

http://doi.org/10.11646/zootaxa.4132.1.4
http://zoobank.org/urn:lsid:zoobank.org:pub:EB53921A-A174-46A3-96B4-84F168969F64

The Westpalaearctic *Lasius paralienus* complex (Hymenoptera: Formicidae) contains three species

BERNHARD SEIFERT^{1,3} & CHRISTOPHE GALKOWSKI²

¹Senckenberg Museum für Naturkunde Görlitz, Am Museum 1, 02826 Görlitz, Germany.
E-mail: bernhard.seifert@senckenberg.de

²104 route de Mounic, 33160 Saint Aubin de Médoc, France. E-mail: chris.gal@wanadoo.fr

³Corresponding author. E-mail: bernhard.seifert@senckenberg.de

Abstract

Application of Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT) demonstrated the existence of three cryptic species within the Westpalaearctic *Lasius paralienus* species complex: *L. paralienus* Seifert, 1992, having a wider European distribution north to Sweden, *L. casevitzi* sp. nov., an endemic of Corsica, and *Lasius bombycinus* sp. nov. from south-east Central Europe, the Balkans and Asia Minor. Hierarchical NC-Ward clustering and non-hierarchical NC-k-means clustering of 16 morphological characters resulted in 98.7% identical classifications within 76 examined nest samples of the three species. The classification error in 180 worker individuals was 0% in a linear discriminant analysis (LDA) and 1.3% in a LOOCV-LDA. Differential characters to other species groups and an identification key of the six European members of the *Lasius alienus* Förster species group are provided.

Key words: numeric morphology-based alpha-taxonomy, nest centroid clustering, sister species, endemic, morphometry

Introduction

The ant genus *Lasius* Fabricius, 1804 has a Holarctic distribution. While the taxonomic situation in the Nearctic region is poorly known and has not been studied during the last 60 years, the Palaearctic fauna is much better investigated and contains, according to the senior author's personal estimate, about 97 species when the large number of delimited but undescribed species stored in the collection of the Senckenberg Museum of Natural History Görlitz are considered. The Palaearctic fauna can be grouped by genetic and morphological criteria in five subgenera (Wilson 1955, Janda *et al.* 2004, Maruyama *et al.* 2008). These subgenera are *Lasius* s.str. Ruzsky, 1913 (about 55 Palaearctic species), *Cautolasius* Wilson, 1955 (7 species), *Dendrolasius* Ruzsky, 1913 (6 species), *Chthonolasius* Ruzsky, 1913 (about 27 species) and *Austrolasius* Faber, 1967 (2 species). Independent colony foundation occurs in *Lasius* s.str. and *Cautolasius* only—the species of all other subgenera are temporary social parasites.

The subgenus *Lasius* s.str. is characterized by large-eyed workers, elongated maxillary palps and more or less intensive above-ground foraging. Within the group of the numerous Palaearctic species with no or very few standing setae on the scape—all lumped by Wilson (1955) under the name *Lasius alienus* Förster, 1850—Seifert (1992) distinguished five Westpalaearctic species: *Lasius alienus*, *Lasius paralienus* Seifert, 1992, *Lasius piliferus* Seifert, 1992, *Lasius psammophilus* Seifert, 1992 and *Lasius obscuratus* Stitz, 1930. *Lasius paralienus* is found in Europe and Asia Minor and can be separated from the other four species by a much denser pubescence on the cuticular surface and on the clypeus in particular. The discovery of high numbers of cryptic species in ants is predictable (Seifert 2009) but it took us by surprise to find two new species related to *L. paralienus* which are separable by comparably simple methods. Through applying Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT), we demonstrate here the separate identity of *Lasius paralienus*, *Lasius casevitzi* sp. nov. from Corsica and *Lasius bombycinus* sp. nov. from the Balkans and Asia Minor.

We follow here the Pragmatic Species Concept (PSC) of Seifert (2014) which avoids any claims about putative

or potential reproductive isolation - a conceptional property which is for several groups of organisms basically not applicable and for sexually reproducing organisms in most practical situations not testable. The PSC is universal by focusing on evolutionary divergence alone. The name "pragmatic" was chosen to indicate that the PSC aims at just that conceptional property which is in practice always measurable and testable—dependent from the mode of reproduction of a particular group of organisms under study and if these are fossil or recent. Seifert formulated his concept in several sentences: "*A species is a cluster of organisms which passed a threshold of evolutionary divergence. Divergence is determined by one or several operational criteria on the basis of an adequate numerics. A single conclusive operational criterion is sufficient. Conflicts between operational criteria require an evolutionary explanation. Thresholds for each operational criterion are fixed by consensus among the experts of a discipline under the principle of avoiding oversplitting. Clusters must not be the expression of intraspecific polymorphism.*" The term "operational criterion" has to be understood as a research field or discipline (e.g., genetics, phenetics, bioacoustics) that uses reproducible numeric data for decisions on the basis of the threshold principle. Seifert proposed for the discipline "multivariate phenetic investigation of ant workers" as a remedy against over-splitting that at least 97% of the classifications by exploratory data analyses should agree with the classifications by linear discriminant analyses that form the final species hypothesis. This threshold was later adjusted by Seifert & Csösz (2015) to >96% (or an error <4%) after evaluation of the performance of two powerful methods of NC-clustering, NC-K-Means and NC-Ward, in the separation of 74 cryptic ant species (Table 1 in Seifert *et al.* 2013).

Material

Material used in the exploratory and hypothesis-driven data analyses. A detailed account of the samples is given below in the following sequence and format: site, date in the format yyyy.mm.dd, sample No [latitude in decimal format, longitude in decimal format, altitude in meters]. The accuracy of coordinates is proportional to the number of decimal points and "xx" in the sampling date sequence mean missing data. In some samples without any direct or derived information on date, the collector is given to allow an approximate conclusion on the time period of collection. Sample numbers are missing in many samples.

Lasius bombycina sp. nov.

A total of 20 nest samples originated from the following countries: Austria 4, Bulgaria 1, Greece 1, Hungary 4, Slovakia 1 and Turkey 9 samples. A total of 53 workers were subjected to NUMOBAT investigation.

AUSTRIA: E Austria, 1989.xx.xx [47.8, 16.8, 150]; Illmitz-Sandeck, 1982.08.28 [47.744, 16.773, 112]; Kalvarienberg near Neusiedl, 1982.08.30 [47.948, 16.873, 168]; Zurndorf, Neusiedler See, 1982.08.31 [47.961, 16.991, 159]. **BULGARIA:** Rozen, 1982.08.26 [41.58, 23.47, 1450]. **GREECE:** Evros, Soufli, 1999.09.03 [41.193, 26.300, 46]. **HUNGARY:** Budapest, 1989.07.xx, samples No 01, No 02 [47.70, 19.10, 100]; Budapest-Göd, 1991.04.26 [47.690, 19.149, 100]; Budapest-Poesmegyer, 1991.04.30 [47.693, 19.112, 103]. **SLOVAKIA:** Zemplinska Sirava, 1983.08.30, sample No 2 [48.621, 21.341, 171]. **TURKEY:** Bitlis-10 km NE, 1993.06.16 [38.486, 42.173, 1800]; Bunyan, 1989.06.27 [38.85, 35.86, 1800]; Cappadoce: Ürgüp, 1997.xx.xx, [38.631, 34.913, 1060]; Kars-25 km SE: Dagpinar, 1993.06.23, sample No 1121 [40.464, 43.324, 2100]; Kastamonu, 1989.07.05 [41.4, 33.8, 1400]; Müezzinler, 2004.06.13, sample No 913 [40.967, 30.317, 68]; Saray-8 km SE, 2001.07.16, sample No 0990, No 1015 [41.412, 27.968, 151]; Ziyarettepesi Gecidi, 1997.05.09, sample No 01 [38.899, 36.830, 1900].

Lasius casevitzi sp.nov.

A total of 6 nest samples originated from France: Corsica. A total of 18 workers were subjected to NUMOBAT investigation.

FRANCE: Bonifatu, 2015.07.12, sample No 1, No 2 [42.440, 8.868, 687]; Poggiolo, 2015.05.05, sample No 1 [42.172, 8.901, 448]; Ponte Novu, 2014.08.18, sample No 3 [42.487, 9.282, 146]; Zonza, 2014.08.25, sample No 4 [41.749, 9.172, 796]; Cappiciolo, 2009.04.14, sample No C [41.697, 8.873, 35].

Lasius paralienus

49 nest samples with 106 workers were subjected to NUMOBAT investigation for all characters. These

originated from the following countries: Austria 1, Bosnia 1, Bulgaria 1, France 1, Germany 30, Italy 13 and Switzerland 2 samples. All seven samples from Sweden were classified by the senior author as *L. paralienus* nine years before but were not available for the current investigation of the complete character set. As these Swedish sites are between 980 km and 1220 km north of the northern distributional border of *L. bombycina* sp.nov., there is no doubt that they belong to *Lasius paralienus*.

AUSTRIA: Arzl, Kalvarienberg, 1994.08.16, sample No K25 [47.283, 11.435, 646]. **BOSNIA:** Prozor, 1902.xx.xx (leg. O. Leonhard) [43.820, 17.610, 764]. **BULGARIA:** Dobrostan, 1982.09.13 [41.92, 24.90, 1400]. **FRANCE:** St. Aubin de Medoc, 2007.09.29 [44.917, -0.724, 32]. **GERMANY:** Alfeld /Leine, 1985.05.28 [52.004, 9.833, 280]; Rudersberg-1.4 km SSW, 2005.05.xx [48.875, 9.519, 300]; Buttenhausen-1.5 km W, Eichhalde, 1991.05.20, sample No 0, No 13, No 37, No 65, No 70, No 91, No 56215, No F [48.370, 9.464, 700]; Gomadingen, Sternberg, 1991.xx.xx, sample No 56851 [48.391, 9.377, 790]; Gross-Saubernitz, 1962.09.12 [51.233, 14.639, 162]; Hohenstein, Bauenofen, Spitzwald, 1991.xx.xx, sample No 54904 [48.353, 9.290, 760]; Lichtenstein, Tobelkopf, 1991.05.19, sample No 24 [48.400, 9.269, 765]; Niedergurig-1 km SW, 1961.05.18 [51.220, 14.471, 170]; Niederoderwitz, 1919.04.05 [50.96, 14.69, 475]; Northeim /Weper, 1982.08.31 [51.70, 10.00, 133]; Oberstetten, 1991.05.19, sample No 10 [48.312, 9.333, 780]; Schönecken 1.5 km ENE, 1991.09.06, sample No 084 [50.164, 6.475, 443]; Schöps, Schanze, 1990.06.30 [51.157, 14.751, 206]; Schöps, Schanze, 1991.07.03, sample No 029, No 043, No 045, No 069, No 082, No 237 [51.157, 14.751, 206]; Spitzkunnersdorf, Grosser Stein, 2011.05.29 [50.941, 14.659, 440]; Weissenberg- 2 km S, Strohmberg, 1991.07.11, sample No 066 [51.175, 14.655, 258]; Trendelburg-2.4 km W, 2012.07.26, sample No SKT18 [51.569, 9.387, 215]; Trochtelfingen, Flachsühl, 1991.05.20, sample No 86 [48.341, 9.929, 736]. **ITALY:** La Cicerana, 2009.07.10, sample No 104, No 107 [41.864, 13.717, 1565]; Pagliara, 2009.07.08, sample No 050 [41.832, 13.780, 1309]; Sorgente Duna, 2009.07.07, sample No 016, No 017 [41.887, 13.755, 1240]; Val Fondillo, 2009.07.07, sample No 023 [41.768, 13.854, 1106]; Val di Giovenco, 2009.07.10, sample No 095 [41.935, 13.745, 1087]; Valone Cavuto, 2009.07.08, sample No 040 [41.817, 13.768, 1316]; Maiella, 1987.08.02 [42.073, 14.026, 1327]; Colle dele Finestre, 2009.05.29 [45.063, 7.033, 1850]; Lazio: Percile, 1991.08.15 [42.097, 12.912, 550]; Lazio: Saracinesco, 1991.03.17 [42.003, 12.954, 867]; Roma, Pantheon, 1991.03.17 [41.899, 12.477, 20]. **SWEDEN:** Gotland: without precise locality, pre 1979.xx.xx [57.5, 18.5, 35]; Öland: Grabborg, 1979.08.16 [56.667, 16.607, 30]; Öland: Räpplinge, 1974.08.11 [56.828, 16.603, 32]; Öland: Möckelmossen, 1992.06.12 [56.532, 16.508, 30]; Öland: Högsrum-2 km NE, 1992.06.13 [56.771, 16.625, 39]; Öland: Bornholm's slot ruin 0.5 km S, 1992.06.13 [56.866, 16.643, 39]; Södermanland: Björnlunda-1.3 km E, 2006.xx.xx [59.065, 17.187, 25] **SWITZERLAND:** Gland, 1990.07.13 [46.426, 6.233, 490]; Jona: Rotenweg, 2000.08.15, sample No 9a [47.246, 8.817, 500].

Lasius paralienus cf.

A problematic nest sample of 6 workers with unclear classification has the collecting data **ITALY:** Sardinia: Macomer, 1991.03.08 [40.308, 8.821, 800].

Type material

Lasius bombycina sp. nov.

Holotype worker labeled "Holotype *Lasius bombycina* Seifert & Galkowski" and "TUR: 38.63°N, 34.91°E, 1060 m, Capadoce: Ürgüp, SSE-facing meadow, S. Aron 1990"; 4 paratype workers on a second pin, one male and a gyne paratype on a third pin, all with the same site labeling as the holotype but labeled "Paratype *Lasius bombycina* Seifert & Galkowski"; Senckenberg Museum für Naturkunde Görlitz. Three paratype workers labelled "Türkei_26, Prov. Kayseri, Ziyarettepesi Gecidi (ca. 130 km E. Kayseri), 1900mH, 09.05.1997, Leg. A.Schulz, K.Vock, M. Sanetra 01"; Naturhistorisches Museum Wien.

Lasius casevitzi sp. nov.

Holotype labeled "Holotype *Lasius casevitzi* Seifert & Galkowski" and "FRA: 42.440°N, 8.868°E, 687 m Corse: Bonifatu, leg. Galkowski 2015.07.12 -1" and three paratype workers with same site labeling as the holotype, stored in Muséum National d'Histoire Naturelle Paris. Three paratype workers from the holotype nest and four paratype workers from another nest at the holotype locality labeled "FRA: 42.440°N, 8.868°E, 687 m Corse: Bonifatu, leg. Galkowski 2015.07.12 -2" in Senckenberg Museum für Naturkunde Görlitz.

Lasius paralienus Seifert, 1992

Holotype labelled "Germania: Kr. Bautzen, 2 km S Weißenberg; N066 11.7.1991, leg. Seifert", "*Lasius paralienus* Seifert Holotypus", "GBIF-D/FoCol 0751 specimen + label data documented"; 4 paratype workers with the same site labelling and "*Lasius paralienus* Seifert Paratypus"; material stored in Senckenberg Museum für Naturkunde Görlitz. The type locality is situated at 51.1752°N, 14.6551°E and 258 m.

Methods

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. The mean relative measuring error over all magnifications was 0.2%. 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 and microsculpture at highest magnifications. 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.

The morphometric characters. Eighteen morphometric characters were investigated in workers. In bilaterally developed characters, arithmetic means of both body sides were calculated. All measurements were made in mounted and fully dried specimens. Measurements of body parts always refer to real cuticular surface and not to the diffuse pubescence surface. Setae, also called pilosity or simply 'hairs', are differentiated from pubescence hairs in having a distinctly larger basal diameter. All seta counts (nSC, nHT, nOCC, nGU, nGEN, nSt) are unilateral numbers (i.e., the arithmetic means of the number on both sides) and are restricted to *standing setae projecting >20 µm from the silhouette of cuticular surface as observed under use of transmitted-light*.

CL	maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of posterior head and/or clypeus reduce CL.
CS	arithmetic mean of CL and CW as less variable indicator of body size.
CW	maximum cephalic width; this is either across, behind, or before the eyes.
dCIAn	shortest distance from posterior clypeal suture (PCS) to <i>upper inner</i> margin of antennal sockets (Fig. 1). If no surface structure indicates the position of PCS, the centre of the dark line is taken as the anterior measuring point.
EYE	eye-size: the arithmetic mean of the large (EL) and small diameter (EW) of the elliptic compound eye under consideration of all structurally visible ommatidia - i.e., including also unpigmented ones.
GUHL	maximum length of setae on underside of head ("gula").
MaDe	number of teeth on masticatory border of mandible. Data of both mandibles are averaged.
MP6	length of the sixth (terminal) segment of maxillary palps.
nGEN	number of setae projecting from the contour line of genae. Counting is done in the measuring position of CW with head in full face view; the bilateral number is halved. Keep care to avoid the parallax error when determining the posterior end of counting line that is at level of anterior eye margin.
nGU	number of setae on underside of head ("gula") as seen in full profile. The bilateral sum is halved.
nHT	setae number on extensor profile of hind tibia under exclusion of the very apical setae. The bilateral sum is halved.
nOCC	setae number projecting from hind margin of vertex frontad to caudal end of eye. Counting is done with head in full face view and by rotating the head within visual plane to avoid a parallax error in estimating the 20 µm projecting distance. Keep care to avoid the parallax error when determining the anterior end of the counting line that is at level of posterior eye margin. The bilateral sum is halved.
nSC	setae number on dorsal plane of scape under exclusion of the most apical setae, counted with view on the small scape diameter. The bilateral sum is halved.
nST	setae number on lateral and caudolateral surface of metapleuron. The upper margin of the counting

	area is an imagined line parallel to the lower straight margin of metapleuron and crossing the lower margin of the cuticular ring of propodeal spiracle. Protective setae fringing the orifice of the metapleural gland are excluded (Fig. 2). The bilateral sum is halved.
PLF	mean length of pubescence hairs on head between the frontal carinae. Seven measurements in each individual are averaged.
PNHL	length of the longest hair on pronotum.
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. 3).
SL	maximum straight line scape length excluding the articular condyle.
sqPDCL	square root of pubescence distance PDCL on clypeus. The number of pubescence hairs crossing or just touching a census line from caudomedian clypeus to lateral clypeal depression (Fig. 4) is counted. Hairs crossing / touching the census line are counted as 1 / 0.5. Erroneous zero counts in surface areas with torn-off pubescence can be avoided when the basal points of the missing hairs can be visualized by adequate illumination and high-resolution optics and when average pubescence hairs length is considered. Square root data transformation is applied to normalize positively skewed distributions.

Removal of allometric variance. There is a strong intraspecific variance of body size in *Lasius* ants which is determined by age and social structure of the colonies and by nutrition. In order to reveal in comparative tables which shape variables differ between the species independent of body size, a removal of allometric variance (RAV) was performed with the procedure described by Seifert (2008). Evaluation of scatter plots suggested a use of linear monophasic allometry functions. RAV was calculated assuming all individuals to have a cephalic size of CS=900 µm. RAV functions were calculated as the arithmetic mean of the species-specific functions of 47 Palaearctic *Lasius* s.str. species with sufficient sample size. The RAV functions of six shape and eight seta and one pubescence character are given in the following.

$$CL/CW_{900} = CL/CW / (-0.2290 \text{ CS} + 1.2804) * 1.0743$$

$$SL/CS_{900} = SL/CS / (-0.2095 * \text{CS} + 1.1771) * 0.9886$$

$$nSC_{900} = nSc / (+13.04 * \text{CS} - 2.50) * 9.23$$

$$nGen_{900} = nGen / (+7.11 * \text{CS} - 1.89) * 4.50$$

$$nOcc_{900} = nOcc / (+9.00 * \text{CS} + 3.27) * 11.37$$

$$nGu_{900} = nGU / (+8.38 * \text{CS} - 1.54) * 6.00$$

$$nHT_{900} = nHT / (+19.02 * \text{CS} - 6.73) * 10.39$$

$$nSt_{900} = nST / (+7.14 * \text{CS} - 2.73) * 3.70$$

$$sqPDCL_{900} = sqPDCL / (-0.992 * \text{CS} + 5.619) * 4.726$$

$$PLF_{900} = PLF / (+21.99 * \text{CS} + 9.49) * 29.19$$

$$PoOc/CL_{900} = PoOc/CL / (-0.0194 * \text{CS} + 0.2517) * 0.2342$$

$$EYE/CS_{900} = EYE/CS / (-0.0704 * \text{CS} + 0.3017) * 0.2383$$

$$dCLAN/CS_{900} = 100 * dCLAN/CS / (+0.287 * \text{CS} + 4.249) * 4.507 \text{ [given in \%]}$$

$$GuHL/CS_{900} = GuHL/CS / (-0.0108 * \text{CS} + 0.1179) * 0.1082$$

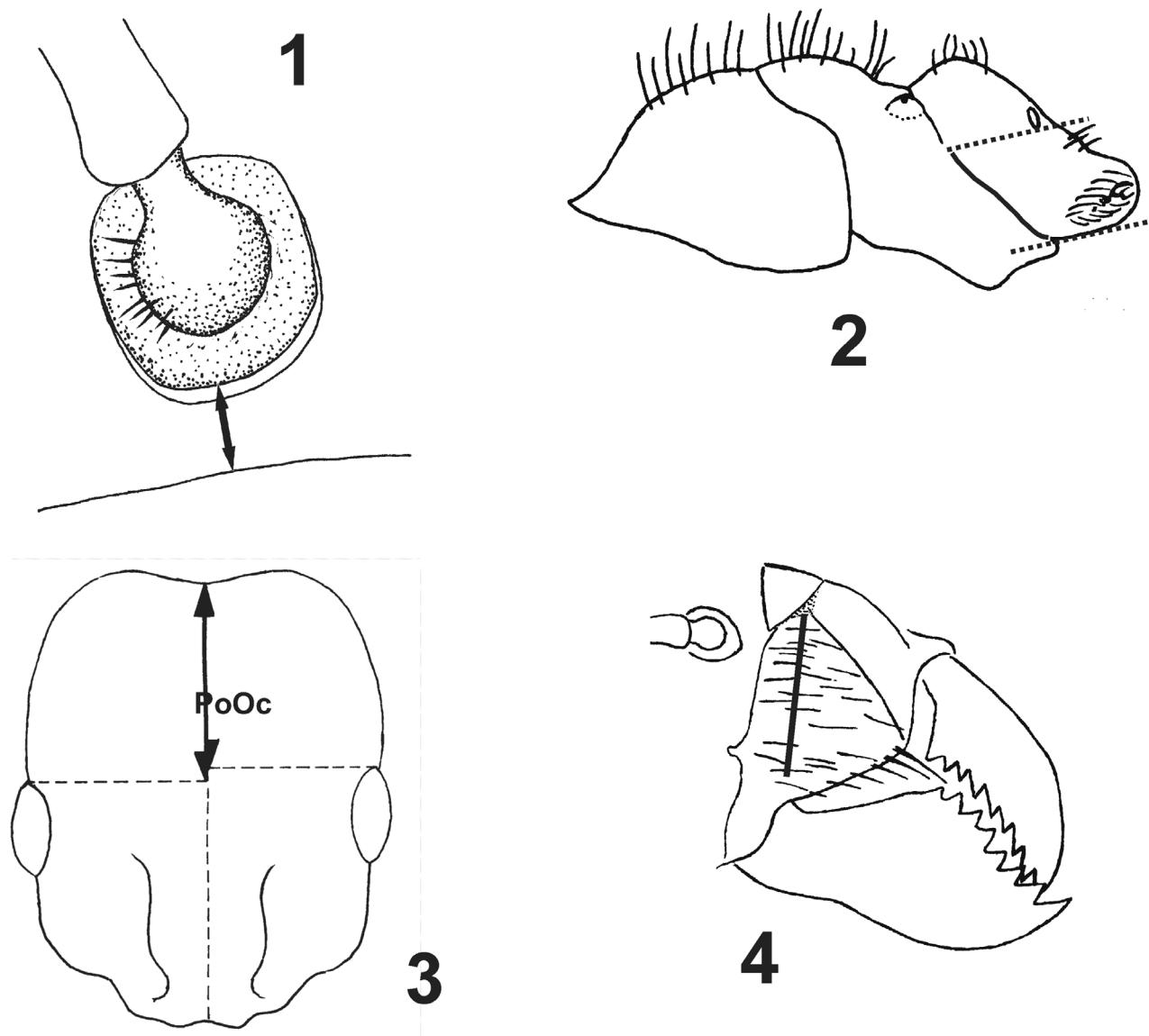
$$PnHL/CS_{900} = PnHL/CS / (+0.0001 * \text{CS} + 0.1425) * 0.1425$$

$$MP6/CS_{900} = MP6/CS / (-0.0705 * \text{CS} + 0.2504) * 0.1870$$

Explorative and hypothesis-driven data analyses and classification. The delimitation of the cryptic species was done by an interaction of Nest-Centroid Clustering (NC clustering) and a controlling linear discriminant analysis (LDA). NC Clustering was run both as hierarchical NC-Ward clustering and non-hierarchical NC-K-means clustering. These methods were described in more detail by Seifert *et al.* (2013) who also provided a script written in R and freely available under the GNU / GPL license from the following website: <http://sourceforge.net/projects/agnesclustering/>.

NC-Ward clustering was run first to indicate the putative number of K main clusters. In the second step, NC-K-Means was performed with the setting of K classes suggested by NC-Ward. Classifications being coincident

between the hierarchical and non-hierarchical clustering formed the hypothesis for the controlling LDA that was subsequently run. Samples with classifications disagreeing between NC-Ward and NC-K-means were run in this LDA as wild-cards. The final classification ("final species hypothesis") was established by the LDA in the iterative procedure described by Seifert *et al.* (2013). There remained no undecided cases also if their posterior probabilities were close to 0.5. The classification of particular type specimens was checked by a 'Leave-One-Out Cross-Validation' analysis LDA (LOOCV-LDA, Lachenbruch & Mickey 1968, Lesaffre *et al.* 1989). LDA, LOOCV-LDA and ANOVA tests were performed with the software package SPSS 15.0.



FIGURES 1–4. Fig. 1: Measuring of antennal socket position dCIAn; Fig 2: Reference area for counting of metanotal setae nSt; Fig 3: Mode of measuring postocular distance PoOc; Fig. 4: Mode of recording mean clypeal pubescence distance PDCL.

Results and discussion

Diagnosis of the *Lasius paralienus* complex and separation from other species

The species of the *Lasius paralienus* complex are separable from related species by the combination of low clypeal pubescence distance, low setae numbers on scape and differences in length of maxillary palp segments. *Lasius alienus*, *L. psammophilus* and *L. piliferus* are the most similar species occurring within the geographic range of the three *L. paralienus* complex species. *L. alienus* differs from the other five species by having strongly reduced setae numbers on the metapleuron below the level of the propodeal spiracle. Nest means of nSt are 0.32 ± 0.39 in *L.*

alienus but 3.79 ± 1.23 in the other five species. *Lasius piliferus* and *L. psammophilus* differ by higher clypeal pubescence distance with sqPDCL_{900} ranging 3.87–5.59 (or PDCL_{900} 14.98–31.25 µm) in 67 nest samples of both species whereas sqPDCL is 3.01–3.78 (or PDCL_{900} 9.06–14.29 µm) in 75 nest samples of the *L. paralienus* complex species. A safe separation of individual workers is possible by a discriminant function considering head width, the length of the terminal maxillary palp segment and clypeal pubescence distance. With CW and MP6 given in mm and PDCL given in µm, the discriminant

$$D(3) = 6.139 \text{ CW} - 62.834 \text{ MP6} + 1.774 \text{ sqPDCL} - 3.422$$

correctly classifies 99.2% of 276 individuals of both groups. The cluster formed by *L. paralienus*, *L. bombycina* sp. nov. and *L. casevitzi* sp. nov. shows $D(3)$ values <0 whereas the *L. psammophilus* / *L. piliferus* cluster shows positive values.

Clear clustering of three species within the *L. paralienus* complex

The hierarchical exploratory data analysis NC-Ward and the non-hierarchical exploratory data analysis NC-k-means clustering considered those 16 NUMOBAT characters for which complete data sets were available. These characters were the absolute size indicator CS, six shape characters, one pubescence character and eight seta characters: CS, CL/CW₉₀₀, SL/CS₉₀₀, MP6/CS₉₀₀, PoOc/CL₉₀₀, EYE/CS₉₀₀, dClAn/CS₉₀₀ [%], sqPDCL_{900} , GuHL/CS₉₀₀, PnHL/CS₉₀₀, nOcc₉₀₀, nGen₉₀₀, nGu₉₀₀, nSc₉₀₀, nHT₉₀₀ and nSt₉₀₀. We got three clearly separate clusters (Fig. 5). NC-Ward and NC-k-means came to coincident classifications in 98.7% of the 76 investigated samples. The only disagreeing sample Italy: Sorgente Duna, 2009.07.07, No 016, was run as wild-card in the controlling LDA and classified as *L. paralienus*. A classification error of 1.3% by exploratory data analyses relative to the controlling LDA is clearly below the 3% threshold recommended by the Pragmatic Species Concept (Seifert 2014) or the 4% threshold later considered to be more adequate for cryptic species (Seifert & Csösz 2015). The classification error of the LDA was 0% in 180 worker individuals of all three species and 1.3% in a LOOCV-LDA. The zoogeographic pattern and a homogenous within-nest phenotype composition rejects the hypothesis that *L. paralienus* and *L. bombycina* sp. nov. could represent an intraspecific dimorphism. The primary NUMOBAT data of all 180 specimens are available from the corresponding author on request.

The identification of the sample from Italy: Sardinia: Macomer, 1991.03.08—classified by the above procedures as *L. bombycina* sp. nov.—seems problematic from a zoogeographic point of view, because there is no *L. bombycina* sp. nov. known from areas west of 16.7°E. A relatedness to *L. casevitzi* sp. nov., which could be supposed from the close geographic neighborhood of Sardinia to Corsica, is clearly rejected by the data shown in Tab. 1. Removing the well-separated *L. casevitzi* sp. nov. from the analysis and running the sample as wild-card in a LDA considering all 16 characters, the posterior probabilities are $p=0.878$ for *L. bombycina* sp. nov. and $p=0.122$ for *L. paralienus*. Yet, running the sample as a wild-card in a stepwise LDA considering the five characters CS, MP6/CS₉₀₀, PnHL/CS₉₀₀, nOcc₉₀₀, nGen₉₀₀, the posterior probabilities are $p=0.266$ for *L. bombycina* sp. nov. and $p=0.734$ for *L. paralienus*. In the absence of a convincing morphological indication, we interpret this sample provisionally as belonging to an aberrant island population of *L. paralienus* that developed a longer pronotal pilosity and more numerous setae on the metapleuron and posterior head (Tab. 1).

Apart from this problematic sample, we have clear identifications over all samples. Most easily separable is the Corsican endemic *Lasius casevitzi* sp. nov. which differs from the sister species by much longer maxillary palps and much more developed pilosity on genae and extensor profile of hind tibia (Tab. 1). A safe separation of *L. paralienus* and *L. bombycina* sp. nov. on an individual worker level requires four characters. With the metric data PnHL and MP6 given in mm, the discriminant

$$D(4) = 55.452 \text{ PnHL} - 101.942 \text{ MP6} + 0.216 \text{ nOcc} + 0.622 \text{ nGen} + 5.762$$

provides 100% correct classification in all 156 investigated workers. *Lasius paralienus* has negative values of $D(4)$ and *L. bombycina* sp. nov. positive ones. If arithmetic means of discriminant values of three workers per nest are considered, only two characters are required: the discriminant

$$D(2) = 77.625 \text{ PnHL} - 107.575 \text{ MP6} + 5.916$$

shows negative values in *L. paralienus* and positive ones in *L. bombycina* sp. nov. This is a surprisingly simple separation if we consider the fact that the separate identity of *L. bombycina* sp. nov. remained undiscovered for more than 25 years of intensive ant research in Central Europe.

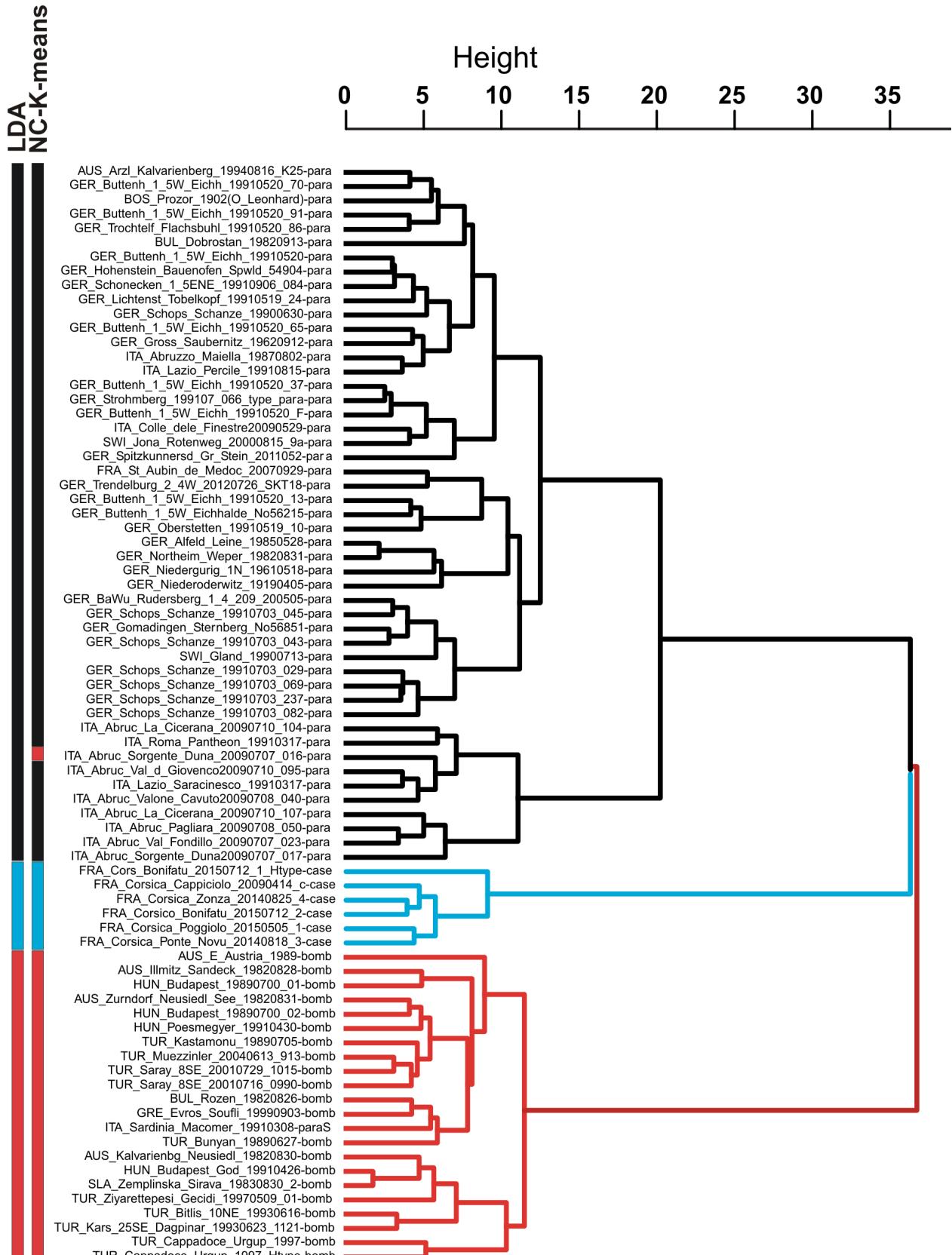


FIGURE 5. Hierarchical NC-Ward clustering of 76 nest samples of the three European species of the *Lasius paralienus* complex considering all 16 morphometric characters unselectively: *L. paralienus* Seifert, 1992, *L. bombycinus* sp. nov. and *L. casevitzi* sp. nov. The vertical bars show the results of the controlling LDA (final species hypothesis) and of non-hierarchical NC-K-Means clustering which disagrees with NC-Ward clustering in one sample (red square within the black bar).

TABLE 1. Nest sample means of absolute cephalic size and of 15 RAV-corrected characters of shape, pubescence and setae of workers of *Lasius paralienus*, *L. bombycina* sp. nov., *L. casevitzi* sp. nov. and an aberrant sample of *L. paralienus* from Sardinia. Removal of allometric variance is performed for the assumption that all individuals have the same head size CS of 900 µm. Means of diagnostic characters are in bold; n= number of nest samples, i= number of individuals.

	<i>L. paralienus</i> (n=49, i=106)	<i>L. paralienus</i> Sardinia (n=1, i=6)	<i>L. bombycina</i> sp. nov. (n=20, i=50)	<i>L. casevitzi</i> sp.nov. (n=6, i=18)
CS [µm]	863 ± 70 [710,1097]	927	891 ± 50 [828,1016]	849 ± 35 [803, 885]
CL/CW ₉₀₀	1.067 ± 0.014 [1.024,1.094]	1.074	1.075 ± 0.008 [1.062,1.086]	1.066 ± 0.010 [1.053,1.078]
SL/CS ₉₀₀	0.986 ± 0.014 [0.953,1.032]	0.998	0.968 ± 0.015 [0.934,0.987]	0.995 ± 0.009 [0.987,1.011]
MP6/CS ₉₀₀	0.184 ± 0.006 [0.170,0.197]	0.176	0.161 ± 0.008 [0.145,0.179]	0.210 ± 0.005 [0.206,0.218]
PoOc/CL ₉₀₀	0.242 ± 0.007 [0.230,0.263]	0.236	0.238 ± 0.006 [0.223,0.250]	0.245 ± 0.003 [0.242,0.248]
EYE/CS ₉₀₀	0.241 ± 0.004 [0.230,0.250]	0.238	0.237 ± 0.004 [0.231,0.244]	0.239 ± 0.002 [0.236,0.241]
dClAn/CS ₉₀₀ [%]	4.08 ± 0.41 [3.30,5.15]	4.39	4.36 ± 0.23 [3.87,4.92]	4.44 ± 0.21 [4.17,4.77]
GuHL/CS ₉₀₀	0.106 ± 0.022 [0.028,0.138]	0.119	0.114 ± 0.009 [0.095,0.128]	0.109 ± 0.003 [0.106,0.114]
PnHL/CS ₉₀₀	0.135 ± 0.010 [0.101,0.160]	0.157	0.162 ± 0.007 [0.152,0.177]	0.157 ± 0.018 [0.121,0.172]
sqPDCL ₉₀₀	3.48 ± 0.17 [3.01,3.78]	3.39	3.44 ± 0.15 [3.21,3.72]	3.31 ± 0.18 [3.12,3.53]
nOcc ₉₀₀	7.57 ± 1.54 [3.3,10.6]	10.20	12.46 ± 1.81 [8.4,16.0]	12.36 ± 1.08 [10.5,13.8]
nGen ₉₀₀	0.20 ± 0.27 [0.0,0.9]	0.40	1.06 ± 0.58 [0.0,2.2]	4.17 ± 0.37 [3.4,4.4]
nGu ₉₀₀	2.37 ± 0.71 [0.2,3.3]	2.38	3.34 ± 0.82 [2.3,5.1]	3.98 ± 0.80 [3.1,5.2]
nSc ₉₀₀	0.21 ± 0.60 [0.0,3.3]	0.15	0.73 ± 0.52 [0.2,2.1]	1.30 ± 0.60 [0.6,2.2]
nHT ₉₀₀	1.81 ± 1.15 [0.0,5.0]	2.32	3.18 ± 1.08 [1.8,6.3]	8.63 ± 1.03 [7.0,10.2]
nSt ₉₀₀	2.90 ± 0.86 [1.0,5.3]	5.98	4.06 ± 0.93 [2.0,6.1]	5.23 ± 0.39 [4.6,5.7]

Key to the members of the *Lasius alienus* group of Europe and Asia Minor

The *Lasius alienus* group within the European species of the subgenus *Lasius* s.str. is characterized by no or very few setae on dorsal plane of the scape (differing from the *Lasius niger* and *Lasius emarginatus* species groups) and by the masticatory margin of mandibles showing ≥ 8 teeth (differing from the *Lasius brunneus* group). The data in the key refer to nest sample means.

- 1a Sparsely haired: nSt 0–1.3, nOcc < 8.0, nGu 0–2.5 , nHT 0–1. Propodeal dome in medium-sized specimens more conic. Scape shorter: SL/CL 0.921 ± 0.019. Body concolorous dark brown *alienus*
- 1b Less sparsely haired: nSt 1.8–8.0, nOcc 5.2–18.0, nGu 0.9–4.8, nHT 0–8. Propodeal dome in medium-sized specimens more hemispheric 2

2a	Pubescence on head and mesosoma more dilute, nest means of sqPDCL > 3.82. Terminal (6 th) segment of maxillary palps shorter: MP6 < 0.140 mm. With CW and MP6 given in mm and PDCL in μm , discriminant 6.164 CW +1.758 sqPDCL -62.482 MP6 -3.718 > 0 [error in 145 samples 0%]	3
2b	Pubescence on head and mesosoma rather dense: nest means of sqPDCL < 3.82. Terminal (6 th) segment of maxillary palps longer: MP6 > 0.140 mm. With CW and MP6 given in mm and PDCL in μm , discriminant 6.164 CW +1.758 sqPDCL -62.482 MP6 -3.718 < 0 [error in 145 samples 0%]	4
3a	With all measurements given in mm, discriminant 24.577 CW -125.645 EYE -7.836 SL +0.477 nGen +31.866 GuHL +7.476 > 0 [error in 12 samples 0%]. Iberia, sympatric with <i>psammophilus</i> in S France	<i>piliferus</i>
3b	With all measurements given in mm, discriminant 24.577 CW -125.645 EYE -7.836 SL +0.477 nGen +31.866 GuHL +7.476 < 0 [error in 58 samples 0%]. Asia Minor and Europe except Iberia	<i>psammophilus</i>
4a	Corsica only. Terminal (6 th) segment of maxillary palps very long, genae with more numerous setae: MP6/CS >0.200, nGen >2.8	<i>casevitzi</i>
4a	European Mainland. Terminal (6 th) segment of maxillary palps shorter, genae with less numerous setae: MP6/CS <0.200, nGen <2.8	5
5a	Terminal (6 th) segment of maxillary palps longer and longest hair on pronotum shorter. With the metric data of PnHL and MP6 given in mm, discriminant 77.625 PnHL - 107.575 MP6 + 5.916 <0 [error 0% in 49 nest samples]. Europe west to S France and north to S Sweden.	<i>paralienus</i>
5b	Terminal segment of maxillary palps shorter and longest hair on pronotum longer. With the metric data of PnHL and MP6 given in mm, discriminant 77.625 PnHL - 107.575 MP6 + 5.916 >0 [error 0% in 20 nest samples]. Asia Minor, Balkans northwest to east Austria and south Slovakia.	<i>bombycina</i>

Zoogeographic aspects

The simple discriminant function using PnHL and MP6 only allowed a time-saving assessment of all collection material stored in the collection of SMN Görlitz and a new interpretation of zoogeographic and biological aspects (Fig. 6). *Lasius paralienus* has a European, north meridional to temperate distribution. In the south it ranges from S France (0.7°W) over all of Italy east to Bosnia and Bulgaria (25°E). The main distribution in Central Europe reaches north to about 52.5°N. North of this line only isolated populations are known in Sweden: Södermanland (59.1°N), Öland (56.7°N) and Gotland (57.4°N). In Germany it ascends to 990 m (at 47.9°N) and at the southern slope of the Alps to 1800 m (at 46.8°N).

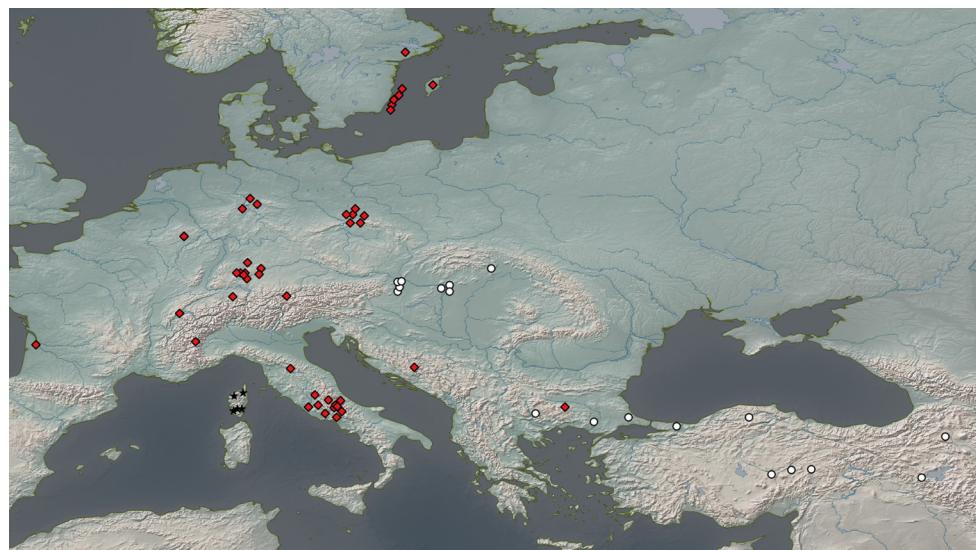


FIGURE 6. Distribution of collecting sites of *Lasius casevitzi* sp. nov. (black stars), *L. paralienus* (red rhombs) and of *L. bombycina* sp. nov. (white discs). For exact coordinates see section 2.1.

L. bombycina sp. nov. has a meridional to submeridional Balkanian-Anatolian distribution. The most northwestern point of its range is in E Austria (48.0°N, 16.7°E). In Asia Minor it goes east to 44°E at least and shows here a very wide altitudinal range from sea level to 2350 m (at 40.6°N). Sympatric occurrence with *L. paralienus* is known so far only in the NW Balkans and Bulgaria.

L. casevitzi sp. nov. is clearly a Corsican endemic. Due to its comparably strong isolation, this Mediterranean island has many endemic insect species with rather strongly deviating characters. Among ants these are *Temnothorax melas* (Espadaler *et al.*, 1984), *Temnothorax corsicus* (Emery, 1895), *Formica corsica* Seifert, 2002 and *Plagiolepis delaubergerrei* Casevitz-Weulersse, 2014.

Description of *Lasius casevitzi* sp.nov.

Etymology. The species is dedicated to Janine Casevitz-Weulersse who investigated the Corsican ant fauna over many years.

Type material. See in section 2.2."Type Material".

Description of worker caste. Figs. 7–9. Numeric data given in the following description are primary data (without removal of allometric variance) of 18 measured individuals.



FIGURE 7. Head of a paratype of *L. casevitzi* sp. nov. from the holotype locality in dorsal view.

Rather small-sized, CS 849 ± 41 [779,912] μm . Basic morphology not different from other members of the *Lasius alienus* group. Head moderately elongated, with convex sides and straight posterior margin of vertex, CL/CW 1.078 ± 0.019 [1.039,1.123]. Scape rather long, SL/CS 1.006 ± 0.016 [0.981,1.036]. Eye medium-sized and moderately distant from posterior margin of head, EYE/CS 0.242 ± 0.004 [0.236,0.248], PoOc/CL 0.247 ± 0.005 [0.239,0.254]. Antennal socket situated rather close to posterior clypeal margin, dCLAn/CS 4.43 ± 0.26 [4.06,4.97]%. Masticatory border of mandibles with 8-9 teeth. Maxillary palps with six segments and much longer than in related species, MP6/CS 0.214 ± 0.009 [0.201,0.235]. Pubescence on whole body surfaces dense and whitish, sqPDCL 3.35 ± 0.20 [3.00,3.72]. Mean length of pubescence hairs between frontal carinae 36.9 ± 2.23 [32.9,40.5] μm . Pilosity compared to next related species rather rich and long—in particular on genae and tibiae. Eyes with several microsetae of 11-16 μm length. Scape with no or very few standing setae: nSc 1.20 ± 0.89 [0.0,3.0]. Genae and extensor profile of hind tibia with more erect setae than in the next related species: nGen 3.86

± 0.71 [2.5,5.0], nHT 7.85 ± 1.73 [5.5,12.5]. Underside of head and hind margin of vertex with rather numerous standing setae: nGu 3.69 ± 0.94 [2.0,6.0], nOcc 11.90 ± 1.83 [9.5,15.5]. Metapleuron below level of propodeal stigma with rather many erect to suberect setae: nSt 4.72 ± 0.87 [3.0,6.0]. Length of setae on pronotum rather large: PnHL/CS 0.157 ± 0.018 [0.107,0.175]. Whole body concolorous medium to dark brown; mandibles, scape and sometimes tibia lighter reddish or yellowish brown. Mesosoma in lateral view with an angulate-convex propodeal dome. Petiole scale low, in frontal view with slightly convex sides converging towards the dorsal crest which is straight or slightly convex.



FIGURE 8. Mesosoma of a paratype of *L. casevitzi* sp. nov. from the holotype locality in lateral view.

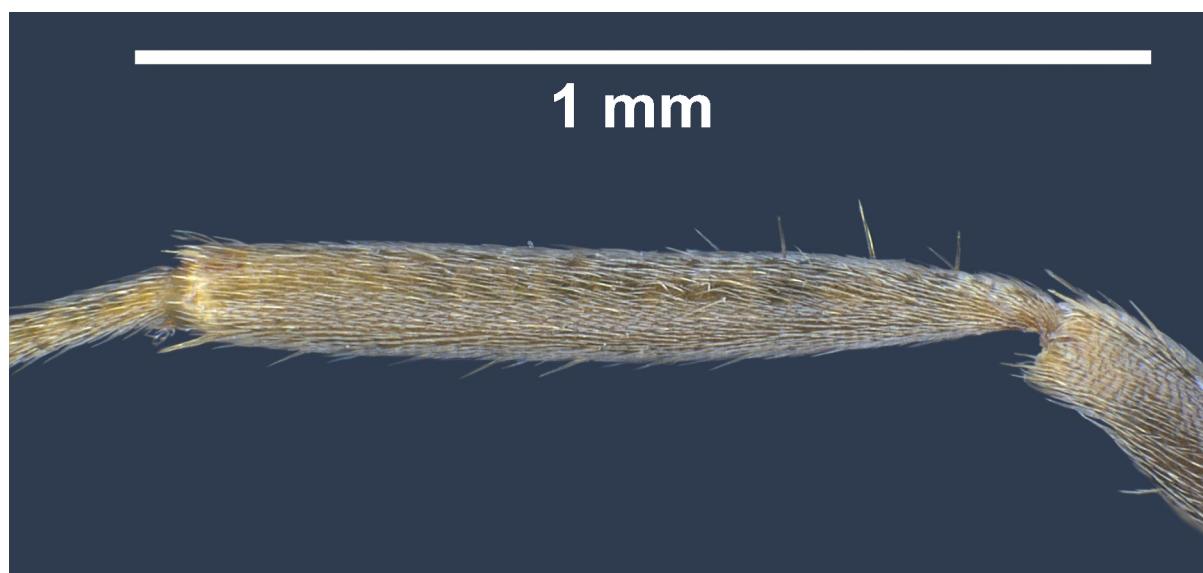


FIGURE 9. Hind tibia of a paratype of *L. casevitzi* sp. nov. from the holotype locality seen in the plane of flexion.

Comments. It is an endemic of Corsica where it is a common species. It does not occur in xerothermous grassland unlike other members of the *L. paralienus* complex and prefers semi- arboreal habitats and dry open forest. Nests are in soil, under trees or under stones. It is absent from the coastal environment and from elevations

above 1100 m. The altitudinal extremes within 50 records were 23 m and 1100 m, with the majority of nests found between 300 m and 800 m. Alates were not observed in the nests during collecting in July and August. The ants behave aggressively during disturbance of the nest by the collector.

Description of *Lasius bombycina* sp.nov.

Etymology. The species epithet refers to the rich, whitish pubescence giving the cuticular surface a silky appearance.

Type material. See in section 2.2."Type Material".

Description of worker caste. Figs. 10–12. Numeric data given in the following description are primary data (without removal of allometric variance) of 50 measured individuals.

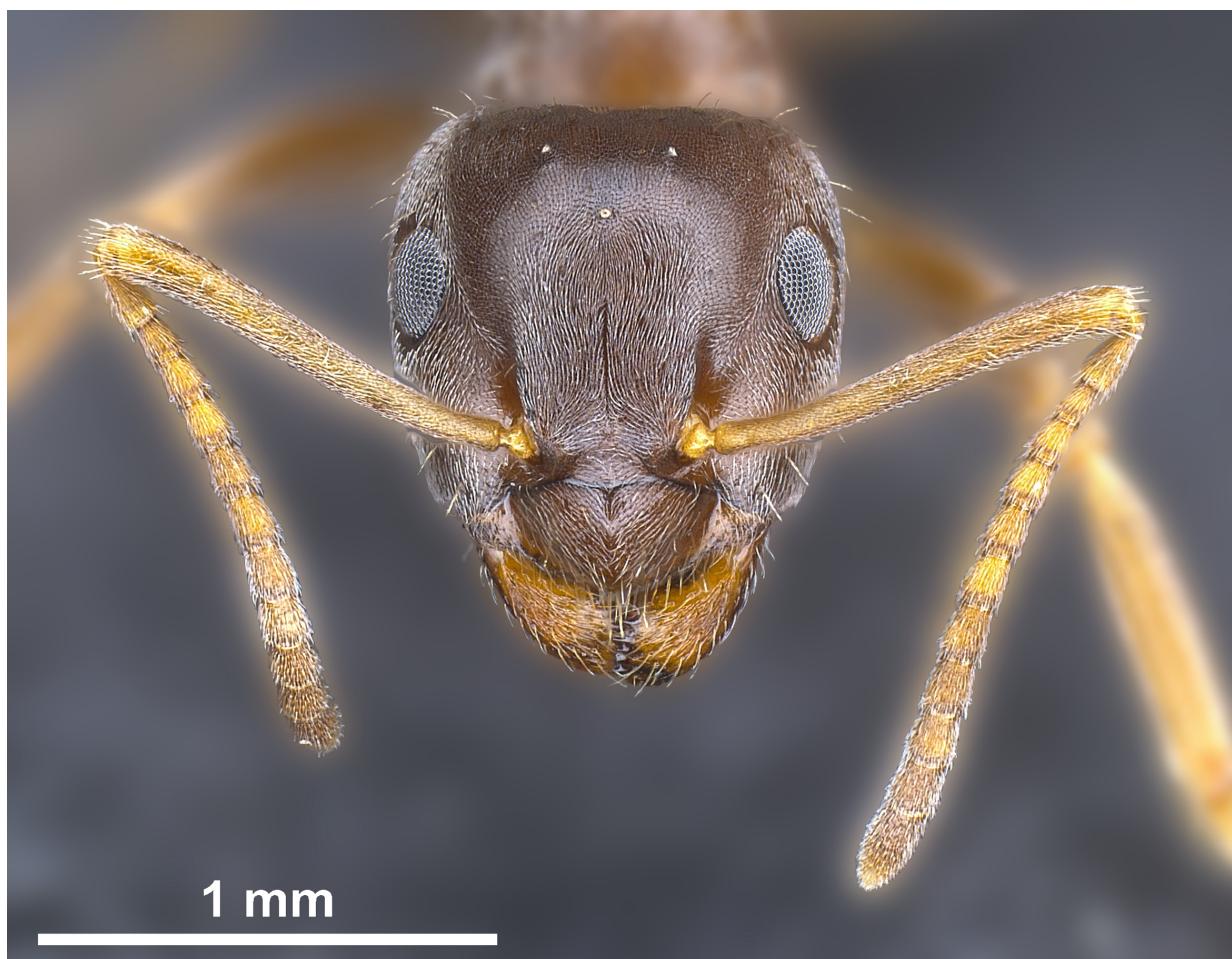


FIGURE 10. Head of the holotype of *L. bombycina* sp. nov. in dorsal view.

Rather large compared to related species, CS 893 ± 68 [744,1076] μm . Basic morphology not different from other members of the *Lasius alienus* group. Head moderately elongated, with convex sides and straight posterior margin of vertex, CL/CW 1.077 ± 0.017 [1.043,1.112]. Scape rather short: SL/CS 0.970 ± 0.018 [0.932,1.008]. Eye medium-sized and moderately distant from posterior margin of head: EYE/CS 0.238 ± 0.007 [0.223,0.253], PoOc/CL 0.239 ± 0.008 [0.224,0.257]. Antennal socket situated rather close to posterior clypeal margin: dCLAn/CS 4.34 ± 0.35 [3.75,5.07]%. Masticatory border of mandibles with 8-9 teeth. Maxillary palps with six segments and comparably short: MP6/CS 0.160 ± 0.009 [0.143,0.176]. Pubescence on whole body surfaces very dense and whitish, sqPDCL 3.42 ± 0.23 [3.05,3.98]. Mean length of pubescence hairs between frontal carinae 33.9 ± 2.38 [29.9,35.8] μm . Pilosity compared to next related species long. Eyes with rather many microsetae of 23.1 ± 1.98 [18,25] μm length (these unusually long microsetae are perhaps diagnostic but this was not thoroughly checked).

Dorsal plane of scape with no or very few standing setae: nSc 0.55 ± 0.63 [0.0,2.5]. Genae with very few erect setae: nGen 1.10 ± 0.80 [0.0,3.0]. Underside of head and hind margin of vertex with rather numerous standing setae: nGu 3.34 ± 1.34 [1.5,8.5], nOcc 12.30 ± 2.66 [4.5,18.0]. Extensor profile of hind tibia with few and metapleuron below level of propodeal stigma with rather many erect to suberect setae: nHT 3.15 ± 1.77 [0.5,8.5], nSt 4.13 ± 1.32 [1.5,7.5]. Setae on pronotum long: PnHL/CS 0.161 ± 0.011 [0.137,0.201]. Whole body concolorous medium to blackish brown; mandibles, scapes and sometimes tibiae lighter reddish or yellowish brown. Mesosoma in lateral view with a rather low and more convex propodeal dome. Petiole scale low, in frontal view with convex sides which strongly converge towards the rather narrow dorsal crest which is slightly emarginate to straight.



FIGURE 11. Mesosoma of the holotype of *L. bombycina* sp. nov. in lateral view.

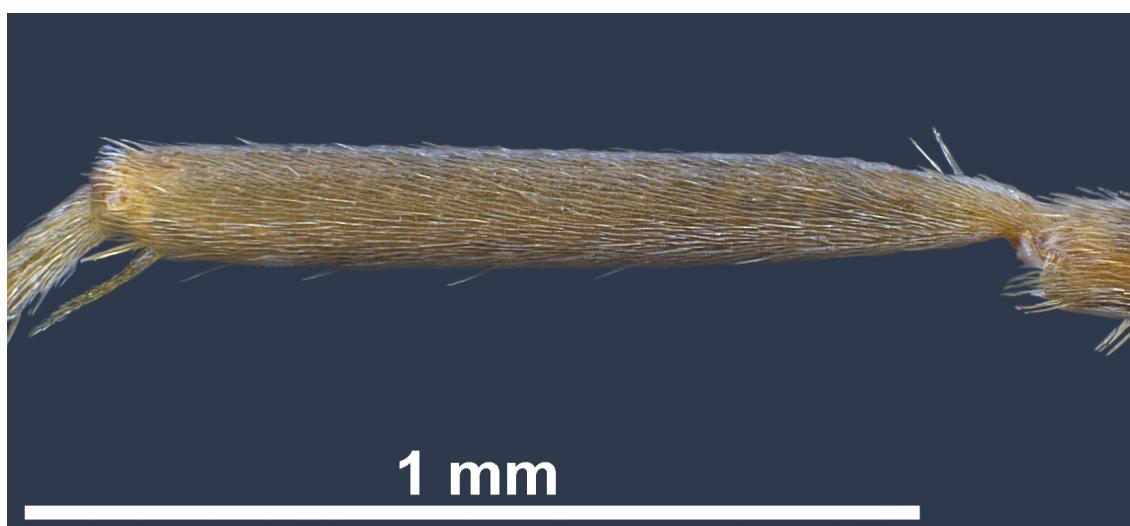


FIGURE 12. Hind tibia of the holotype of *L. bombycina* sp. nov. seen in the plane of flexion.

Comments. For the geographic distribution see section 4.4. The main habitats are different kinds of xerothermous grassland, but in Turkey this species was also found in an urban garden. It frequently builds more hidden soil nests but in the steppes of the Neusiedler See region conspicuous soil mounds overgrown by herbs were observed which seems to be a difference to *L. paralienus*. Over the whole range, alates are observed inside the nests or swarming rather late: 9 September ± 26 d [1 August – 21 October] n=7. Oviposition of foundresses usually does not start before the next spring and workers develop until July. Gynes are on average larger than in *alienus* and *psammophilus*. In a laboratory nest, a founding gyne reared 26 workers without feeding (Andrasfalvy 1961; named by Andrasfalvy "*Lasius alienus*", recent determination as *L. bombycina* sp. nov. by geographic indication, swarming time and verbal description). Above-ground foraging was observed on the soil surface, in the herb layer and on bushes. There is trophobiosis with subterranean and epigaean Aphidae and tending of Lycaenidae caterpillars (*Plebejus sephirus*, *Plebejus argus*). *Lasius bombycina* is zoophagous on small invertebrates.

Acknowledgements

We wish to thank Roland Schultz, SMN Görlitz for producing the distribution map and the z-stack photographs of the type specimens.

References

- Andrasfalvy, A. (1961) Mitteilungen über Daten des Hochzeitsfluges verschiedener Ameisenarten in Ungarn und Ergebnisse von Versuchen der Koloniegründung im Formicar bei diesen Arten. *Insectes Sociaux*, 8 (4), 299–310.
<http://dx.doi.org/10.1007/BF02226553>
- Janda, M., Folková, D. & Zrzavý, J. (2004) Phylogeny of *Lasius* ants based on mitochondrial DNA and morphology, and the evolution of social parasitism in the Lasiini (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution*, 33, 595–614.
<http://dx.doi.org/10.1016/j.ympev.2004.07.012>
- Lachenbruch, P. & Mickey, M. (1968) Estimation of error rates in discriminant analysis. *Technometrics*, 10, 1–11.
<http://dx.doi.org/10.1080/00401706.1968.10490530>
- Lesaffre, E., Willems, J.L. & Albert, A. (1989) Estimation of error rate in multiple group logistic discrimination. The approximate leaving-one-out method. *Communication in Statistics Theory and Methods*, 18, 2989–3007.
<http://dx.doi.org/10.1080/03610928908830072>
- Maruyama, M., Steiner, F., Stauffer, C., Akino, T., Crozier, R.H. & Schlick-Steiner, B.C. (2008) A DNA and morphology based phylogenetic framework of the ant genus *Lasius* with hypotheses for the evolution of social parasitism and fungiculture. *BioMed Central Evolutionary Biology*, 8, 237.
<http://dx.doi.org/10.1186/1471-2148-8-237>
- R Development Core Team (2012) R: a language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria. Available from: <http://www.R-project.org/> (accessed 31 December 2012)
- Seifert, B. (1992) A taxonomic revision of the Palaearctic members of the ant subgenus *Lasius* s.str. (Hymenoptera: Formicidae). *Abhandlungen und Berichte des Naturkundemuseums Görlitz*, 66 (5), 1–67.
- Seifert, B. (2008) Removal of allometric variance improves species separation in multi-character discriminant functions when species are strongly allometric and exposes diagnostic characters. *Myrmecological News*, 11, 91–105.
- Seifert, B. (2009) Cryptic species in ants (Hymenoptera:Formicidae) revisited: we need a change in the alpha-taxonomic approach. *Myrmecological News*, 12, 149–166.
- Seifert, B. (2014) A pragmatic species concept applicable to all eukaryotic organisms independent from their mode of reproduction or evolutionary history. *Soil Organisms*, 86, 85–93.
- Seifert, B. & Csösz, S. (2015) *Temnothorax crasecundus* sp. n. – a cryptic Eurocaucasian ant species (Hymenoptera, Formicidae) discovered by Nest Centroid Clustering. *Zookeys*, 479, 37–64.
<http://dx.doi.org/10.3897/zookeys.479.8510>
- Seifert, B., Ritz, M. & Csösz, S. (2013) Application of Exploratory Data Analyses opens a new perspective in morphology-based alpha-taxonomy of eusocial organisms. *Myrmecological News*, 19, 1–15.
- Wilson, E.O. (1955) A monographic revision of the ant genus *Lasius*. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 113, 1–201.