to misplaced samples which are rectified if run as wild-cards in a controlling linear discriminant analysis.

explorative and hypothesis-driven data analyses and we can also state here that *M. r. var. reticulata* is a junior synonym of *M. spinosior*.

#### **Separation of *Myrmica martini* from *M. scabrinodis* by geometric morphometrics and NC-clustering**

**a) NC-clustering considering the first ten Relative Warps:** Geometric morphometrics (GM) was performed in a total of 23 nest samples with 84 worker specimens of *Myrmica martini* sp.n. and 83 samples with 252 workers of *M. scabrinodis*. The GM analysis extracted a total of 316 Relative Warps (RWs). NC-clustering considering all 316 RWs did not show a clear and reasonable structure. Therefore, we restricted the analysis to the first ten RWs which describe 63.3% of variance in the data set. NC-Ward clustering of RWs 1 - 10 showed two clearly different main branches (Fig. 9) and the controlling LDA was run with this hypothesis – with exception of a wild-card setting in the sample St.-Martin-Vésudie-No 2. NC-Ward positioned this sample within *M. scabrinodis* but there was a strong geographic counter-indication because all samples from this spot should belong to *M. martini*. The two-class LDA changed the classification of St.-Martin-Vésudie-No 2 to *M. martini* but also that of the sample Tourettes-sur-Loup 1955.06 to *M. scabrinodis*. As a consequence, the clustering error of NC-Ward on the two-class level was 1.9% if the LDA indication is considered as the deciding system. The lectotype series of *M. scabrinodis* var. *rugulosoides* and of *M. piloscapus* were clearly allocated by the LDA to *M. scabrinodis* with the sample means of posterior probabilities being  $p = 0.960$  and  $p = 0.973$  respectively.

NC-Ward clustering suggested the existence of three groups – *Myrmica martini* and two entities within *M. scabrinodis*. Accordingly, we ran the controlling method NC-K-means clustering assuming three groups. This run showed an error of 0% against the two-class LDA classification: no *M. martini* sample was allocated to one of the two *M. scabrinodis* clusters and, conversely, no *M. scabrinodis* sample to *M. martini*. Hence, the agreement of NC-K-means and NC-Ward was 98.1% on the two-class level.

**b) NC-clustering considering the most diagnostic Relative Warps:** Taking the hypotheses formed above and in order to possibly improve the success of NC-clustering, we ran a stepwise LDA considering all 316 RWs. This resulted in a reduction to 14 RWs: 1, 2, 4, 7 - 10, 12, 14, 18, 22, 23, 25 and 28. Considering these 14 characters, NC-Ward clustering (Fig. 10) and the controlling LDA agreed by 100% – even the samples St.-Martin-Vésudie-No 2 and Tourettes-sur-Loup 1955.06 were correctly placed by the explorative data analysis. The lectotype series of *Myrmica scabrinodis* var. *rugulosoides* and of *M. piloscapus* were clearly allocated by the LDA to *M. scabrinodis* with the nest means of posterior probabilities being  $p = 0.996$  and  $p = 0.999$ .

Similar to the analysis with RWs 1 - 10, NC-Ward clustering suggested the existence of three groups and we ran NC-K-means clustering under this assumption. The run showed an error of 0% against the two-class LDA classification: no *Myrmica martini* sample was allocated to one of the two *M. scabrinodis* clusters and, conversely, no *M. scabrinodis* sample to *M. martini*. Accordingly, the agreement of NC-K-means, NC-Ward and LDA was 100% on the two-class level. A leave-one-out-cross-validation LDA

showed the same result which is expected as the number of cases in the smallest class ( $n = 84$  in *M. martini*) was sixfold larger than the number of considered characters. Over all samples, the LDA classified 95.9% of the worker individuals correctly with 82.3% of individuals being classified with posterior probabilities of  $p > 0.95$ .

#### **Conventional linear morphometrics**

FL / FR is clearly the most discriminative shape component of conventional linear morphometrics (Tab. 1). Yet, even the best character is weak: on the worker individual level and without removal of allometric variance, 57.2% of specimens are found in the interspecific overlap range. This illustrates the extreme similarity of *Myrmica martini* sp.n. and *M. scabrinodis*. We used the species hypothesis found by geometric morphometrics (GM) as input for a LDA considering all 19 characters of conventional linear morphometrics (CLM). NC Ward clustering (Fig. 11) classified 2.8% of the samples in disagreement with the classification by GM (Fig. 10). The LDA confirmed these three classifications, however with insignificant posterior probabilities: St.-Martin-Vésudie No 2 was classified as *M. scabrinodis* ( $p = 0.556$ ) whereas Tourettes-sur-Loup 1955.06 and Sur-2010.07.15 No 17 were changed to *M. martini* ( $p = 0.821$  and  $p = 0.632$ ). The type samples of *M. piloscapus* and *M. s. rugulosoides* were allocated to the *M. scabrinodis* cluster with  $p = 0.988$  and  $p = 0.671$ . Over all samples and after accepting those three changes, the LDA classified 94.2% of the worker individuals correctly with 71.0% of individuals being classified with posterior probabilities  $> 0.95$ .

A stepwise LDA reducing the number of characters to seven (SL / CS, FL / CS, FR / CS, PPHL / CS, PoOc / CL, SW / SL, FL / FR) did not basically change the situation: The posterior probabilities were  $p = 0.671$  in St.-Martin-Vésudie No 2,  $p = 0.863$  in Tourettes-sur-Loup 1955.06 and  $p = 0.671$  in Sur-2010.07.15 No 17 whereas the type samples of *Myrmica piloscapus* and *M. s. rugulosoides* were allocated to the *M. scabrinodis* cluster with  $p = 0.983$  and  $p = 0.622$ . Also using the reduced character set, NC-Ward performed the same classification changes described in the previous section (Fig. 11) while NC-K-means repeated these three changes and added another one: St.-Martin-Vésudie No 10 was classified as *M. scabrinodis*.

#### **The type series of *Myrmica scabrinodis* NYLANDER is clearly different from *M. martini* sp.n.**

The type series of *Myrmica scabrinodis* NYLANDER, 1846 – consisting of three workers labeled "Kuusamo, W.Nyland. and Mus. Fenn." – was not ordered from Helsinki for this study because there was no danger of a synonymy with *M. martini* sp.n. In 1995, the senior author studied this series by subjective eye-inspection and performed a few simple measurements in one specimen. In overall impression, all three type specimens fully matched the typical *M. scabrinodis* phenotype. Furthermore, FL / FR was 1.465 in the single measured specimen which is clearly above the range of variation known in 84 workers of *M. martini*:  $1.291 \pm 0.048$  [1.167, 1.397]. These data are normally distributed and there is a probability of  $p < 0.00015$  for *M. martini* to achieve a FL / FR value of 1.465 or larger. The third strong argument against a synonymy is the distance

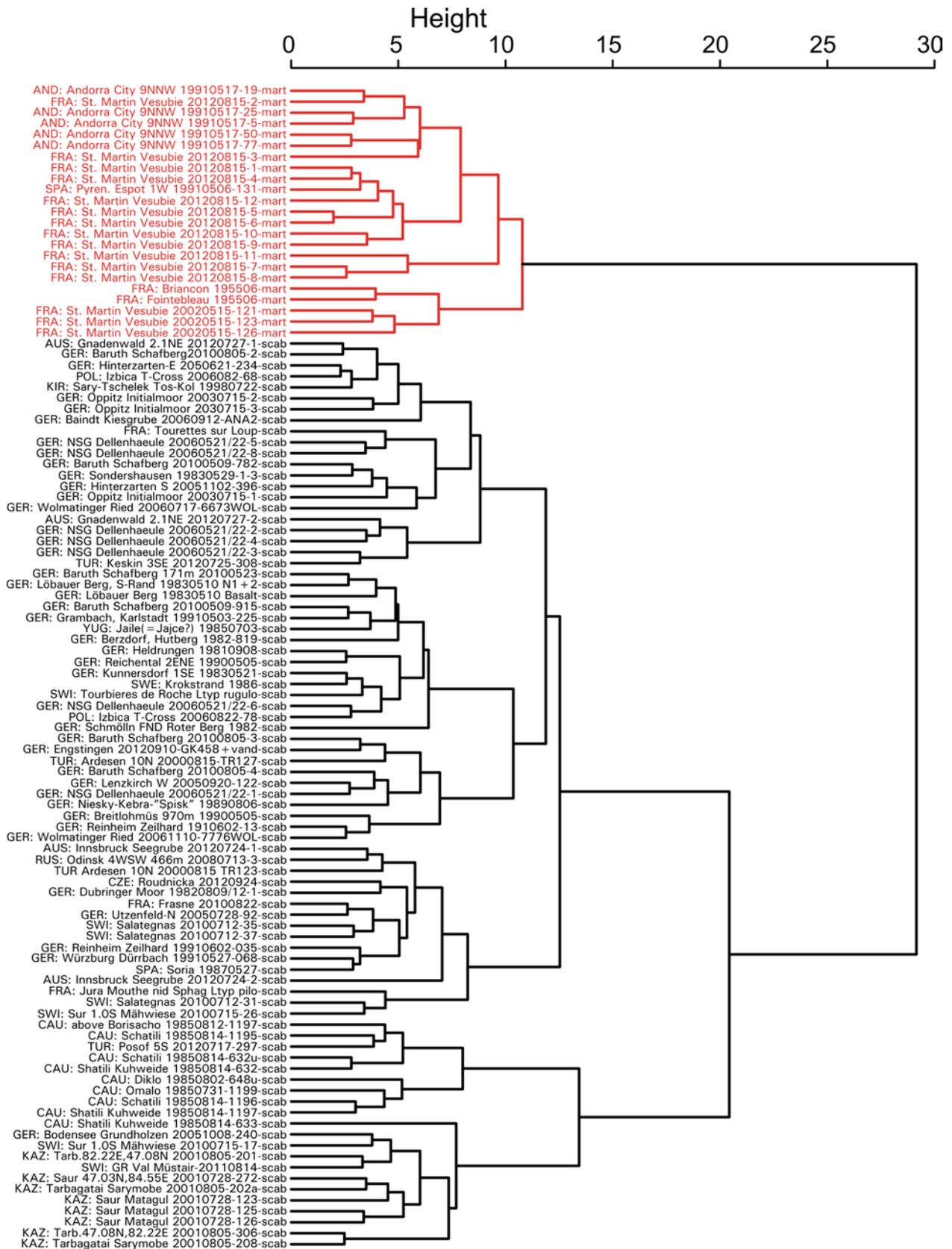


Fig. 10: NC-Ward clustering of the 14 most diagnostic relative warps separating *Myrmica martini* sp.n. (red) and *M. scabrinodis* (black). This classification is fully confirmed by NC-K-means and NC-NMDS-K-means clustering and the controlling linear discriminant analysis.

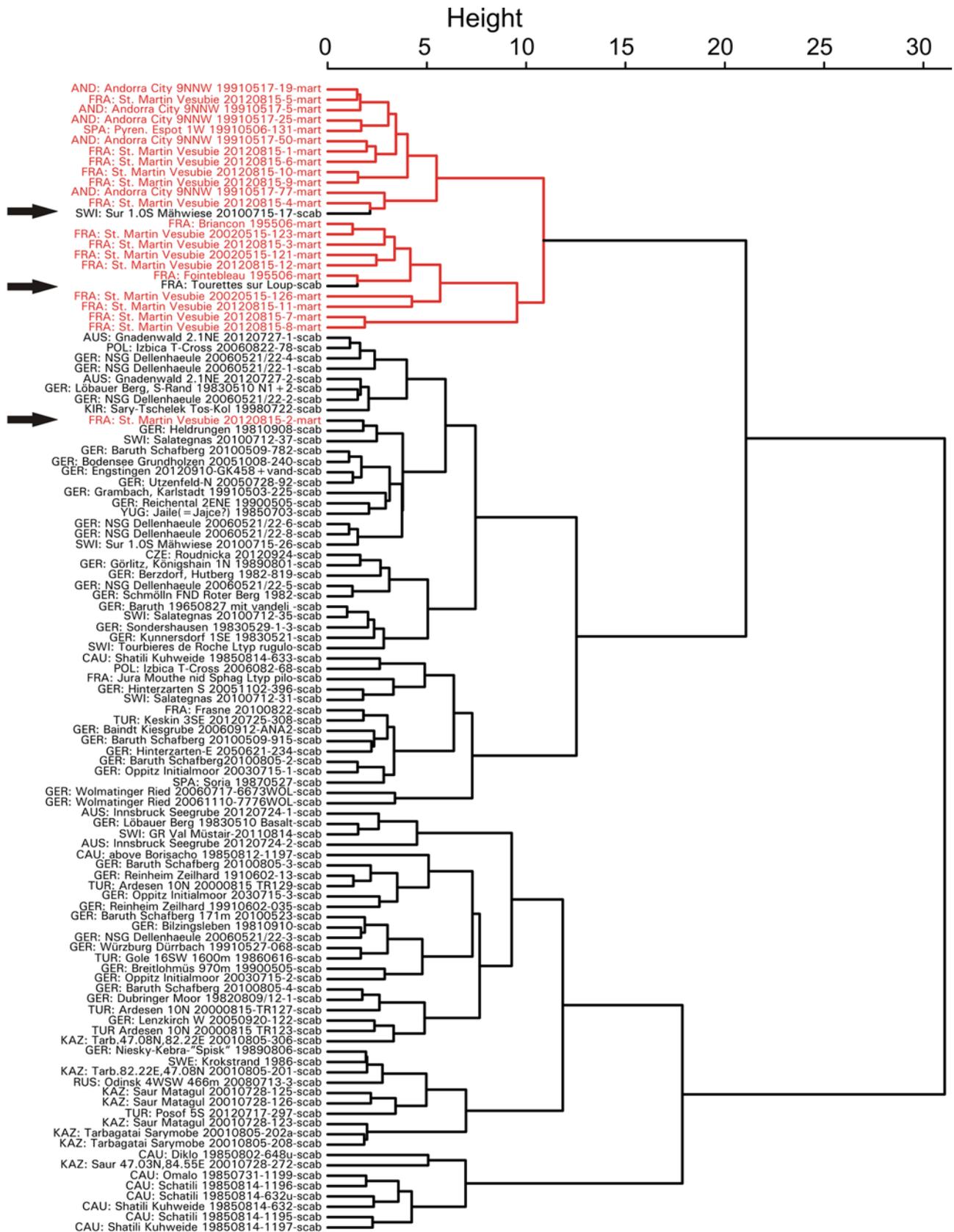


Fig. 11: NC-Ward clustering of the seven most diagnostic characters of conventional linear morphometrics to separate *Myrmica martini* sp.n. (red) and *M. scabrinodis* (black). Arrows point to samples positioned in disagreement with the clustering based on geometric morphometrics (Fig. 9).

of 2700 km between the boreal type locality of *M. scabrinodis* and the next known site of *M. martini* sp.n.

### A simpler way to discriminate *M. martini* from *M. scabrinodis*

The data presented above show an extreme similarity of the two species. Accordingly an easy way of determination is not possible. We tried to simplify the species delimitation procedure by using absolute linear measurements only and by reducing the number of characters for the condition that the error at nest sample level was < 5%. We extracted a morphometric method requiring 8 - 10 minutes per specimen. With all measurements recorded in millimetres, a linear discriminant function

$$D(5) = 0.466 * SL - 37.204 * FL + 45.95 * FR + 42.11 * PPHL - 16.92 * SW - 4.103$$

resulted in an error of 3.6% on the nest sample level while the number of misclassified worker individuals was 9.4%. Nest samples or worker individuals with  $D(5) < 0.633$  are classified as *M. scabrinodis*, those above this threshold as *M. martini*. Within the range of  $D(5) < 0.186$  and  $D(5) > 0.825$ , the classification error on the individual level falls below 2%.

### Final conclusions

Using GM, we found a clear morphological separation of *Myrmica martini* sp.n. from *M. scabrinodis*. The independent system of CLM basically confirmed this classification and disagreed by 2.8% only. For the reasons given below, we consider GM as the more powerful system and the leading indicator. The morphological classification is highly correlated with a geographical pattern: *Myrmica martini* sp.n. was found in the submontane to subalpine zones of the Pyrenees and the French Alps whereas *M. scabrinodis* is found in temperate and subboreal Eurasia outside this region. The two *M. scabrinodis* samples from Tourettes-sur-Loup are very close to the localities of *M. martini* in the French Alps. The data we have so far indicate no or only minimal range overlap between the two species and we consider them as parapatric species with probably significant reproductive barriers. It remains to be studied by means of nuDNA markers how strong these barriers really are. The geographic distribution of *M. martini* strongly suggests that it survived the last glaciation in a lowland refuge south of the Pyrenees or between the Pyrenees and French Alps. The main postglacial spreading was probably a movement up into the montane and subalpine zones of these mountains but there is no information on the situation in the planar and colline regions of South and Central France.

We suppose a lower performance of CLM compared to GM. This is indicated by two classifications being in strong disagreement with zoogeographic arguments. The hypothesis of a lower performance is also supported by the lower percentage of individual workers classified with posterior probabilities of  $p > 0.95$ . Furthermore, BAGHERIAN YAZDI & al. (2012), investigating an interspecific hybrid scenario in *Myrmica* and running CLM and GM on identical samples in parallel, also suggested a slightly lower performance of CLM. However, there is also an advantage of CLM: the much lower processing time. Recording 19 characters in CLM needs about 50 minutes per worker specimen whereas the whole GM procedure needs 160 minutes at the cur-

rent state of imaging technology and software development. This means more than a threefold processing time, summing up to eight hours for a standard sample of three workers, and prevents the present application of GM in routine investigations of thousands of samples. However, 165 minutes required from specimen preparation to the final computation result is very little compared to the complete processing time of other advanced methods in taxonomy such as microtomography or Next Generation Sequencing. This important disadvantage is usually not communicated.

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