SOIL ORGANISMS **96** (3) · December 2024 pp. 183–194

Lasius frequens n.sp. – a sister species of the supercolonial pest ant *Lasius neglectus* showing a differing biology (Hymenoptera: Formicidae)

Bernhard Seifert

Department of Entomology, Senckenberg Museum of Natural History Görlitz, Am Museum 1, 02826 Görlitz, Germany
E-mail: bernhard.seifert@senckenberg.de

Received 17 September 2024 | **Accepted** 7 October 2024
Published online at www.soil-organisms.org 1 December 2024 | **Printed version** 15 December 2024
DOI https://doi.org/10.25674/435

Abstract

A new sister species of the supercolonial invasive pest ant *Lasius neglectus* Van Loon et al., 1990 is described based on a broad sample from the Iran. It is named *Lasius frequens* n. sp. due to its abundance in that region. The strongest difference to the next similar species *Lasius turcicus* Santschi, 1921, *L. neglectus* Van Loon et al., 1990 and *L. precursor* Seifert, 2020 are distinctly longer scapes and terminal segments of maxillary palps. In order to disentangle the complicated situation within this species complex, species hypotheses were generated by exploratory and hypothesis-driven data analyses using numeric recordings of 16 morphological characters within a total of 255 nest samples with 782 worker individuals. The exploratory data analyses NC-part.kmeans and NC-NMDS.kmeans indicated the existence of five clusters. These classification hypotheses were confirmed through a controlling linear discriminant function (LDA) by 99.2% in NC-part.kmeans and by 97.2% in NC-NMDS.kmeans. As NC-clustering does not expose hybrid samples, the spatial distribution of the clusters was checked in the simple vectorial space by 2-dimensional plotting of cluster triples in a LDA. There was no indication for substantial interspecific hybridization in any triple plotted. The clusters 1, 2, 3 and 4 could be attributed to the four above-mentioned taxa by clear allocation of the type samples. A fifth, strongly separated, morphological cluster was not described as a separate species but was assumed to represent a setae morph of *Lasius turcicus*. *Lasius frequens* n. sp. differs from *L. neglectus* in having its main distribution in natural habitats but 28% of the samples were also found in rural and urban habitats. Gynes of *L. frequens* n. sp. have about 160% of the mesosoma volume of *L. neglectus* gynes which indicates strongly developed flight muscles. This morphological trait and the wide distribution over most different habitat types indicate that this species should perform, in contrast to *L. neglectus*, a normal nuptial flight with long-range flight-dispersal and independent single-queen colony foundation.

Keywords Invasive ants | *Lasius turcicus* species complex | cryptic species |
numeric morphology-based alpha-taxonomy | stereomicroscopy

1. Introduction

Ants of the subgenus *Lasius* s. str. are important elements of open land and woodland ecosystems of the Holarctic. One species, the supercolonial invasive pest ant *Lasius neglectus* Van Loon et al., 1990 has explosively spread over urban regions of Europe after about 1985 where it is causing much havoc (e.g. Seifert 2000, Espadaler et al. 2007, Ugelvig et al. 2008). It belongs to the *Lasius turcicus* complex which is well separable from other members of the subgenus by having a smaller number of mandibular dents, by a petiole scale appearing in

ISSN 1864-6417 · **eISSN** 2509-9523

lateral view thin and forming an acute tip and with a dorsal crest being in anterior view straight or slightly emarginate, by a very dilute clypeal pubescence and by absence of setae on dorsal plane of scape (Seifert 2020a). The members of the *L. brunneus* complex fully match this character combination but are well separable from those of the *L. turcicus* species complex by much lower setae numbers on posterior margin and underside of head and on metapleuron. Six species of the *L. turcicus* species complex were recognized in the taxonomic revision of Palaearctic species (Seifert 2020a): *Lasius austriacus* Schlick-Steiner, 2003, *L. tapinomoides* Salata & Borowiec, 2018, *L. israelicus* Seifert, 2020, *Lasius turcicus* Santschi, 1921, *L. neglectus* Van Loon et al., 1990 and *L. precursor* Seifert, 2020. The latter three species are closely related and difficult to separate and the question arises which species is the next relative of the infamous *Lasius neglectus*. A recent analysis of 120 nest samples of *Lasius* s. str. ants from the Iran added a new element to the story by discovering a fourth cryptic species.

The analysis of Iranian material resulted in the separation of at least 11 species of the subgenus *Lasius* s. str. Surprisingly, a single so far undescribed species made up 55 % of the total Iranian material. This species, named here *Lasius frequens* n. sp. due to its abundance in that region, is by worker morphology very close to *L. neglectus*. In contrast to *L. neglectus* which has so far not been observed in natural habitats, *L. frequens* n. sp. has its main habitat in the Iran in natural broad-leafed Caspian forest. Furthermore it was not observed by the different Iranian collectors to be supercolonial and it has gynes with much larger mesosoma volume than *L. neglectus* indicating a stronger potency for dispersal flight and independent single-queen colony foundation. This allowed a spreading also into rural and urban habitats with some greenery where some 28 % of all findings were made. As a whole, *L. frequens* n. sp. seems to be, in its natural history, quite a “normal” *Lasius* s. str. ant.

Thanks to the large sample size now available it was possible to clearly demonstrate by exploratory and hypothesis-driven data analyses the separate identity of *Lasius frequens* n. sp. from the next similar species *L. turcicus*, *L. neglectus* and *L. precursor.* Describing this species and showing the complicated morphological structures and relations between these four most related taxa is the purpose of this paper.

2. Material

Excluding single-specimen samples and those with an incompletely recorded character set, NUMOBAT data of the four considered species were evaluated in a total of 255 nest samples with 782 worker individuals. With the exception of samples of special relevance, data of this large material are not presented in detail in the main text of this paper but listed up in the supplementary information SI1 and SI2. Material of *Lasius frequens* n. sp. was available in 67 samples – 2 from Turkey and 65 from the Iran. *Lasius turcicus* material was available in 76 samples – 8 from Greece, 60 from Turkey and 8 from Iran. Material of *L. precursor* was available in 52 samples – 1 from Greece and 51 from Turkey. Material of the invasive species *L. neglectus* was investigated in 50 samples – from Belgium (3), Bulgaria (1), France (5) Georgia (4) , Germany (4), Hungary (1), Kyrgyztan (4), Poland (1), Romania (2), Spain (4) and Turkey (17).

The following type series were available for study:

***Lasius turcicus* Santschi, 1921**

Lectotype worker on the same pin with a *Prenolepis* gyne, labelled “Asie min. Angora G.d.Kerville”, “Lasius turcicus Sant SANTSCHI det.1920”, “lectotype € desig. by E.O.Wilson”, ANTWEB CASENT 0912297; 2 paralectotype workers on another pin labelled “Asie min. Angora G.d.Kerville)”, “Lasius turcicus Sant type SANTSCHI det.1920”, “K. 201”; depository NHM Basel.

***Lasius neglectus* Van Loon et al., 1990:**

7 paratype workers from the holotype colony labelled „HUNGARY Budapest 1. VII 1988“; depositories: NHM London, SMN Görlitz.

***Lasius precursor* Seifert, 2020**

Holotype worker plus 4 paratype workers and 3 alate gynes labelled “TUR:39.795° N,26.681°E, Üzümlü-1.1 km N, 167 m, rural grassland, leg. Cremer et al. 2004.06.08 -712”; 5 paratype workers plus 2 males labelled “TUR:39.795° N,26.682°E, Üzümlü-1.1 km N, 160 m, rural grassland, leg. Cremer et al. 2004.06.08-710”; depository SMN Görlitz.

***Lasius frequens* n. sp.**

Holotype worker plus 2 paratype workers labelled “IRAN: 37.46261°N, 49.33553°E

Abkenar, -30 m, horticultural area in humid lagoon, A.Yazdi 2016.06.15 -392”; further paratype samples see under species description; depository SMN Görlitz.

3. Methods

Species separation was based on the Gene and Gene Expression (GAGE) species concept which derivation is explained in detail elsewhere (Seifert 2020b). Equipment, recording of morphological characters, removal of allometric variance, running of explorative and supervised data analyses, classification and statistical testing is thoroughly explained in Seifert (2020a). Only those statements, which are essential for understanding the species description, the main text and the evaluation procedures of this paper, are repeated here.

Sixteen thoroughly recorded morphometric characters (seven shape, seven seta and one pubescence character as well as absolute size (indicated by CS) used for characterization of workers are defined below including three mesosoma measures only used in gynes. Figures assisting the definition of these characters are given in Seifert (2018). All bilateral characters were recorded as arithmetic mean of both sides. MaDe, the number of mandibular dents, is an accessory character which was not used in the multiple data analyses as it was not recordable in each specimen because of a partially concealed masticatory border due to mandible adduction. Seta counts only include seta projecting at least 20 µm from cuticular surface. Here, a parallax error in estimating this distance may occur due the different viewing angles of the left and right eye. Assessing this distance has to be done with the measuring scale kept perpendicular in the visual field and the respective spot of cuticular surface adjusted in a right angle to the scale which often needs to rotate the measured object in the visual plane. This is crucial in counting postocular setae numbers: if the measuring scale but also the median line of head are kept perpendicular in the visual field and the measuring scale is placed in the left ocular, setae distance on the right head side will be underestimated and too low seta numbers will be counted.

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.

dClAn: Torulo-clypeal distance: the shortest distance from posterior clypeal suture (PCS) to inner margin of antennal torulus (socket). The right spatial adjustment is given when upper and lower portions of this inner margin superimpose. If no surface structure indicates the position of PCS, the center 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 mandibular dents; suggested denticles may score in the count as 0.5.

MH: Mesosoma height in gynes: longest section line directed perpendicular to the straight dorsal profile line of mesosoma (formed by mesonotum and scutellum). The lower measuring point is usually the lowest part of mesopleuron.

ML: Mesosoma length from caudalmost point of mesosoma (either median near to petiolar junction or more lateral at propodeal lobe) to the most distant point of steep anterior pronotal face.

MP6: Length of the sixth (terminal) segment of maxillary palps.

MW: Maximum mesosoma width anterior of the tegulae.

nGen: With head in full face view, number of setae on head sides frontal of anterior eye margin (“gena”). The bilateral sum is halved.

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 also 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. The bilateral sum is halved.

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.

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 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 hair length is considered. Square root data transformation is applied to normalize positively skewed distributions.

Removal of allometric variance (RAV) was performed following the basic 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 (Seifert 2020a).

Linear discriminant analysis (LDA) without and with stepwise character reduction as well as ANOVA tests were run with the SPSS 15.0 software package.

Four different forms of exploratory data analyses (EDA) were run using nest centroids as input data (NC clustering, Seifert et al. 2013). These were hierarchical NC-Ward clustering, hierarchical NC-part.hclust and the iterative vector-quantization method NC-part.kmeans – both implemented in partitioning algorithms based on recursive thresholding (for details see Csösz & Fisher, 2015). The fourth EDA used was NC-NMDS.kmeans – a nonmetric multidimensional scaling combined with iterative vector-quantization (Seifert et al. 2013).

Checking samples with controversial classifications was done by an interaction of NC clustering and a controlling linear discriminant analysis (LDA) in which these samples were run as wild-cards, following the rationale described in Seifert et al. (2013). The final classification (“final species hypothesis”) was established by the LDA in an iterative procedure and there remained no undecided cases even if their posterior probabilities were close to 0.5.

The different variants of NC-clustering – in particular NC-part.kmeans and NC-NMDS.kmeans clustering – are excellent methods for hypothesis formation in taxonomic data sets. However, there is a danger. When substantial numbers of hybrids are in a data set, these methods may suggest a very clear species separation when such a strong separation does really not exist. Examples are reported for members of the *Formica rufa* group (Seifert 2021) and in *Myrmica* (Seifert 2024). In other words, hybrid clusters are not or only tentatively exposed by NC-Clustering because these methods tend to allocate hybrid samples to either parental cluster. For this reason even seemingly well separate NC-clusters have to be checked by assessing their spatial distribution in the simple vectorial space. This was done here by plotting of cluster triples in the 2-dimensional LDA space.

4. Results and Discussion

4.1 Delimitation and naming of species clusters

Four variants of NC-clustering were run in 255 worker samples using 15 characters: absolute head size CS and the RAV-corrected characters CL/CW_{900}, SL/CS_{900}, $dCLAN/CS_{900}$, $PoOc/CL_{900}$, EYE/CS_{900}, $MP6/CS_{900}$, $GuHL/CS_{900}$, $PnHL/CS_{900}$, nHT_{900}, $nOcc_{900}$, $nGen_{900}$, nGu_{900}, nSt_{900} and $sqPDCL_{900}$. The number of scape setae nSc was removed from evaluation because it did not provide information for species delimitation. The clustering results of NC-Ward, NC-part.kmeans and NC-NMDS.kmeans, are shown in Fig. 1. All three methods indicated the existence of five clusters. The disagreement between the controlling LDA, that fixed the final species hypothesis, and the classification by the exploratory data analyses were 10.2 % in NC-Ward, 0.8 % in NC-part.kmeans and 2.8 % in NC-NMDS. kmeans. Based on the clear placement of type series, the five clusters were named *Lasius frequens* n. sp., *L. neglectus*, *L. precursor*, *L. turcicus* normal morph and *L. turcicus* morph RI from Rhodes and the Iran. Tab. 1 shows the posterior probabilities of the type series in a LDA with K=5 considered classes.

The high overall agreement of the NC-part.kmeans and NC-NMDS.kmeans classifications with the final species hypothesis shown above might indicate a rather clear taxonomic situation. However, as NC-clustering is in danger to conceal hybrid samples, it is necessary to check the delimitation of the clusters in the simple vectorial space. This was done here by two-dimensional LDA-plots of species triples (Figs 2–5). With exception of the plots of *L. precursor* against the normal morph of *L. turcicus* (Figs 2 and 4), any other combination of morphological clusters is separated by gaps. Hence, the morphological distinctness between *L. frequens* n.sp., *L. neglectus* and *L. turcicus* morph RI – and each of

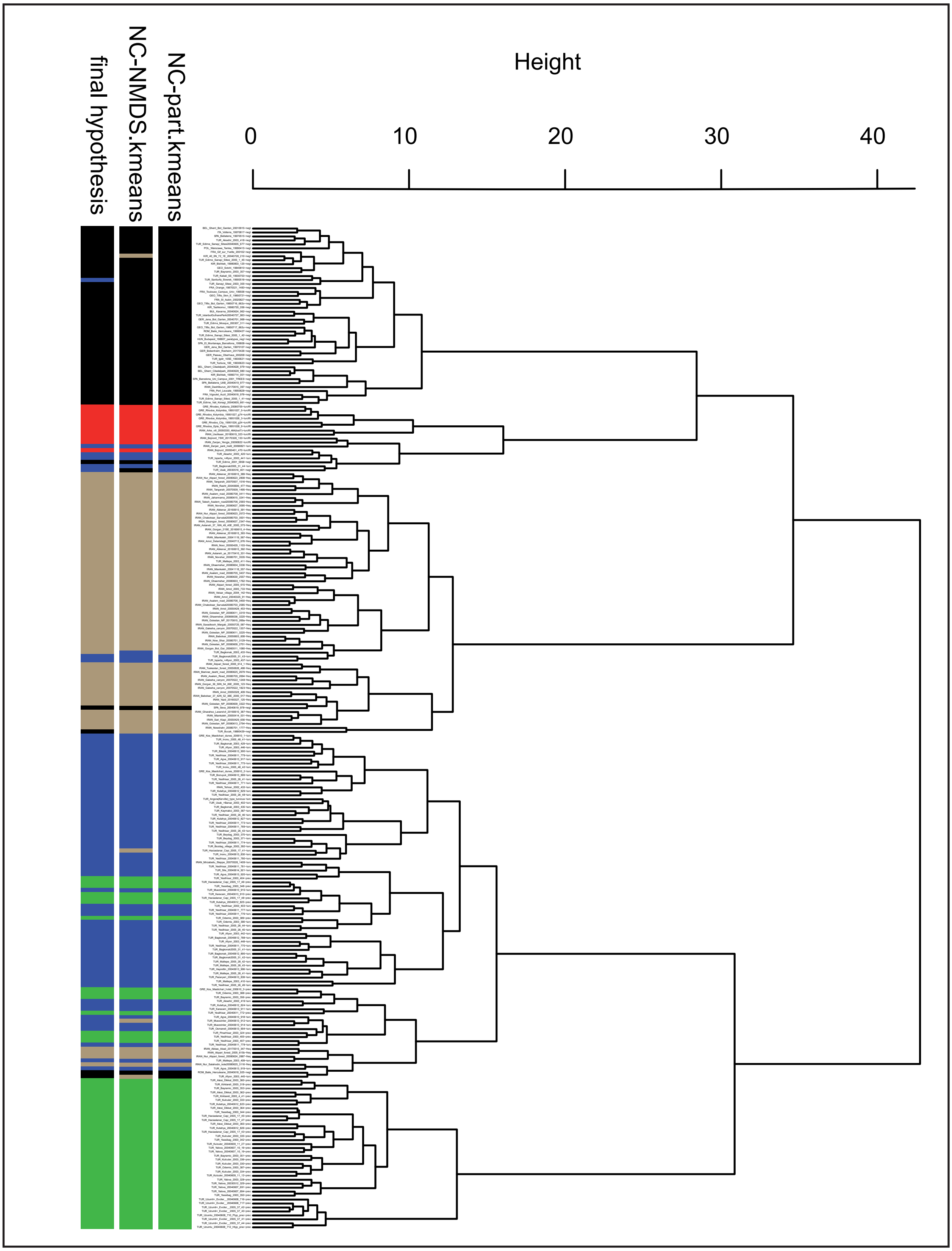

Figure 1. Demonstration of five clusters within the *Lasius turcicus* species complex by three variants of NC-clustering. The final classification hypothesis was fixed by a controlling linear discriminant function (LDA). The agreement of the exploratory data analysis with the final classification was 89.8 % in NC-Ward (tree shown), 99.2 % in NC-part.kmeans and 97.2 % in NC-NMDS.kmeans. Marking of bars: *L. neglectus* – black, *L. turcicus* morph RI – red, *L. frequens* n.sp. – greyish brown, *L. turcicus* – blue, *L. precursor* – green.

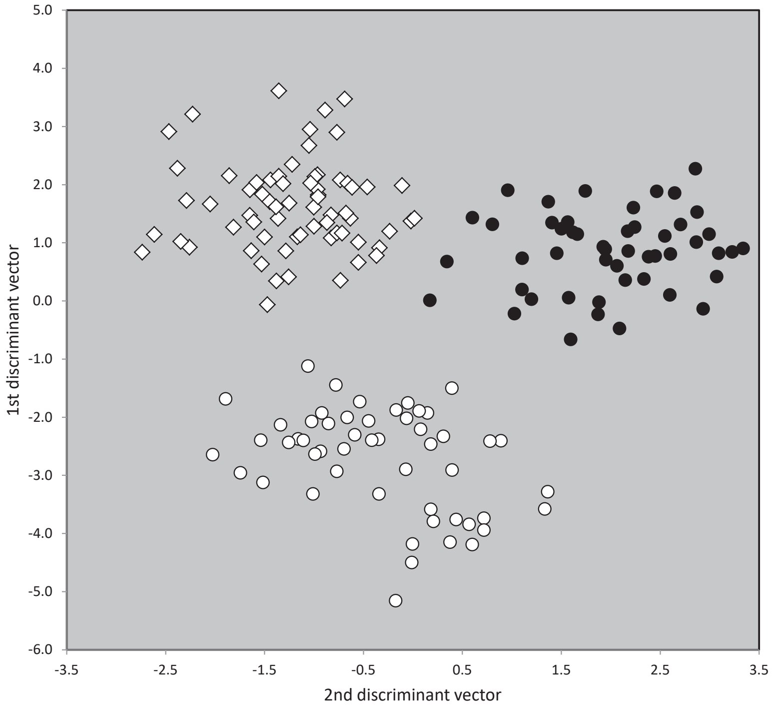

Figure 2. Plotting of nests sample means of discriminant vectors in *Lasius frequens* n.sp. (white rhombs), *L. precursor* (white dots) and *L. neglectus* (black dots).

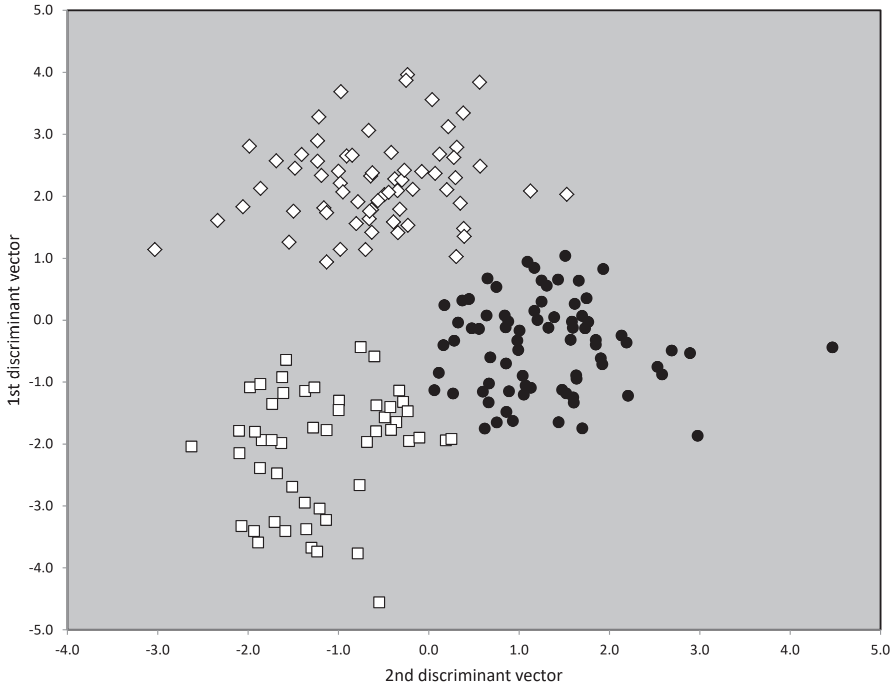

Figure 3: Plotting of nests sample means of discriminant vectors in *Lasius frequens* n.sp. (white rhombs), *L. precursor* (white squares) and *L. turcicus* (black dots).

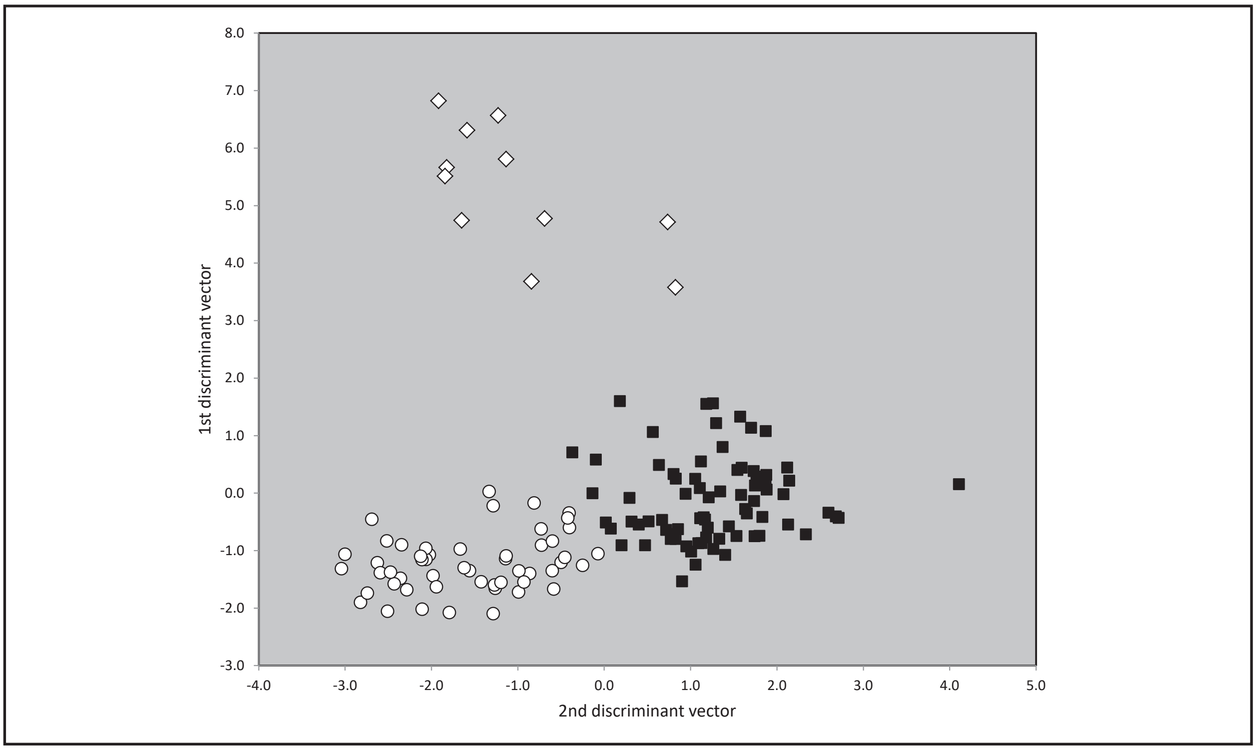

Figure 4. Plotting of nests sample means of discriminant vectors in *Lasius turcicus* morph RI (white rhombs), *L. precursor* (white dots) and *L. turcicus* (black squares).

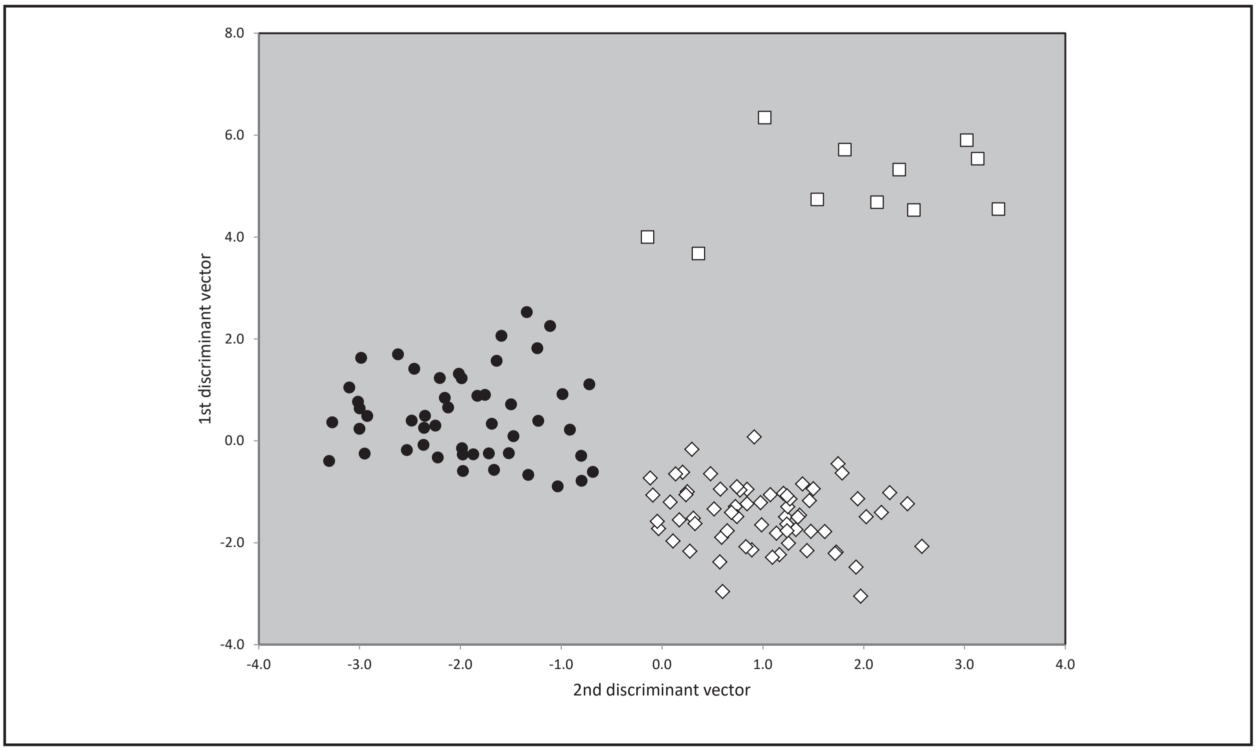

Figure 5. Plotting of nests sample means of discriminant vectors in *Lasius turcicus* morph RI (white squares), *L. neglectus* (black dots) and *L. frequens* n. sp. (white rhombs).

Table 1. Posterior probabilities of the type series of four taxa of the *L. turcicus* species complex in allocation to five distinguished morphological clusters.

type series	**p (freq)**	**p (negl)**	**p (prec)**	**p (turc)**	**p (turc RI)**
L. frequens n.sp., Abkenar / Iran	**0.9674**	0.0307	0.0000	0.0018	0.0000
L. neglectus, Budapest / Hungary	0.0321	**0.9475**	0.0092	0.0112	0.0000
L. precursor, Üzümlü / Turkey	0.0000	0.0001	**0.9997**	0.0001	0.0000
L. turcicus , Angora /Turkey	0.0039	0.0010	0.0020	**0.9931**	0.0000
L. turcicus morph RI, undescribed	n.d.	n.d.	n.d.	n.d.	n.d.

Table 2. Head size and RAV-corrected data of shape, setae and pubescence characters in worker individuals of five morphological clusters in the *L. turcicus* complex. Arrangement of data: arithmetic mean ± standard deviation [minimum, maximum]. The full length of the masticatory border of mandibles is often concealed in mounted specimens. Thus, MaDe was recordable in only that number of specimens given after the maximum value.

	precursor (n=194)	***neglectus* (n=143)**	**frequens (n=196)**	***turcicus* (n=237)**	***turcicusRI* (n=42)**
code	**prec**	**negl**	**freq**	**turc**	**turcRI**
CS [µm]	770 ± 51 [656,887]	767 ± 43 [620,907]	785 ± 61 [610,921]	861 ± 59 [682,1019]	835 ± 47 [697, 943]
CL/CW (900)	1.074 ± 0.018 [1.038,1.124]	1.091 ± 0.012 [1.054,1.123]	1.092± 0.014 [1.048,1.133]	1.083± 0.015 [1.025,1.130]	1.075 ± 0.014 [1.047,1.110]
SL/CS (900)	0.946 ± 0.018 [0.891,1.002]	0.966 ± 0.014 [0.924,0.999]	0.995 ± 0.017 [0.952,1.043]	0.964 ± 0.017 [0.889,1.019]	0.969 ± 0.013 [0.931,0.999]
MP6/CS (900)	0.183 ± 0.010 [0.156,0.209]	0.191 ± 0.007 [0.173,0.210]	0.206 ± 0.009 [0.177,0.235]	0.192 ± 0.009 [0.168,0.215]	0.194 ± 0.007 [0.178,0.208]
PoOc/CL (900)	0.237 ± 0.008 [0.219,0.257]	0.228 ± 0.006 [0.211,0.251]	0.225 ± 0.006 [0.210,0.246]	0.229 ± 0.007 [0.210,0.258]	0.234 ± 0.007 [0.213,0.247]
EYE (900)	0.236 ± 0.005 [0.223,0.248]	0.240 ± 0.005 [0.228,0.254]	0.239 ± 0.005 [0.222,0.253]	0.234 ± 0.006 [0.211,0.252]	0.231 ± 0.007 [0.217,0.242]
dClAn/CS (900)[%]	3.58 ± 0.34 [2.84,4.56]	4.26 ± 0.35 [3.39,5.27]	3.90 ± 0.33 [2.83,4.78]	4.03 ± 0.41 [2.80,5.53]	3.85 ± 0.27 [3.30,4.52]
MaDe (900)	7.61 ± 0.44 [7.0,8.1] n=90	7.33 ± 0.53 [6.0,8.3] n=90	7.49 ± 0.49 [6.1,9.1] n=102	7.73 ± 0.53 [6.0,9.0] n=113	7.32 ± 0.49 [7.0,8.5] n=19
sqPDCL (900)	5.11 ± 0.47 [4.19,6.78]	5.38 ± 0.51 [4.35,6.85]	5.26 ± 0.45 [3.90,6.55]	5.33 ± 0.55 [3.76,6.81]	5.25 ± 0.50 [4.35,6.48]
GuHL/CS (900)	0.126 ± 0.010 [0.103,0.153]	0.116 ± 0.011 [0.091,0.146]	0.114 ± 0.009 [0.087,0.148]	0.128 ± 0.010 [0.094,0.154]	0.129 ± 0.011 [0.110,0.155]
PnHL/CS (900)	0.128 ± 0.009 [0.097,0.152]	0.125 ± 0.009 [0.096,0.149]	0.126 ± 0.012 [0.082,0.154]	0.127 ± 0.008 [0.102,0.152]	0.143 ± 0.011 [0.116,0.166]
nOcc (900)	5.8 ± 1.6 [1.7, 9.6]	9.6 ± 2.3 [4.6,15.3]	6.6 ± 1.9 [0.0,11.0]	6.6 ± 2.1 [2.5,15.0]	11.6 ± 2.4 [6.6,16.3]
nGen (900)	1.4 ± 0.9 [0.0, 4.2]	2.2 ± 1.5 [0.0, 7.6]	1.0 ± 0.9 [0.0, 7.5]	1.9 ± 1.1 [0.0,7.9]	2.3 ± 1.7 [0.0,5.4]
nGu (900)	3.4 ± 0.9 [1.2,6.7]	3.0 ± 1.0 [0.6,6.7]	2.9 ± 0.8 [1.1,5.2]	4.0 ± 1.2 [0.0,8.6]	3.8 ± 1.1 [2.3,6.6]
nSc (900)	0.04 ± 0.25 [0.0,2.3]	0.21 ± 0.73 [0.0,5.4]	0.02 ± 0.17 [0.0,1.8]	0.05 ± 0.20 [0.0,1.8]	0.0 ± 0.2 [0.0,1.1]
nHT (900)	0.15 ± 0.33 [0.0,1.4]	0.40 ± 0.59 [0.0,2.5]	0.11 ± 0.29 [0.0,2.1]	0.28 ± 0.48 [0.0,7.4]	2.8 ± 1.0 [1.0,5.7]
nSt (900)	3.9 ± 1.1 [0.7,7.1]	3.5 ± 1.1 [0.0,6.5]	3.6 ± 1.1 [0.5,6.1]	4.2 ± 1.3 [0.0,7.4]	5.3 ± 1.2 [2.9,9.2]

these to *L. precursor* and *L. turcicus* – is well supported.

The absence of a clear gap between the clusters of *Lasius precursor* and *L. turcicus* needs to be commented. *Lasius precursor* has a small geographic range apparently restricted to Western Asia Minor (Seifert 2020a) – at least it is quite sure that it does not reach east to the Iran. This small range is fully embedded within the larger range of *L. turcicus*. Despite significant differences in vertical distribution, closely-neighbored occurrence of the two entities is rather frequent. Microsatellite data, mating experiments, colony demography and data on cuticular hydrocarbons provided by Cremer et al. (2008) basically support the morphological species classification. However, according to mating experiments, moderate (unidirectional) gene flow might possibly occur: *Lasius precursor* males mated *L. turcicus* gynes in 38% of 29 tests whereas *L. turcicus* males never mated *L. precursor* gynes in 51 tests (Cremer et al. 2008). The issue requires further studies.

Problematic is the interpretation of the cluster named here *L. turcicus* morph RI. The morphological separation to any of the other four clusters is very strong (Figs 4 and 5) and suggests to describe it as distinct species. However, most disturbingly, this cluster is composed of six samples from the Island of Rhodes and five samples from the Iran with a gap of 1800 km in between. In the absence of conclusive genetic data, there is no place here to argue if we have widely disjunct conspecific populations or two different species showing morphological convergence. It seems better to consider this cluster for now as a morph or mutant of *Lasius turcicus* which shows longer pronotal setae and higher setae number on posterior vertex and hind tibia (Tab. 2).

4.2 Description of *Lasius frequens* n. sp. and remarks on its biology

Lasius frequens n. sp.

Etymology: Meaning “frequent”, named so because it was by far the most abundant *Lasius* s. str. species collected in the Iran.

Type material: Holotype worker plus 2 paratype workers labelled “IRAN: 37.46261°N, 49.33553°E

Figure 6. Head of the holotype worker of *Lasius frequens* n. sp. in full face view.

Figure 7. Lateral aspect of the holotype worker of *Lasius frequens* n. sp.

Abkenar, -30 m, horticultural area in humid lagoon, A.Yazdi 2016.06.15 -392"; 3 paratype workers labelled "IRAN: 37.46261°N, 49.33553°E, Abkenar, -30 m, horticultural area in humid lagoon, A.Yazdi 2016.06.15 -393"; 4 paratype workers labelled „IRAN: 37.95453°N, 49.33342°E, Abkenar, -21 m, village with a lot of greenery, A.Yazdi 2016.06.15 -391; 3 paratype workers labelled „IRAN:37.95453°N, 49.33342°E, Abkenar,-21 m, village with a lot of greenery, A.Yazdi 2016.06.15 -389"; depository SMN Görlitz.

All material examined: A total of 67 nest samples with 196 workers –two from Turkey and 65 from the Iran – was subject to numeric morphological analysis. For details see supplementary information SI1.

Geographic range: So far only known from Turkey and the Iran between 30 and 56° E and 31 and 39° N. The altitudinal distribution ranges from -30 m to 1463 m.

Description: Worker (Tab. 2, Figs 6–8; in contrast to table 2, this description uses mean values without removal of allometric variance): Body size small (CS 785 µm). Head rather long (CL / CW 1.119). Scape and terminal segment of maxillary palps longer than in all closely related species (SL / CS 1.019, MP6 / CS 0.217). Postocular and torulo-clypeal distance rather small (PoOc / CL 0.227, dClAn / CS 3.87 %). Eye moderately large (EYE / CS 0.247). Number of mandibular dents low (MaDe 7.4). Clypeal pubescence very dilute (sqPDCL 5.38). Pronotal setae rather short (PnHL / CS 0.126), slightly longer than gular setae (GuHL / CS 0.115). Setae numbers on hind vertex, underside of head, genae and metapleuron low (nOcc 6.0, nGu 2.5, nGen 0.8, nSt 2.8). Dorsum of scape and extensor side of hind tibia almost always without setae. Propodeum in lateral view with a rather shallow dome. Petiole scale in profile view rather thin with an acute dorsal tip. Pubescence hairs on frons rather long (PLF 31.0 µm). Head dark to medium brown but mandibles and lateral parts of clypeus yellowish. Mesosoma in small and medium-sized specimens light brown with a typical yellowish component which is usually not seen in *L. neglectus*. Gaster medium brown. Antennae, metatarsae and tarsae yellowish.

Figure 8. Anterior head of the holotype worker of *Lasius frequens* n. sp. in dorsofrontal view.

Biology. All but one of the Iranian findings were made along the humid Caspian zone between 35.5 and

38.6° N and at elevations between minus 30 and 1463 m. Extremely dry habitats are avoided. The exceptional finding in the city of Yazd (31.890°N, 54.356°E, 1224 m), located within a hot desert zone, was made in a park with irrigation. The habitat distribution of 65 Iranian nest samples was: 53.8 % inside humid Caspian broad-leafed forest, 12.3 % in cities or villages with gardens, 12.3 % in cities with few greeneries, 9.2 % in ecotones between Caspian broad-leafed forest and open land, 4.6 % in humid grassland with shrubs, 3.1 % in horticultural fields in a humid lagoon, 3.1 % at river banks and 1.5 % on open Caspian Sea shore. The nest microhabitats of 26 reported cases were 57.7 % under stones, 19.2 % in rotten log, 15.4 % in soil, and 3.8 % each in leaf litter and in a wall.

Contrasting the small mean worker size, the four available winged gynes of *L. frequens* n. sp. are rather large and have about 160 % of the mesosoma volume of *L. neglectus* gynes (Tab. 3) which indicates strongly developed flight muscles. This morphological trait and the wide distribution over natural, rural and urban habitats indicate that this species should perform, in contrast to *L. neglectus*, a normal nuptial flight with long-range flight-dispersal and independent single-queen colony foundation.

The biology reported above raises suspicion that parts

Table 3. Head size and shape, setae and pubescence characters in gyne individuals of four species of the *L. turcicus* complex. Arrangement of data: arithmetic mean ± standard deviation [minimum, maximum]. Numbers given after the maximum value indicate incomplete data sets.

	precursor (n=29)	***neglectus* (n=37)**	**frequens (n=4)**	*turcicus* **(n=48)**
code	**prec**	**negl**	**freq**	**turc**
CS [µm]	1331 ± 37 [1271,1410]	1267 ± 36 [1200,1375]	1398 ± 44 [1349,1446]	1483 ± 38 [1388,1550]
ML*MW*MH	5.528 ± 0.598 [4.330,7.024]	4.883 ± 0.537 [4.050,7.047]	7.754 ± 0.928 [6.599,8.794]	8.740 ± 0.886 [6.690,10.245]
CL/CW	0.887 ± 0.018 [0.843,0.919]	0.900 ± 0.015 [0.877,0.932]	0.896 ± 0.006 [0.891,0.904]	0.873 ± 0.015 [0.838,0.913]
SL/CS	0.789 ± 0.010 [0.772,0.807]	0.829 ± 0.019 [0.782,0.868]	0.815 ± 0.015 [0.797,0.833]	0.793 ± 0.021 [0.766,0.866]
ML/CS	1.846 ± 0.048 [1.774,1.964]	1.938 ± 0.076 [1.808,2.114]	2.002 ± 0.010 [1.994,2.016]	1.923 ± 0.052 [1.774,1.964]
MW/CS	1.311 ± 0.076 [1.143,1.411]	1.258 ± 0.065 [1.141,1.379]	1.379 ± 0.041 [1.328,1.419]	1.370 ± 0.068 [1.153,1.460]
MH/CS	0.968 ± 0.041 [0.885,1.048]	0.985 ± 0.048 [0.901,1.110]	1.026 ± 0.028 [1.004,1.066]	1.014 ± 0.036 [0.922,1.086]
dClAn/CS [%]	3.22 ± 0.32 [2.73,3.88]	4.19 ± 0.30 [3.77,4.84]	3.78 ± 0.32 [3.38,4.04]	3.70 ± 0.43 [2.66,4.37] n=44
MaDe	7.58 ± 0.69 [6.5,9.0] n=15	7.44 ± 0.50 [7.0,8.0] n=24	no data	8.11 ± 0.79 [6.0,9.0] n=26
sqPDCL	4.00 ± 0.45 [3.24,5.24]	4.72 ± 0.52 [3.71,6.15]	4.45 ± 0.35 [3.99,4.81]	4.34 ± 0.52 [3.34,5.32]
GuHL/CS	0.124 ± 0.010 [0.104,0.150]	0.135 ± 0.014 [0.091,0.165]	0.127 ± 0.017 [0.105,0.143]	0.124 ± 0.009 [0.102,0.149] n=44
PnHL/CS	0.110 ± 0.008 [0.094,0.126]	0.129 ± 0.011 [0.111,0.153]	0.128 ± 0.012 [0.111,0.138]	0.113 ± 0.011 [0.086,0.137]
nOcc	5.6 ± 3.3 [1.0,13.0]	9.2 ± 4.2 [2.5,20.0]	4.0 ± 2.3 [2.0, 6.0]	8.1 ± 4.0 [0.0,16.5]
nGen	1.9 ± 1.6 [0.0, 6.5]	2.2 ± 1.1 [0.5, 5.5]	1.6 ± 0.5 [1.0, 2.0]	2.5 ± 1.3 [0.0, 5.0] n=44
nGu	6.5 ± 2.2 [3.0,13.5}	5.5 ± 1.4 [3.0, 9.0}	7.1 ± 1.8 [5.0, 9.0}	8.9 ± 2.7 [2.5,16.0]
nSc	2.8 ± 3.5 [0.0,13.5]	1.2 ± 1.9 [0.0, 6.0]	0.9 ± 0.8 [0.0, 1.5]	3.2 ± 3.3 [0.0,15.0]
nHT	1.6 ± 1.8 [0.0,8.0]	1.1 ± 1.9 [0.0,9.5]	0.2 ± 0.3 [0.0,0.5]	4.4 ± 2.9 [0.0,17.0]
nSt	10.3 ± 2.8 [5.5,16.5]	8.8 ± 2.5 [5.0,18.0]	10.5 ± 1.4 [8.5,11.5]	15.4 ± 2.7 n=44 [9.5,20.5]

of an ant population from Uzbekistan, identified by Stukalyuk et al. (2020) as *Lasius neglectus*, could really refer to *L. frequens* n. sp.

These authors wrote: “We recently found numerous populations of this species in Uzbekistan, where it lives in the natural habitats throughout the country except for arid zones as the Kyzylkum desert and the Ustyurt plateau. *Lasius neglectus* inhabits here mesophytic, moderately humidified biotopes at altitudes from 91 to 1982 m a. s. l., but is also common in urban areas. Colonies of *L. neglectus* in the natural habitats are polygynous, but monocalic, it has a true nuptial flight, its workers are aggressive toward conspecific workers from other nests.”

5. Conclusion

The clear separation in exploratory and hypothesis-driven data analyses of morphometric data, the differing habitat selection and differing gyne morphology indicating a high potency for single-gyne flight dispersal, confirm that *Lasius frequens* n.sp. is heterospecific to *Lasius neglectus.* Studying the biological properties of the new species and its genetic relations to the other three species would be an interesting field of future investigation.

6. Acknowledgements

I wish to thank the Iranian colleagues Omid Paknia, Ali Bagherian-Yazdi, Sakine Hossein Nezhad, Afsane Hossein Nezhad, Zahra Rahmani and Zeynab Heydati for provinding so many samples from their country. The equipment for the morphological studies was co-financed by tax money on the basis of the state budget passed by the Sächsischer Landtag according to the Antragsnummer 100590787 of the Sächsische Aufbaubank issued 3 August 2021.

7. References

Cremer, S., Ugelvig, L.V., Drijfhout, F.P., Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Hughes, D.P., Schulz, A., Petersen, K.S., Konrad, H., Stauffer, C., Kiran, K., Espadaler, X., d’Ettorre, P., Aktaç, N., Eilenberg, J., Jones, G.R., Nash, D.R., Pedersen, J.S. & Boomsma, J.J. (2008): The evolution of invasiveness in garden ants. PLoS ONE 3(12): e3838. https://doi.org/10.1371/journal.pone0003838.

Csősz, S. & Fisher, B.L. (2015): Diagnostic survey of Malagasy Nesomyrmex species-groups and revision of hafahafa group species via morphology-based cluster delimitation protocol. ZooKeys 526: 19–59. https://doi.org/10.3897/zookeys.526.6037.

Espadaler, X., Tartally, A., Schultz, R., Seifert, B. & Nagy, C. (2007): Regional trends and preliminary results on the local expansion rate in the invasive garden ant, Lasius neglectus (Hymenoptera, Formicidae). Insectes Sociaux 54: 293–301.

Seifert, B. (2000): Rapid range expansion in Lasius neglectus (Hymenoptera, Formicidae) – an Asian invader swamps Europe. Deutsche Entomologische Zeitschrift 47(2): 173–179.

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. (2018): The ants of Central and North Europe. Lutra Verlags- und Vertriebsgesellschaft, Tauer (Germany), 425 pp.

Seifert, B. (2020a): A taxonomic revision of the Palaearctic members of the subgenus Lasius s. str. (Hymenoptera, Formicidae). Soil Organisms 92(1): 15–94.

Seifert, B. (2020b): The Gene and Gene Expression (GAGE) Species Concept: an universal approach for all eukaryotic organisms. Systematic Biology 69(5): 1033–1038. https://doi.org/10.1093/sysbio/syaa032.

Seifert, B. (2021): A taxonomic revision of the Palaearctic members of the Formica rufa group (Hymenoptera: Formicidae) – the famous mound-building red wood ants. Myrmecological News 31: 133–179. https://doi.org/10.25849/myrmecol.news_031:133.

Seifert, B. (2024): Pleistocene population differentiation in the ant Myrmica scabrinodis (Hymenoptera: Formicidae) – a taxonomic borderline case. Soil Organisms 96(1): 11–21. https://doi.org/10.25674/357.

Seifert, B., Ritz, M. & Csősz, S. (2013): Application of exploratory data analyses opens a new perspective in morphology-based alpha-taxonomy of eusocial organisms. Myrmecological News 19: 1–15.

Stukalyuk, S.V., Radchenko, A.G., Akhmedov, A. & Reshetov, A.A. (2020): Uzbekistan — the alleged native range of the invasive ant Lasius neglectus (Hymenoptera, Formicidae): geographical, ecological and biological evidences. Zoodiversity 54(2): 111–122. https://doi.org/10.15407/zoo2020.02.111.

Ugelvig, L.V., Drijfhout, F.P., Kronauer, D.J.C., Boomsma, J.J., Pedersen, J.S. & al. (2008): The introduction history of invasive garden ants in Europe: integrating genetic, chemical and behavioural approaches. BMC Biology 6: 11. https://doi.org/10.1186/1741-7007-6-11.