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Social parasites in evolutionary transition: *Lasius* (*Chthonolasius*) *castor* n.sp. and *L. (Chthonolasius) pollux* n.sp. – two hidden ant species with unusual biology

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Abstract

Lasius castor n.sp. and *L. pollux* n.sp., two new species of the ant subgenus *Chthonolasius*, are described and separated by high-resolution stereomicroscopy, Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT), analysis of nest sample compositions and zoogeography. With a total of 922 nest samples of 11 *Chthonolasius* species, the study attempted to investigate all available nest samples found in the geographic range of the new species. The subgenus-specific mode of temporary socially parasitic colony foundation in *Lasius* s. str. species was observed in only about 6% of the *L. castor* n.sp. and *L. pollux* n.sp. nest populations and is considered a behavioral relict. In contrast, 94% formed mixed nest populations with the *Chthonolasius* species *L. umbratus*, *L. distinguendus* and *L. sabularum.* As much as 15% of the nests of the latter species contained workers of *L. castor* n.sp. or *L. pollux* n.sp. The new species differ in pilosity characters, zoogeography and selection of habitat. The mixed populations are long-term stable and contain on average 40% of fully functional workers of *L. castor* n.sp. or *L. pollux* n.sp. The new species actively search for association with a *Chthonolasius* host. The observations indicate an ongoing evolutionary transition from temporary social parasitism in *Lasius* s.str. to a form of association with closely related *Chthonolasius* species. We discuss the unresolved question of whether this transition represents (i) a switch to permanent social parasitism, (ii) a switch to parabiosis or (iii) a transition by establishment of male-transmitted genetic lineages. Deviating from the normal *Chthonolasius* morphology, the stouter body shape,

appendage shortening and change of petiole shape in both new species, could signalize a beginning development to a degenerate parasite morphology.

Key words
Colony foundation strategy, numeric morphology-based alpha-taxonomy, parabiosis, social parasitism, male-transmitted genetic lineage.

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Introduction

It is a common thinking among myrmecologists that socially parasitic species are trapped in their particular life histories without having an exit. We describe here a situation in two new species of the subgenus *Chthonolasius* Ruzsky 1913 in which an evolutionary transition from temporary social parasitism to an alternative life history is happening. We will discuss if this transition is either a switch from temporary social parasitism in *Lasius* s.str. species to parabiosis with related *Chthonolasius* species, a switch from temporary social parasitism in *Lasius* s.str. species to permanent social parasitism in related *Chthonolasius* species or a transition by establishment of male-transmitted genetic lineages.

All species of the subgenus *Chthonolasius* found their nests by temporary social parasitism in species of the subgenera *Lasius* s.str. Fabricius 1804 and *Cautolasius* Wilson 1955 (summarized in Seifert 2018). Species-level taxonomy of *Chthonolasius* is far from being sufficiently resolved, there are no comprehensive genetic studies considering whole genomes and the current species delimitations are solely based on numeric morphology-based alpha-taxonomy (NUMOBAT) supplemented by ecological and zoogeographical information (Schlick-Steiner et al. 2002a, 2002b, 2008; Seifert 1982, 1988, 1990, 1997, 2018; Seifert & Buschinger 2001). Morphological crypsis of worker samples and a multitude of special biological factors causing mixed compositions of nest populations makes the taxonomy of this subgenus to perhaps the most difficult one of any ant group in Europe. According to Seifert (2018), the main biological factors responsible for the worker classification dilemma are the following:

(a) The temporal and spatial separation of mating may be weak in several. For example, mating swarms of *Lasius umbratus* (Nylander, 1846), *L. jensi* Seifert, 1982 and *L. meridionalis* (Bondroit, 1920) may assemble at the same hour of the day at the same spot (Seifert 2006).

(b) Social cleptogamy (Seifert 2006), also called asymmetric social hybridogenesis (Parker 2004) or sperm parasitism (Umphrey 2006), is frequent and produces nest populations of hybrid workers.

(c) Multiple mating of gynes with both heterospecific and conspecific males may result in worker offspring of both hybrid and pure species identity (unpublished cases of mixed nest samples of *Lasius jensi* and *L. jensi x umbratus* in the collection of SMN Görlitz identified by the senior author).

(d) Socially parasitic colony foundation may be pleometrotic (Dekoninck et al. 2004, Schlick-Steiner et al. 2002a, Seifert & Buschinger 2001). From this and factor (a) allopleometrotic foundation in the same host nest cannot be excluded. Heterospecific gynes with both conspecific and heterospecific mates may simultaneously invade a host colony and may avoid mortal fight by separating in remote parts of the nest (oligogyny).

(e) As a hypothetic prediction, take-over of an orphaned *Chthonolasius* colony by a mated gyne of a different *Chthonolasius* species may result in temporarily mixed samples.

(f) The phenotype of hybrid workers may approach in a fair number of characters to one of the parental species instead of being intermediate. This is often observed in hybrids *L. jensi* x *umbratus* or *L. meridionalis* x *umbratus* (Seifert 2006, this publication).

We present here astonishing observations referring to a morphologically well separable entity that has been provisionally named and characterized in Seifert (2016, 2018) as Compacta morph of *Lasius umbratus*. The argumentation why it is no morph of *L. umbratus* and why this entity can be divided in a species *Lasius castor* n.sp. and a species *L. pollux* n.sp. which differ in morphology, parabiosis / or host species partners, zoogeography and habitat selection will be presented in this paper. The case appears extraordinary because these two species each follow two alternative strategies of colony foundation. The first strategy, the original *Chthonolasius* mode of temporary social parasitism in ants of the subgenera *Lasius* s.str. or *Cautolasius*, is much rarer than the alternative strategy and possibly a behavioral relict. The second strategy to live in association with related *Chthonolasius* species is clearly dominating and is an evolutionary transition so far unknown for socially parasitic ants. As a rare and lucky situation within the subgenus *Chthonolasius*, the identification of nest populations mixed with *Lasius castor* n.sp. and *L. pollux* n.sp. was unusually clear because both species are separable from any related species or hybrid occurring in the study area even on the level of individual workers. The other side of the coin is that samples of these hidden ants are not easily collected in sufficient numbers and that there are big difficulties to locate queens in the extended subterranean nest space. Furthermore a long-term culturing of colonies and their observation in the laboratory is challenging. We hope that these conditions will not scare away

105 future investigators from launching comprehensive genetic and ethological studies to address the
106 many open questions which emerged as a result of this paper.
107
108
109 **Material**
110 The study attempted to thoroughly investigate all available nest samples of *Chthonolasius* species
111 found in the geographic distribution area of *Lasius castor* n.sp. and *L. pollux* n.sp. (Supplementary
112 information SI1). Evaluated were only samples with at least two workers per nest, this was a total of
113 922 nest samples – largely gathered over the last five decades by the authors of this study. The
114 largest species cluster, named here *Lasius umbratus* aggr. contained 350 nest samples. This collective
115 cluster, consisting of *Lasius umbratus* (Nylander, 1846), hybrids of *L. umbratus* with *L. jensi* Seifert,
116 1982 and of hybrids of *L. umbratus* with *L. meridionalis* (Bondroit, 1920), was formed because hybrid
117 workers are difficult to separate from *L. umbratus* but are clearly distinct from the parental species *L.*
118 *jensi* and *L. meridionalis.* A cluster of 127 nest samples, named here *Lasius distinguendus* aggr.,
119 comprised the species *L. balcanicus* Seifert, 1988 and *L. distinguendus* (Emery, 1916). It was formed
120 because worker samples of these species are morphologically unseparable in contrast to the very
121 distinct gynes. *Lasius castor* n.sp. was represented by 67 nest samples and *L. pollux* n.sp by 28 nest
122 samples. The number of nest samples investigated in the other species was 104 in *L. jensi*, 71 in *L.*
123 *sabularum* (Bondroit, 1918), 57 in *L. meridionalis*, 54 in *L. nitidigaster* Seifert, 1996, 39 in *L. mixtus*
124 (Nylander 1846) and 22 in *L. citrinus* Emery, 1922. About 60% of this material is stored in
125 Senckenberg Museum of Natural History Görlitz, about 37% in the private collections of Petr Werner,
126 Herbert Wagner and Jørn Bittcher, and 3% in different collections of other museums.
127 Species delimitation was done in 600 samples with 1567 workers by B. Seifert through analysis
128 of numerically recorded characters (Supplementary information SI2). Subjective determinations were
129 done in 322 samples containing only clear *Lasius umbratus* phenotypes and more easily determinable
130 species (*L. jensi*, *L. meridionalis*, *L. nitidigaster*, *L. mixtus*, and *L. citrinus*). The study concentrated on
131 Central and West Europe with 82% of the material collected in this region. The source countries are
132 given in alphabetic order: Armenia (1 sample), Austria (156), Azerbaijan (1), Belgium (7), Bosnia &
133 Herzegovina (3), Bulgaria (37), China (1), Croatia (7), Czechia (159), Denmark (49), Finland (3), France
134 (5), Great Britain (21), Georgia (2), Germany (258), Greece (10), Iran (1), Italy (18), Kazakhstan (1),
135 Montenegro (2), North Makedonia (1), Norway (1), Poland (13), Romania (6), Russia (6), Slovakia
136 (113), Slovenia (3), Spain (8), Sweden (4), Switzerland (12), Turkey (3) and Ukraine (3). The number
137 of gynes investigated was 32 in *L. sabularum*, 69 in the collective cluster *Lasius castor* n.sp. et
138 *umbratus*, and 48 in the collective cluster *Lasius pollux* n.sp. et *distinguendus* (Supplementary
139 information SI3, for explanation of cluster formation in gynes see below).

Methods

Recording of NUMOBAT characters

A pin-holding stage, permitting full rotations around X, Y, and Z axes, and a Leica M165C high-performance stereomicroscope equipped with a 2.0 x planapochromatic objective (resolution 1050 lines/mm) was used for spatial adjustment of specimens at magnifications of 120-360 x. 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 parallax error, its measuring line was constantly kept vertical within the visual field. Fourteen morphometric or meristic characters, nine shape, two seta, two pubescence characters, and absolute size, indicated by CS, were investigated and are described below. All bilateral characters were recorded as arithmetic mean of both sides.

Any metric measurement refers to real cuticular surface and not to the diffuse pubescence surface (most important in CW, Smax, Smin, HTmax). All setae counts include setae projecting > 10 µm from the cuticular profile, recorded with no change of the standardized viewing position. In cases of ambiguous projecting distance or unclear hair type (is it a fine seta or only a larger pubescence hair?) countings of 0.5 were applied. All morphometric data were taken by B. Seifert.

CL – maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of posterior vertex reduce CL.

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

CW – maximum cephalic width – either across, anterior, or posterior of eyes, whichever yields the maximum measurement.

F2L – (only gynes) median length of second funiculus segment in dorsal view which is given when the swiveling plane of 1st funiculus segment is positioned in the visual plane.

F2W – (only gynes) maximum width of second funiculus segment in dorsal view in the same spatial adjustment as for F2L.

GHL – length of longest seta on dorsal plane of 1st gastral tergite excluding setae near the posterior margin. The anterior area of dorsal plane where it slopes down to anterior face is included, but no areas sloping > 50° (here setae are significantly longer).

HTmax – maximum width of hind tibia at midpoint. The reference points to determine the midpoint are the distal end of tibia and the point of strongest constriction of the tibia's flexor side at the tibio-femoral joint (arrow in Fig. 1).

IFu2 – (only gynes) length index of 2nd funiculus segment: F2L / F2W.

MnHL – (only gynes) length of the longest seta on mesonotum.

nGen – number of setae projecting more than 10 µm above the profile of head capsule anterior of the eyes.

nHT – number of setae projecting more than 10 µm above the extensor profile of hind tibia. Apical setae at distal end are excluded.

PEAS – bilateral height asymmetry of petiolar crest. Height difference of the corners of petiolar crest. If only one side shows a corner, height measurement on the other side is performed at a sagittal level which distance from the median is equal to that of the sagittal level of the corner from the median (Fig. 2).

PECR – width of petiolar crest. In case of an average *Chthonolasius* scale shape (Fig. 3), PECR is measured from corner center to corner center. If the crest is straight, PECR is measured between the lateralmost points of the straight section (Fig. 4). If only one side shows a corner in asymmetric scales, PECR is twice the distance a of the corner center from the median (Fig. 5). PECR is 0 in case of symmetric crests without any corners (rounded or tipped crests as typical for *L. jensi*).

PEH – petiolar height measured from center of spiracle to dorsalmost point of petiolar crest. In case of asymmetric crests, the average of both sides is taken (Fig. 2).

PEINC – depth of incision of petiolar crest.

PEW – maximum width of petiole.

ScHD (only gynes) **–** maximum perpendicular projection of the longest hair from dorsal plane of scape with the smallest median diameter of scape in visual plane.

SL – maximum straight line scape length excluding the articular condyle.

Smax – maximum scape diameter at midpoint.

Smin – minimum scape diameter at midpoint.

sqPDF – square root of transverse pubescence distance on vertex frontal of mid ocellus. To avoid damaged and oily surface areas, countings are done as average of three or four shorter sections of 90 µm. A section crossing the frontal line is always included. Hairs crossing / just touching the 90 µm reference lines are counted as 1 / 0.5. Use objectives with numeric apertures > 0.2. The square root transformation is applied to normalize positively skewed distributions.

sqPDG – square root of transverse pubescence distance on dorsum of first gastral tergite. Measuring is basically done as in sqPDF. In species with high pubescence distance and undamaged, clean

surfaces, larger sections of up to 400 µm should be combined but do not exceed this length to minimize errors caused by surface convexity.

Species discrimination was improved through increase of measuring accuracy by considering the conditions reported in Seifert (2002) and by removal of allometric variance (RAV, Seifert 2008). RAV has no influence on the classifications by linear discriminant functions (LDA) but is important to increase the performance of principal component analyses (PCA) and to show in comparative tables which interspecific differences are independent from body size. Removal of allometric variance was performed in workers by overall functions computed as average of species-specific functions of 11 *Chthonolasius* entities with sufficient sample size: *Lasius citrinus*, *Lasius castors* n.sp., *L. distinguendus*, *L. jensi*, *L. meridionalis*, *L. mixtus*, *L. nitidigaster*, *L. pollux* n.sp., *L. sabularum*, *L. umbratus*, and *L. jensi* x *umbratus.* This overall correction was preferred to enable a direct comparison of many species in tables and because pair-specific corrections did not provide significant improvement. RAV transforms the data for the assumption of each worker individual having a head size CS of 1.05 mm:

CL/CW_{COR} = CL/CW / (-0.1385 * CS + 1.1934) * 1.0480

SL/CS_{COR} = SL/CS / (-0.0466 * CS + 0.9311) * 0.8822

GHL/CS_{COR} = GHL/CS / (-0.06201 * CS + 0.15831) * 0.09319

$Smax/CS_{COR}$ = Smax /CS / (+0.00303 * CS + 0.09236) * 0.09555

$Smax/Smin_{COR}$ = Smax/Smin / (+0.4018 * CS +1.0450) * 1.4670

$HTmax/CS_{COR}$ = HTmax /CS / (-0.00068 * CS + 0.13837) * 0.13766

$sqPDF_{COR}$ = sqPDF / (-0.0063 * CS + 2.758) * 2.752

$sqPDG_{COR}$ = sqPDG / (-1.0971 * CS + 4.117) * 2.965

$nGen_{COR}$ = nGen / (+13.87 * CS – 7.75) * 6.81

nHT_{COR} = nHT / (+25.56 * CS – 12.52) * 14.33

$PECR/CS_{COR}$ = PECR /CS / (+0.0767 * CS + 0.0035) * 0.0840

$PEAS/CS_{COR}$ = PEAS/CS / (+0.318 * CS - 0.127) * 0.461

PEW/CS_{COR} = PEW/CS / (-0.0011 * CS + 0.2858) * 0.2847

PEH/CS_{COR} = PEH/CS / (+0.0841 * CS + 0.1583) * 0.2466

RAV was performed in gynes based on data derived from the gyne clusters A and B (see below) and for the assumption of all specimens having a cephalic size of 1.57 mm

CL/CW_{COR} = CL/CW / (-0.0828 * CS + 0.9878) * 0.8577

SL/CS_{COR} = SL/CS / (-0.1935 *CS + 1.1169) * 0.813

$Smax/CS_{COR}$ = Smax/CS / (-0.00565 * CS + 0.0980) * 0.0891

$Smax/Smin_{COR}$ = Smax/Smin / (-0.0334 *CS + 1.5944) * 1.544

$HTmax/CS_{COR}$ = HTmax/CS / (0.00917*CS+0.12934) * 0.1437

$IFu2_{COR}$ = IFu2 / (0.2845 * CS + 1.0996) * 1.546

$nGen_{COR}$ = nGen / (13.052 * CS-9.10) * 11.39

nHT_{COR} = nHT / (31.43 * CS - 28.50) * 20.8

GHL/CS_{COR} = GHL/CS / (-0.00883*CS+0.07421) * 0.0603

$MnHL/CS_{COR}$ = MnHL/CS / (-0.01643 * CS + 0.07592) * 0.0501

$ScHD/CS_{COR}$ = ScHD/CS / (-0.00769 * CS + 0.03405) * 0.0220

$PeInc/CS_{COR}$ = PeInc/CS / (7.393 * CS - 7.949) * 3.658

PeW/CS_{COR} = PeW/CS / (0.0619 * CS + 0.3162) * 0.4133

$PeCr/CS_{COR}$ = PeCr/CS / (0.1440 * CS + 0.0117) * 0.2378

PeH/CS_{COR} = PeH/CS / (0.0977 * CS + 0.1193) * 0.2727

$sqPDF_{COR}$ = sqPDF / (0.4324 * CS + 1.871) * 2.55

$sqPDG_{COR}$ = sqPDG / (0.3605*CS + 1.484) * 2.05

Species delimitation and statistical testing

Four morphologically separable entities were found in association with *Lasius castor n.sp.* and *pollux* n.sp.: *L. umbratus* aggr., *L. distinguendus* aggr., *L. sabularum*, and *L. citrinus*. The latter species is easily recognized by subjective eye inspection. Hypotheses on species identity of the former three entities were made by principal component analysis (PCA) and there after checked by iterative runs of linear discriminant functions (LDA) in which the classification proposed by the LDA was adopted in the next run. The analysis was terminated when the classification error reached a minimum. PCA, LDA, and ANOVA tests were performed with the software package SPSS 15.0.

Results and discussion

Morphometric analysis of workers

As the first step of analysis workers of *Lasius castor* n.sp. and *L. pollux* n.sp., were collected in the *L. castor & pollux* aggr. This resulted in the separation of four main clusters hereafter named *L. castor & pollux* aggr., *L. distinguendus* aggr., *L. umbratus* aggr., and *L. sabularum*. This analysis included cephalic size CS and the 13 RAV-corrected characters CL/CW_{COR}, SL/CS_{COR}, GHL/CS_{COR}, $Smax/CS_{COR}$, $Smax/Smin_{COR}$, $HTmax/CS_{COR}$, $sqPDF_{COR}$, $sqPDG_{COR}$, nHT_{COR}, $PeAs/CS_{COR}$, $PeCr/CS_{COR}$, PeW/CS_{COR}, and

PeH/CS_{COR}. Species hypotheses were formed on the basis of sample means of a PCA. The PCA revealed four well-separable clusters (Figs. 6-8) which were taken as hypotheses for a LDA. The following LDA runs of 1117 worker individuals achieved identic classifications independently of whether primary or RAV-corrected characters were used (see Supplementary information SI2, Figs. 9-11). None of the 195 specimens belonging to the *L. castor & pollux* aggr. and of the 125 *L. sabularum* was misclassified whereas 0.9% of 213 *L. distinguendus* aggr. and 0.7% of 594 *L. umbratus* aggr. were misclassified. This strong discrimination of the four clusters on individual level allowed to correctly determine the composition of mixed samples.

For the reasons mentioned in the section Material, discrimination of species and hybrid entities within the *L. distinguendus* aggr. and *L. umbratus* aggr. clusters is insufficiently solved and often tentative. The missing gap between *L. umbratus* and *L. jensi* x *umbratus* or *L. umbratus* and *L. meridionalis* x *umbratus* samples is probably not only caused by morphological intermediates but also by multiple mating of the colony founding gyne by a conspecific and heterospecific male leading to a mixture of pure species and hybrid workers in the same nest. The skewed demarcation line between the hybrid samples and *L. umbratus* (Figs. 12, 13) is very unusual for a 3-class LDA and could be an expression of these more complicated mixtures of nest populations. Up to the present, no gyne suspicious to represent *Lasius jensi* x *umbratus* have been found by the first author among 99 gynes of *L. jensi* caught after swarming or among more than 500 gynes showing a *L. umbratus* morphology. As a consequence, the case is interpreted as a form of social cleptogamy (Seifert 2006) in which no hybrid gyne is produced and the queen can only propagate her genes by producing males. In contrast, many hybrid gynes of *L. meridionalis* x *umbratus*, which are clearly separable from both parental species (Seifert 2006, 2018), have been found. This is interpreted as a less effective case of social cleptogamy because the costly production of useless hybrid gynes is not abolished.

The workers of *Lasius castor* & *pollux* aggr. are a unique combination of small scape length, more cylindrical cross-section of scape, small width of hind tibia, wide petiolar crest and petiole, small petiole height, and long gastral setae (Tab. 1). The ants as a whole are more thickset than any known *Chthonolasius* species.

The differentiation between *L. castor* n.sp. and *L. pollux* n.sp. is more problematic, as they are most similar in size and shape characters (Tab. 2), because 42% of the 95 nest samples consisted of only a single worker, and because just the four samples found in an early stage of socially parasitic colony foundation with *Lasius* s.str. (sample numbers 77, 189, 497, 595; Supplementary information SI2) thoroughly consisted of dwarf workers, in which the differential characters separating *L. castor* n.sp. and *L. pollux* n.sp. are less clearly expressed. As a consequence, no form of exploratory data analysis provided clearly separated clusters. However, there was a strong correlation between

hairiness, biogeography, habitat selection and species identity of the associate species. These data were used in the following way to form an initial hypothesis for the LDA:

(a) populations living in association with *L. umbratus* aggr. species or *L. sabularum* and living in more humid woodland, meadow or bog habitats were assumed to be *L. castor* n.sp.

(b) the northernmost populations with a higher number of genal setae – i.e., those from Great Britain, Denmark, Germany, Poland and northern Czechia – were supposed to be *L. castor* n.sp.

(c) all populations living in dryer steppe habitats in association with *L. distinguendus* aggr. species and having a lower number of genal setae were supposed to be *L. pollux* n.sp.

(d) all lowland populations from Croatia, Hungary, Italy and Greece were supposed to be *L. pollux* n.sp.

In the first run of the LDA with nGen added as 15th character, the four dwarf-worker samples were set as wild-cards. In the following LDA runs the hypotheses were changed based on nest sample means of posterior probabilities until a stable state was achieved. The final classification error was 1.5% in 195 worker individuals with 3 specimens of *Lasius castor* n.sp. allocated to *L. pollux* n.sp. Based on nest sample means there is full separation of the two species but there are no clear gaps between the clusters (Fig. 14.). *Lasius castor* n.sp. and *L. pollux* n.sp. are highly significantly different in seta length and seta number but are almost equal in shape variables and absolute size (Tab. 2).

Morphometric analysis of gynes

The morphometric analysis of gynes considered the 18 characters CS, CL/CW, SL/CS, IFu2, Smax/CS, Smax/Smin, HTmax/CS, PeW/CS, PeCr/CS, PeH/CS, PeInc/CS, MnHL/CS, GHL/CS, nGen, nHT, ScHD/CS, sqPDF and sqPDG (Tab. 2, Supplementary information SI3). As a combination of large CL/CW, Smax/CS, HTmax/CS and low GHL/CS, the separation of *Lasius sabularum* from gynes of the other entities was clear on individual level in any multivariate analysis (see also Fig. 16).

Yet, there are problems regarding the two gyne morphologies called here gyne cluster A and B. Cluster A combines 69 gynes of *Lasius umbratus* and *L. castor* n.sp. whereas cluster B combines 48 gynes of *L. distinguendus* and *L. pollux* n.sp. A principal component analysis considering the RAV-corrected characters $nGen_{COR}$, $ScHD/CS_{COR}$, $MnHL_{COR}$, $Smax/Smin_{COR}$, nHT_{COR} and GHL/CS_{COR} offered a sufficient separation of clusters A and B with one specimen of cluster B allocated to cluster A (Fig. 15). This separation was confirmed with 98.3% by an LDA using the 15 characters CS, CL/CW_{COR}, SL/CS_{COR}, $Smax/CS_{COR}$, $Smax/Smin_{COR}$, $HTmax/CS_{COR}$, PeW/CS_{COR}, $PeCr/CS_{COR}$, PeH/CS_{COR}, $PeInc/CS_{COR}$, $ScHD/CS_{COR}$, $MnHL/CS_{COR}$, GHL/CS_{COR}, $nGen_{COR}$, nHT_{COR}. The two gynes from Thuringia / Germany, allocated by the LDA to cluster B, should really belong to cluster A because *L. distinguendus* n.sp. and *L. pollux* n.sp. should not occur there.

Although individual cluster A gynes could not be assigned morphologically to *L. umbratus* and *L. castor* n.sp., one nest sample of 5 alate gynes of cluster A (SaNo 604) could be assigned as *L. castor* n.sp. based on the following rationale: these cluster A gynes emerged from a mixed nest population composed of *L. sabularum* and *L. castor* n.sp. workers and therefore should not belong to *L. umbratus*. The separation of these *L. castor* n.sp. gynes from *L. sabularum* and cluster B gynes is perfectly clear in an LDA considering the characters CL/CW, Smax/ Smin, IFu2, nGen, nHT, MnHL/CS, GHL/CS, HTmax/CS and PeCr/CS (Fig. 16). The identification of 4 alate gynes of cluster B from another nest sample (SaNo 531) as *L. pollux* n.sp. could be made on a similar basis: these cluster B gynes emerged from a nest composed of *Lasius pollux* n.sp. and *L. umbratus* aggr. workers and therefore should not belong to *L. distinguendus*. The allocation of one of these four gynes to cluster A (Fig. 16) is interpreted as a morphological outlier.

The morphological data in Table 2 show that cluster A and B gynes are almost equal in absolute size and all shape characters, which indicates a close phylogenetic relatedness. Highly significant differences occur in seta number and length. It is remarkable that the pattern of similarities and differences observed between A and B gynes is repeated in the *Lasius castor* n.sp. and *L. pollux* n.sp. workers (Tab. 2). The quotients of the mean values between A and B gynes and of the mean values between *L. castor* n.sp. and *L. pollux* workers show for 14 characters a Pearson's correlation coefficient of 0.990 ($p << 0.001$).

Colony founding strategies and composition of mixed populations

Regarding the choice of partners in mixed colonies, the data seem to indicate strongly different species preferences between *L. castor* n.sp. and *L. pollux* n.sp. but no strict species-specificity in each of the species. These differences, however, are more probably a consequence of the different habitat selection of the associate species: *Lasius umbratus* aggr. species and *L. sabularum* are much more abundant in the moister and more oligothermous habitats preferred by *Lasius castor* n.sp. whereas *L. distinguendus* aggr. species dominate in the xerothermous habitats preferred by *L. pollux* n.sp. There are 65 samples in *L. castor* n.sp. and 27 in *L. pollux* n.sp. where an interpretation of the colony founding strategy appeared possible (Supplementary information SI4). In *L. castor* n.sp., 7.7% of colony foundations were done by temporary social parasitism and 92.3% in association with other species of the subgenus *Chthonolasius* – explicitly 50 (=76.9%) with *L. umbratus* aggr. species, 9 (=13.8%) with *Lasius sabularum*, and 1 (=1.5%) with *L. citrinus*. Proof for socially parasitic founding in *L. platythorax* was given in 3 samples and in *L. paralienus* in one sample (sample number 77).

In *Lasius pollux* n.sp., one nest population (=3.7%) was in the state of founding by temporary social parasitism in *Lasius platythorax* (sample number 497) and 96.3% in association with other

383 species of the subgenus *Chthonolasius* – explicitly 20 (=74.1%) with *Lasius distinguendus* aggr.
384 species, 4 (=14.8%) with *L. umbratus* aggr. species and 1 (=3.7%) with *L. sabularum*. The data given
385 above show that the spectrum of the associated *Chthonolasius* species is significantly different
386 between *Lasius castor* n.sp. and *L. pollux* n.sp. (p = 0.00001 in Fisher's exact test) but, as mentioned
387 above, these differences are more likely to be a reflection of different habitat preferences rather
388 than being a consequence of different host species selection.
389 All four directly observed and one likely socially parasitic colony foundation in *Lasius* s.str.
390 (Supplementary information SI4) thoroughly consisted of dwarf-workers. It is not surprising that
391 mean worker size in colony foundations is smaller than the species average, which mainly derives
392 from data of mature nests, but there is a difference to other *Chthonolasius* species. In 8 colony
393 foundations of *Lasius meridionalis*, *L. mixtus*, and of *L. umbratus* aggr. species, mean head size of the
394 foundation samples divided by the overall species mean was 0.9485 ± 0.1086 (Supplementary
395 information SI5). Pooling the nest means of the four directly observed *Lasius castor* n.sp. and *L.*
396 *pollux* n.sp. foundations, we got 0.7933 ± 0.0344. This is significantly smaller ($F_{1,10}$ 7.393, p = 0.022).
397 Adding the likely socially parasitic foundation (sample number 595) we get 0.7903 ± 0.0306, $F_{1,11}$
398 9.737, p = 0.010. Reduced body size and the very low frequency of socially parasitic colony
399 foundations in *Lasius* s.str. species could indicate that *Lasius castor* n.sp. and *L. pollux* n.sp. are no
400 longer well adapted to temporary social parasitic founding in *Lasius* s.str. which might be considered
401 a behavioral relict.
402 The mean percentage of the *L. castor* n.sp. and *pollux* n.sp. workers within the mixed
403 populations with other *Chthonolasius* species are (in the sequence arithmetic mean ± standard
404 deviation [minimum, maximum] number of samples): 39.7 ± 23.2 [10.0, 91.3] n = 60 in the former
405 and 39.1 ± 17.4 [10.0, 68.8] n = 26 in the latter (Supplementary information SI4). These differences
406 are not significant in any test. The overall mean of both species is 39.5% against 60.5% in the four
407 associate species. The reasons for this difference remain unclear and may be multiple. Possible are
408 (a) lower queen fecundity, (b) a time lag between earlier colony establishment in the associate and
409 later adoption of *L. castor* n.sp. and *L. pollux* n.sp. queens in combination with lower life expectancy
410 in the latter, (c) differences in brood care, (d) a relatively higher investment of *L. castor* n.sp. and *L.*
411 *pollux* n.sp. into sexual instead of worker production, as well as other factors.
412 The observation of a mature unmixed colony with large workers (sample number 484,
413 Supplementary information SI4), of a further three colonies with large workers with some likelihood
414 to be pure (sample numbers 437, 680, 681) and the high share of 40% in the worker force of mixed
415 colonies indicates that the workers of *Lasius castor* n.sp. and *L. pollux* n.sp. are capable to perform
416 any task needed and it is unlikely that these species represent inquilines in *Chthonolasius* species.
417 The data in Tab. 3 show that *Lasius castor* n.sp. and *L. pollux* n.sp. actively search for associate

partners whereas species of *L. distinguendus* aggr., *L. umbratus* aggr. and *L. sabularum* do not do so. No *L. castor* n.sp. or *L. pollux* n.sp. workers were found in 104 *L. jensi*, 57 *L. meridionalis*, 54 *L. nitidigaster* and 39 *L. mixtus* nests.

The following record makes it likely that *Lasius castor* n.sp. may also form mixed colonies with *L. citrinus*: In the ant collection of the Universalmuseum Joanneum, we detected 6 *L. citrinus* and 1 *L. castor* n.sp. worker from 1980 mounted on the same card (SaNo 574). We cannot verify whether this sample represents a true nest sample or whether the collector E. Kreissl mixed workers of different nests from the same locality. However, among 22 *Chthonolasius* worker samples, only two contained different species mounted on the same card (Wagner 2020): one combining *L. citrinus* with *L. castor* n.sp., and one *L. umbratus* with *L. castor* n.sp. This makes it likely that also the former represents a true mixed colony rather than a sampling artefact.

Parabiosis or permanent social parasitism ?

We cannot answer the question whether the two new species found via parabiosis or are permanent social parasites. The behavioral steps leading to the development of so many mixed colonies cannot be observed in the field and are extremely difficult to be observed in the laboratory. Several avenues appear possible and many of these cannot be checked. Stable, long-lived parabioses of two basically independent (self-sustaining) species are so far only known for ants which are not closely related, usually belonging to different subfamilies. Examples are parabioses between *Crematogaster* Lund, 1831 and *Camponotus* Mayr, 1861 species (Menzel et al. 2008, Menzel et al. 2014), *Crematogaster* with *Dolichoderus* Lund, 1831 (Forel 1898, Wheeler 1921), *Pseudomyrmex* Lund, 1831 with *Camponotus* (Gallego-Ropero & Feitosa 2014), and *Camponotus* with *Colobopsis* (Zettel et al. 2018). The most specialized example is the association between *Camponotus femoratus* (Fabricius, 1804) and *Crematogaster levior* Longino, 2003, which live in shared nests on trees; *Crematogaster* workers discover the prey, *Camponotus* workers build the carton nests, and both species together defend their nests (Wheeler 1921, Weber 1943, Swain 1980, Vantaux & al. 2007). Parabiotic species share nest space, foraging trails and food sources, but keep their brood chambers strictly separate. If representing a true parabiosis, the case reported here would be extraordinary because the parabiosis partners are closely related, share the same food sources and have both retained strong, fully functional worker populations. In the following, we discuss four explanations for the formation of the permanently mixed *Chthonolasius* colonies considered here.

Explanation (1): The most likely explanation appears to be penetration of an established queen-right colony of the associate species by *L. castor* n.sp. or *L. pollux* n.sp. gynes without killing the gyne of the associate. We documented that the holotype colony remained mixed at least from July 2021 to July 2025, meaning that it comprised heterospecific queens simultaneously. The big spatial

extension of the subterranean *Chthonolasius* nests should prevent any direct encounter of the heterospecific queens, while their worker populations freely mix in the galleries of the foraging space. This explanation is strongly supported by the high mean share of 39.5% of *L. castor* n.sp. and *L. pollux* n.sp. workers in the mixed colonies.

Explanation (2). Penetration of an orphaned *Chthonolasius* colony cannot be excluded but appears less likely than explanation (1) due to the large longevity of *Lasius* queens – 29 years are confirmed (Kutter 1969). Assuming a mean queen lifespan of 20 years, only 5% of the *Chthonolasius* colonies in an area would be queenless but we found 15.4% of the *Lasius umbratus* aggr., 16.5% of the *L. distinguendus* aggr. and 14.1% of the *L. sabularum* nests to be mixed with either *L. castor* n.sp. or *L. pollux* n.sp. (Tab. 3).

Explanation (3). One possible starting point for mixed colonies may be allopleometric colony foundation in a *Lasius* s.str. host after which the founding queens separate in remote parts of the nest, avoiding mortal fight. This mode of colony foundation was reported by Waloff (1957) and Pontin (1960) for the more distantly related, non-parasitic, subterranean ant *L. flavus* (Fabricius, 1782). Allopleometrotic colony foundations *Lasius* s.str. cannot be excluded but appear much less likely because of the very low frequency of temporary socially parasitic foundings which is about 7% as average over both species.

Explanation (4). Penetration into a *Chthonolasius* colony with killing of the host queen appears very unlikely considering the high share of *L. castor* n.sp. & *L. pollux* n.sp. aggr. workers in the mixed populations and the strong deviation from the frequency pattern in scenarios of temporary social parasitism. For *Lasius* species always founding their nests by temporary social parasitism and considering a larger geographic range, Seifert (2016) has shown that mixed host-parasite nests are much rarer than pure parasite nests and the latter themselves are much rarer than pure host nests. The reported ratio of pure parasite vs. mixed parasite host vs. pure host nests was 11.6 vs. 0.48 vs. 1000, but in our case it is 15.1 vs. 183.6 vs. 1000 when pure *L. compactus* n.sp. and *L. pollux* n.sp. nests are considered to be pure parasite nests.

When explanation (1) is the most probable avenue to the formation of mixed colonies, the question arises if we have here a transition from temporary social parasitism in *Lasius* s. str. to a parabiosis with related *Chthonolasius* species or a transition from temporary social parasitism in *Lasius* s. str. to permanent social parasitism in related *Chthonolasius* species. In a parabiosis scenario, the selective advantage for *Lasius castor* n.sp. and *L. pollux* n.sp. consists in using a pre-established system of foraging routes and food sources which then can be extended by the own worker force. However, under genetic fitness calculations, the parabiosis scenario requires that there is no damage or even an advantage for the associate. This scenario would require the evolutionary step to develop a surface chemistry and adaptive behavior allowing tolerance by host workers when the gynes

inavade the host colony. The permanent social parasite scenario with host-queen tolerance provides the same 'logistic' advantages and requires, in addition to changed queen surface chemistry, the second evolutionary step of developing a system suppressing the production of sexuals in the host. The deviations of body shape in *Lasius castor* n.sp. and *pollux* n.sp. workers from the normal *Chthonolasius* situation are significant but not extreme. Yet, the stouter body shape, appendage shortening and longer setae could signalize a beginning movement to a degenerate parasite morphology. This observation and the fact that permanent parabioses are only known so far for species involving different subfamilies (Seifert 2016) suggest the permanent social parasite scenario to be more likely.However parabioses between closely related species might have been undetected on a global scale due to failing species separation.

The male-transmitted genetic lineage hypothesis

Above we have attempted to present explanations for the very complex observations in our data set under the assumption that true *L. castor* n.sp. and *L. pollu*x n.sp. queens exist and that no complicated genetic mechanisms are involved. However, the gyne determination dilemma casts doubt on these hypotheses. We have shown that the supposed gynes of *Lasius castor* n.sp. are inseparable from those of its main host *L. umbratus* whereas the workers are clearly separable. Conversely, the supposed gynes of *Lasius pollux* n.sp. are inseparable from those of *L. distinguendus* and again the workers of these two species are clearly separable (Figs. 9, 15). This raises suspicion that *L. castor* n.sp. and *pollux* n.sp. gynes do not exist and that the gynes of *L. umbratus* and *distinguendus* could only be the vehicles for propagation of two separate genetic lineages which are embodied by males and workers only. This hypothesis requires postulating complicated genetic mechanisms of which we know many astonishing variants in ants (e.g., Juve et al. 2025). One scenario would be in our case that gynes of *L. umbratus* are mated by both conspecific and *L. castor* n.sp. males. These gynes could then produce conspecific workers, gynes and males. *Lasius castor* n.sp. males would be produced by removal of the *umbratus* genome from a fertilized egg whereas production of *castor* n.sp. workers requires the same mechanism plus subsequent doubling of the *castor* n.sp. chromosomes (but see below). Colonies with only *L. castor* n.sp. workers are then explained by a *L. umbratus* founding gyne having only *L. castor* n.sp. sperm. The frequency relation of colonies with only *umbratus* workers, of those mixed with *L. umbratus* and *castor* n.sp. workers, and of those with only *L. castor* n.sp. workers is in our data set 296 : 50 : 9. The corresponding relation between *L. distinguendus* aggr. and *L. pollux* n.sp. is 106 : 21 : 2 (Tab. 3). The male-lineage hypothesis would nicely explain the much higher abundance of mixed colonies against pure *L. castor* n.sp. and *pollux* n.sp. colonies. The supposed genetic mechanism of removal of heterospecific

genomes from the fertilized egg requires the recognition of the non-*castor* and non-*pollux* genomes. In case of *L. castor* n.sp. this mechanism must work with similar efficiency against the *L. umbratus* aggr., *L. sabularum* and *L. citrinus* genomes and in *L. pollux* n.sp. against the *L. distinguendus* aggr., *L. umbratus* aggr. and *L. sabularum* genomes.

The observation that an equal pattern of similarities and differences between cluster A (=*umbratus*-like) gynes and cluster B (=*distinguendus*-like) gynes is repeated in the *Lasius castor* n.sp. and *L. pollux* n.sp. workers (Tab. 2, Pearson's correlation coefficient r =0.991, n=14, $p < 0.001$) is intriguing. In the context of the male-transmitted genetic lineage hypothesis this observation might suggest that *L. castor* n.sp. workers are hybrids *L. castor* n.sp. x *umbratus* whereas those of *Lasius pollux* n.sp. are hybrids *L. pollux* n.sp. x *distinguendus* aggr. This explanation would replace the hypothesis that workers of the two new species are produced by removal of the heterospecific genome in the zygote and subsequent gene doubling. However, the hybridization hypothesis is unlikely given that *L. castor* n.sp. workers associated with *sabularum* by no means converge towards *L. sabularum* – i.e., they do not have larger CL/CW, Smax/Smin and smaller PeH/CS, GHL/CS, and nHT than those associated with *umbratus* aggr. species (Tab. 4). The correlation of the quotients of the mean values between *L. sabularum* and *umbratus* and of the quotients of the mean values between *L. castor* n.sp. workers in *sabularum* nests and *castor* n.sp.workers in *umbratus* nests is even negative ($r=–0.695$, n=14, $p < 0.006$).

Conclusions

We have discussed here three pathways to explain the mixed worker populations each of which would constitute a remarkable evolutionary transition: (1) a switch from temporary social parasitism in *Lasius* s.str. to permanent social parasitism in related in *Chthonolasius* species, (2) a switch from temporary social parasitism in *Lasius* s.str. to parabiosis with related *Chthonolasius* species and (3) a male-transmitted genetic lineage scenario. For each of the proposed explanations caveats exist, making a clear decision impossible. We tend to prefer hypothesis (1) because it is the most parsimonious explanation of a very complex situation. Very advanced genetic investigations comparable with those in the exemplary study of Juve et al. (2025) are required to achieve well founded answers. The description of two new species presented below is not touched by the question which biological phenomenon can explain our observations. Even if explanation (3) should apply, the International Code of Zoological Nomenclature would not prohibit the description of separate genetic lineages as species.

Lasius (*Chthonolasius*) *castor* n.sp.

Derivatio nominis:

The twin brothers Castor and Pollux are elements of the ancient Greek and Roman mythology. The name is given here because of the high similarity to its sibling species *Lasius pollux* n.sp.

Type material

Holotype worker labelled "AUS: 46.8767°N, 15.3967°E, Gantschenberg, 365 m, Haus mit Garten, unter Terasse, H. Wagner 2021.07.09-13" and "Holotype *Lasius castor* Seifert et al."; two paratype workers from the holotype nest, labelled "AUS: 46.8767°N, 15.3967°E, Gantschenberg, 365 m, Haus mit Garten, unter Terasse, H. Wagner 2021.07.09-13" and "Paratypes *Lasius castor* Seifert et al. 2026"; depository SMN Görlitz. Twelve paratype workers from the holotype nest in the collection of Herbert C. Wagner are labelled as “AUS: 46°52’36’’N, 15°23’48’’E, Gantschenberg, Spiegelgarten, 365 m, unter Terrasse, H. C. Wagner leg., 8.VII.2021 WAG2885” and “AUS: 46°52’36’’N, 15°23’48’’E, Gantschenberg, Spiegelgarten, 365 m, unter Terrasse, H. C. Wagner leg., 9.VII.2021, WAG2899”. For non-type material see Supplementary information SI1.

Geographic distribution

Lasius castor n.sp. is largely known from regions of Europe north of 46° N which are influenced by Atlantic climate. Sympatric occurrence with *L. pollux* n.sp. is known so far from south Czechia, Slovakia and Austria (Fig. 17 map). The altitudinal distribution of 68 sites is 274 ± 208 [5, 760] m.

Description

--worker (Figs. 18-20, Tab. 2): This description uses RAV-corrected data as a prediction for individuals with CS = 1050 µm. Medium-sized, CS 1049 ± 102 µm. Head and scape shorter than in *umbratus*, CL/CW_{COR} 1.029 ± 0.015, SL/CS_{COR} 0.853 ± 0.018. Scape less flattened than in *umbratus* and maximum diameter at midpoint smaller, $Smax/Smin_{COR}$ 1.227 ± 0.066, $Smax/CS_{COR}$ 8.81 ± 0.33%. Hind tibia width smaller than in *umbratus*, $HTmax/CS_{COR}$ 12.59 ± 0.48%. Setae on 1st gastral tergite longer than in *umbratus* and *pollux* n.sp., GHL/CS_{COR} 10.32 ± 0.56. Number of setae on genae and extensor profile of hind tibia much larger than in *pollux* n.sp., $nGen_{COR}$ 12.1 ± 4.7, nHT_{COR} 14.3 ± 7.8. Frons anterior of midocellus and dorsum of 1st gaster tergite with very dense pubescence, $sqPDF_{COR}$ 2.50 ± 0.18, $sqPDG_{COR}$ 2.35 ± 0.19. Petiole scale in frontal view wider and much lower than in *umbratus*, PeW/CS_{COR} 0.307 ± 0.016, PeH/CS_{1050} 0.215 ± 0.023, its dorsal crest usually wider than in *umbratus* and often rather linear (compare Figs. 20 and 21) but notched dorsal crests may occur, $PeCr/CS_{1050}$

0.109 ± 0.032. All body parts usually amber yellowish. Semierect to erect setae present on all surfaces.

Identification of *L. castor* n.sp. and *pollux* n.sp.

The separation of *Lasius castor* n.sp. and *pollux* n.sp. from all other *Chthonolasius* species can be done in many cases by careful inspection of petiole scale but this requires some experience. In addition to scale shape differences, discovering the presence of *L. pollux* n.sp. in nests of the *L. distinguendus* aggr. species is comparably easy by its much lower pubescence density on gaster tergites. If a case is unclear, careful recording of NUMOBAT characters and mounting of the specimen in a way allowing a free frontal view on petiole scale are essential. Provided the recording of NUMOBAT data is sufficiently accurate, *Lasius castor* n.sp. and *pollux* n.sp. cannot be confused with any other *Chthonolasius* species. Yet, really challenging is the separation of our two focal species. Including problematic dwarf workers and single-specimen samples, and with all measurements in mm, a discriminant 0.049*nHT +0.201*nGen+13.39*CL-23.32*SL+82.26*Smin +19.35*PeW-16.04*PeH-51.03*HTmax +3.340 offers a full separation on the nest-sample level. All 28 samples means of *Lasius pollux* n.sp. had values < 0 and all 69 samples of *L. castor* n.sp. values > 0. The percentage of misclassification on individual level was 4.1% in 195 specimens. The different habitat preferences (see below) may provide accessory hints for identification.

Biology

Lasius castor n.sp. prefers habitats with some moisture which are basically the same as in its supposed main hosts *Lasius umbratus, L. jensi x umbratus* and *sabularum.* Within 60 nest finds with information on habitat, 23 were found in broad-leafed woodland (ranging from forest margins to dark inner parts and from floodplains to colline regions), 8 in settlements with gardens, 8 in grassland or heath with scattered trees (including orchard meadows), 4 in conifer or conifer-broad-leafed forests, 4 in moist grassland, 2 in tree rows in agricultural land, 2 in city parks with trees and each one in a boulder stripe with trees, in a moist *Erica tetralix* heath and in a bog with *Comarum palustre*, *Eriophorum* and dead *Betula pendula*. About 9% of *L. castor* n.sp. nests were founded by temporary social parasitism in *Lasius* s.str. – with 3 confirmed cases in *L. platythorax* and 1 in *L. paralienus*. However, 91% of the nest populations were mixed with species of the subgenus *Chthonolasius* – 50 (=75.8%) with *L. umbratus* aggr. species, 9 (=13.63%) with *Lasius sabularum*, and 1 (=1.5%) with *L. citrinus*. We suppose that these mixed nest populations indicate an evolutionary transition to permanent social parasitism of *L. castor* n.sp. in related *Chtonolasius* species with still having a numerous, fully functional worker population. We cannot rule out that these mixed

populations alternatively represent a parabiotic relation or might even be the consequence of a male-transmitted propagation of a genetic lineage (for discussion see above).

Lasius (*Chthonolasius*) *pollux* n.sp.

Derivatio nominis:

The name is given because of the high similarity to its sibling species *Lasius castor* n.sp.

Type material

Holotype worker labelled "AUS: 48.20°N, 15.63°E, 275 m, St. Pölten, kiesige trockene Wiese in Flussaue, Seifert 1995.07.12-863", "Holotype (top), Paratype (bottom), *Lasius pollux* Seifert et al." 3 mounted paratypes from the holotype nest, labelled "Paratypes *Lasius pollux* Seifert et al.", 5 paratypes from the holotype nest in ethanol; 3 mounted paratypes, labelled " AUS: 48.129°N, 16.940, 450 m Hundsheim - 1.3 km NE, NSG Hundsheimer Berge, Seifert 2000.06.18-229" and "Paratypes *Lasius pollux* Seifert et al.", 22 paratype workers from the same nest in ethanol; depository SMN Görlitz. For non-type material see Supplementary information SI1.

Geographic distribution

Lasius pollux n.sp. is a more southern and more continental species than *castor* n.sp. with sympatric occurrence known so far from only from south Czechia, Slovakia and Austria (Fig. 17 map). The altitudinal distribution of 28 sites is 365 ± 288 [56, 1297] m.

Description

--worker (Figs. 22-24, Tab. 2): This description uses RAV-corrected data as a prediction for individuals with CS = 1050 µm. Medium-sized, CS 1046 ± 76 µm. Head and scape shorter than in *distinguendus* aggr., CL/CW_{COR} 1.034 ± 0.015, SL/CS_{COR} 0.860 ± 0.017. Scape less flattened than in *distinguendus* aggr. and maximum diameter at midpoint smaller, $Smax/Smin_{COR}$ 1.231 ± 0.074, $Smax/CS_{COR}$ 8.71 ± 0.25%. Hind tibia width smaller than in *distinguendus* aggr., $HTmax/CS_{COR}$ 12.81 ± 0.59%. Setae on 1st gastral tergite shorter than in *castor* n.sp., GHL/CS_{COR} 9.77 ± 0.56. Number of setae on genae and extensor profile of hind tibia much lower than in *castor* n.sp., $nGen_{COR}$ 2.3 ± 2.3, nHT_{COR} 4.6 ± 3.6. Pubescence on frons anterior of midocellus dense, $sqPDF_{COR}$ 2.50 ± 0.16, on dorsum of 1st gaster tergite very dense, much denser than in *distinguendus* aggr., $sqPDG_{COR}$ 2.33 ± 0.17. Petiole scale in frontal view much wider and much lower than in *umbratus* aggr. or *distinguendus* aggr., PeW/CS_{COR} 0.301 ± 0.012, PeH/CS_{1050} 0.225 ± 0.018, its dorsal crest usually much wider than in *umbratus* and

often rather linear (compare Figs. 24 and 21), notched dorsal crests occur more frequently than in *castor* n.sp., PeCr/CS_{1050} 0.124 ± 0.024. All body parts usually amber yellowish.

Identification of *L. castor* n.sp. and *pollux* n.sp.

See above under *Lasius castor* n.sp.

Biology

Lasius castor n.sp. prefers dry steppe habitats as does its supposed main host *Lasius distinguendus* aggr. Within 23 nest finds with information on habitat, 11 were found in open steppe or dry grassland habitats, 5 in the ecotone between dry grassland and broad-leafed woodland or in dry grassland with scattered trees, 2 in broad-leafed woodland, 2 in a *Pinus* wood with *Fagus*, and each one in a woodland stripe in a settlement, a south-facing vineyard and a moist grassland with apple trees. There is only one documented case of temporary socially parasitic colony foundation and this was in *Lasius platythorax* (Sa No 497) which is certainly not the major host species. Concluded from the their dominance in the steppe ecosystems inhabited by *Lasius pollux* n.sp. in E Austria, Slovakia and Hungary, these should be *L. paralienus* and / or *L. bombycina*. Above we have shown that composition of the associated *Chthonolasius* species is significantly different from *Lasius castor* n.sp. Within a total of 26 nest populations mixed with *Chthonolasius*, 80.8% contained *Lasius distinguendus* aggr. species, 15.4% *L. umbratus* aggr. species and 3.8% *L. sabularum*. The question if these mixed populations have to be interpreted as permanent social parasitism in related *Chtonolasius* species or as a parabiotic relation with these species, or if they might be the consequence of a male-transmitted propagation of a genetic lineage is not resolved. Permanent social parasitism, with the parasite still having a fully functional and numerous worker population, apears to be the most parsimonious explanation with the lowest number of caveats.

References

Dekoninck W, Boer P, Maelfait JP (2004) *Lasius platythorax* Seifert, 1991 as a host of several *Chthonolasius* species, with remarks on the colony foundation of the parasites (Hymenoptera: Formicidae). Myrmecologische Nachrichten 6: 5-8.

Forel AH (1898): La parabiose chez les fourmis. Bulletin de la Société Vaudoise des Sciences Naturelles 34: 380-384.

Gallego-Ropero MC, Feitosa RM (2014) Evidences of Batesian mimicry and parabiosis in ants of the Brazilian Savanna. Sociobiology 61: 281-285.

696 Juvé Y, Lutrat C, Ha A et al. (2025) One mother for two species via obligate cross-species cloning in
697 ants. Nature 646**:** 372–377. https://doi.org/10.1038/s41586-025-09425-w
698 Kutter H, Stumper R (1969) Hermann Appel, ein leidgeadelter Entomologe (1892-1966). Proceedings
699 of the Sixth International Congress of the International Union for the Study of Social Insects
700 (Bern), pp. 275-279.
701 Menzel F, Blüthgen N, Schmitt T (2008): Tropical parabiotic ants: highly unusual cuticular substances
702 and low interspecific discrimination. Frontiers in Zoology 5: art. 16.
703 Menzel F, Kriesell H, Witte V (2014) Parabiotic ants: the cost and benefits of symbiosis. Ecological
704 Entomology 39: 436-444.
705 Parker JD (2004) A major evolutionary transition to more than two sexes? Trends in Ecology and
706 Evolution 19: 83-86.
707 Pontin AJ (1960) Field experiments on colony foundation by *Lasius niger* (L.) and *L. flavus* (F.) (Hym.,
708 Formicidae). Insectes Sociaux 7(3): 227-230.
709 Schlick-Steiner BC, Steiner FM, Seifert B, Straka U(2002a) *Lasius platythorax* as a host of *Lasius*
710 *distinguendus* (Hymenoptera, Formicidae). Insectes Sociaux 49(3): 299.
711 Schlick-Steiner BC, Steiner FM, Seifert B (2002b) *Lasius flavus* – a host species of *Lasius mixtus*
712 (Hymenoptera:Formicidae). Sociobiology 39: 141-143.
713 Schlick-Steiner BC, Steiner FM, Conrad H, Seifert B, Christian E, Moder K, Stauffer C, Crozier RH
714 (2008) Specificity and transmission mosaic of ant nest wall fungi. PNAS 105(3): 941-944.
715 Seifert B (1982) *Lasius* (*Chthonolasius*) *jensi* n.sp. – eine neue temporär sozialparasitische Ameise aus
716 Mitteleuropa. Reichenbachia 20(10): 85-96.
717 Seifert B (1988) A revision of the European species of the ant subgenus *Chthonolasius*.
718 Entomologische Abhandlungen des Staatlichen Museums für Tierkunde Dresden 51(8): 143-180.
719 Seifert B (1990) Supplementation to the revision of the European species of the ant subgenus
720 *Chthonolasius* Ruzsky, 1913. Doriana VI-N. 271: 1-13.
721 Seifert B (1997) *Lasius nitidigaster* n.sp. - a new ant of the Subgenus *Chthonolasius* Ruzsky
722 (Hymenoptera: Formicidae). Annales Zoologici 46: 201-205.
723 Seifert B (2006) Social cleptogamy in the ant subgenus *Chthonolasius* – survival as a minority.
724 Abhandlungen und Berichte des Naturkundemuseums Görlitz 77: 251-276.
725 Seifert B (2008) Removal of allometric variance improves species separation in multi-character
726 discriminant functions when species are strongly allometric and exposes diagnostic characters.
727 Myrmecological News 11: 91-105.
728 Seifert B 2016: Analyzing large-scale and intranidal phenotype distributions in eusocial Hymenoptera
729 – a taxonomic tool to distinguish intraspecific dimorphism from heterospecificity. Myrmecological
730 News 23: 41-59.

731 Seifert B, Buschinger A (2001) Pleometrotische Koloniegründung von *Lasius meridionalis* (Bondroit,
732 1920) bei *Lasius paralienus* Seifert, 1992, mit Bemerkungen über morphologische und
733 ethologische Anpassungen an die sozialparasitische Koloniegründung (Hymenoptera: Formicidae).
734 Myrmecologische Nachrichten 4: 11-15
735 Seifert B (2016) Analyzing large-scale and intranidal phenotype distributions in eusocial Hymenoptera
736 – a taxonomic tool to distinguish intraspecific dimorphism from heterospecificity. Myrmecological
737 News 23: 41-59
738 Seifert B (2018) The Ants of Central and North Europe. Lutra Verlags- und Vertriebsgesellschaft,
739 Tauer, pp 281-290
740 Swain RB (1980) Trophic competition among parabiotic ants. Insectes Sociaux 27: 377-390.
741 Umphrey GJ (2006) Sperm parasitism in ants: selection for interspecific mating and hybridization.
742 Ecology 87(9): 2148-2159.
743 Vantaux A, Dejean A, Dor A, Orivel J (2007) Parasitism versus mutualism in the ant-garden parabiosis
744 between *Camponotus femoratus* and *Crematogaster levior*. Insectes Sociaux 54: 95-99.
745 Wagner HC (2020) The geographic distribution of ants (Hymenoptera: Formicidae) in Styria (Austria)
746 with a focus on material housed in the Universalmuseum Joanneum. Joannea Zoologie 18: 33-152.
747 Waloff N (1957) The effect of the number of queens of the ant *Lasius flavus* (Fab.) (Hymenoptera:
748 Formicidae) on their survival and on the rate of development of the first brood. Insectes Sociaux
749 4: 391-408.
750 Weber NA (1943) Parabiosis in Neotropical “ant gardens.” Ecology 24: 400-404.
751 Wheeler WM (1921) A new case of parabiosis and the “ant gardens” of British Guiana. Ecology 2: 89-
752 103.
753 Zettel H, Baláka P, Yamane S, Laciny A, Lim L,Druzhinina IS (2018) New mimetic ants from Southeast
754 Asia – the *Camponotus* (*Myrmamblys*) *inquilinus* group (Hymenoptera: Formicidae: Camponotini).
755 Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen 70: 125-174.
756

Tab. 1: RAV-corrected morphometric data of workers in the sequence arithmetic mean ± standard deviation [maximum, minimum]. The columns with data of a one-tailed ANOVA are placed between the compared entities.

	sabularum (n = 125)	ANOVA $F_{1,309}$, p	***castor* & *pollux*** aggr. (n = 195)	ANOVA $F_{1,777}$, p	***umbratus*** **aggr.** (n = 594)	ANOVA $F_{1,796}$, p	***distinguendus*** **aggr.** (n=213)
CS [µm]	1073 ± 59 [862,1175]	7.09 0.008	1048 ± 95 [770,1250]	0.27 n.s.	1052 ± 97 [780,1256]	0.42 n.s.	1057 ± 66 [860,1211]
CL/CW_{COR}	1.070 ±0.013 [1.044,1.102]	**611.6** 0.000	1.030 ±0.015 [0.987,1.065]	130.8 0.000	1.045 ±0.016 [0.989,1.085]	14.45 0.000	1.050 ±0.015 [0.999,1.082]
SL/CS_{COR}	0.870 ±0.016 [0.827,0.918]	60.86 0.000	0.854 ±0.018 [0.815,0.905]	**357.3** 0.000	0.880 ±0.016 [0.834,0.926]	209.7 0.000	0.898 ±0.017 [0.853,0.949]
Smax / $Smin_{COR}$	1.239 ±0.048 [1.104,1.350]	1.90 n.s.	1.229 ±0.069 [1.088,1.474]	**966.1** 0.000	1.435 ±0.084 [1.206,1.755]	5.31 0.021	1.420 ±0.075 [1.203,1.621]
$Smax/CS_{COR}$ [%]	9.22 ± 0.30 [8.57,10.01]	**156.2** 0.000	8.78 ± 0.31 [7.63,9.50]	**651.0** 0.000	9.48 ± 0.34 [8.29,10.95]	169.8 0.000	9.13 ± 0.33 [8.34,10.36]
$HTmax/CS_{COR}$ [%]	13.37 ± 0.45 [12.67,14.85]	**155.1** 0.000	12.66 ± 0.52 [11.33,14.43]	**459.8** 0.000	13.57 ± 0.51 [12.25,16.00]	0.08 n.s.	13.55 ± 0.48 [12.22,15.17]
GHL/CS_{COR} [%]	7.56 ± 0.60 [6.03,9.21]	**1389.7** 0.000	10.16 ± 0.61 [8.45,11.98]	167.2 0.000	9.45 ± 0.68 [7.21,11.36]	**1219.0** 0.000	11.39 ± 0.74 [9.26,13.66]
nHT_{COR}	9.2 ± 4.4 [0.0,21.1]	27.01 0.000	13.6 ± 9.0 [0.0,42.9]	44.0 0.000	17.6 ± 6.6 [1.2,37.7]	**712.1** 0.000	5.2 ± 2.7 [0.0,15.5]
$sqPDF_{COR}$	2.56 ± 0.16 [2.26,3.25]	9.80 0.002	2.50 ± 0.17 [2.07,3.13]	125.1 0.000	2.66 ± 0.17 [2.26,3.32]	207.1 0.000	2.86 ± 0.17 [2.39,3.46]
$sqPDG_{COR}$	2.34 ± 0.16 [2.03,2.83]	0.11 n.s.	2.34 ± 0.18 [1.95,2.88]	178.3 0.000	2.57 ± 0.21 [1.99,3.37]	**1578.6** 0.000	3.27 ± 0.26 [2.58,3.86]
$PeCr/CS_{COR}$	0.093 ±0.018 [0.050,0.151]	45.21 0.000	0.114 ±0.031 [0.000,0.188]	**316.7** 0.000	0.081 ±0.018 [0.028,0.134]	88.29 0.000	0.094 ±0.012 [0.057,0.126]
PeW/CS_{COR}	0.289 ±0.011 [0.266,0.326]	**104.8** 0.000	0.305 ±0.015 [0.276,0.373]	**472.7** 0.000	0.281 ±0.013 [0.238,0.323]	141.2 0.000	0.270 ±0.011 [0.243,0.319]
PeH/CS_{COR}	0.221 ±0.016 [0.171,0.259]	1.90 n.s.	0.217 ±0.022 [0.138,0.267]	**618.0** 0.000	0.257 ±0.018 [0.186,0.326]	5.16 0.023	0.254 ±0.016 [0.208,0.309]
$PEAS/CS_{COR}$ [%]	0.40 ± 0.45 [0.00,1.72]	0.06 n.s.	0.41 ± 0.53 [0.00,2.18]	13.14 0.000	0.60 ± 0.64 [0.00,4.17]	14.93 0.000	0.41 ± 0.49 [0.00,2.81]

763 Tab. 2: Morphometric data of gynes and RAV-corrected morphometric data of workers in the
764 sequence arithmetic mean ± standard deviation [maximum, minimum]. The columns with data of a
765 one-tailed ANOVA are placed between the compared entities. The ratios A/B and ca/po denote the
766 relation of the mean values of cluster A and B gynes and of *L. castor* n.sp. and *L. pollux* n.sp. workers.

	gynes					**workers**				
	sabularum (n=32)	***cluster A*** (n=69)	ANOVA $F_{1,115}$,p	***cluster B*** (n=48)	**ratio A/B**		***castor*** n.sp. (n=138)	ANOVA $F_{1,193}$,p	***pollux*** n.sp. (n=57)	**ratio ca/po**
CS [µm]	1542 ± 25 [1466,1582]	1575 ± 48 [1473,1699]	n.s.	1565 ± 55 [1456,1672]	1.006	CS [µm]	1049 ± 102 [785,1250]	n.s.	1046 ± 76 [770,1189]	1.003
CL/CW	0.898 ± 0.009 [0.885,0.927]	0.854 ± 0.014 [0.811,0.888]	8.01 0.005	0.862 ± 0.015 [0.836,0.893]	0.991	CL /CW_{COR}	1.029 ± 0.015 [0.991, 1.065]	4.42 0.037	1.034 ± 0.015 [0.987, 1.061]	0.995
SL/CS	0.810 ± 0.012 [0.788,0.836]	0.811 ± 0.017 [0.767,0.843]	n.s.	0.816 ± 0.022 [0.776,0.888]	0.994	SL /CS_{COR}	0.853 ± 0.018 [0.815,0.904]	4.06 0.045	0.858 ± 0.018 [0.825,0.905]	0.994
IFu2	1.395 ± 0.052 [1.312,1.524]	1.549 ± 0.077 [1.414,1.809]	n.s.	1.542 ± 0.065 [1.432,1.726]		IFu2	no data		no data	
Smax/CS [%]	9.28 ± 0.42 [7.68, 9.82]	8.91 ± 0.30 [8.07,9.57]	n.s.	8.91 ± 0.33 [8.20,9.58]	1.000	Smax /CS_{COR} [%]	8.81 ± 0.33 [7.63, 9.50]	n.s.	8.71 ± 0.25 [8.03, 9.42]	1.011
Smax/Smin	1.257 ± 0.074 [1.074,1.387]	1.308 ± 0.055 [1.183,1.437]	n.s.	1.299 ± 0.067 [1.142,1.488]	1.007	Smax /$Smin_{COR}$	1.227 ± 0.066 [1.054, 1.403]	n.s.	1.231 ± 0.074 [1.120, 1.474]	0.997
HTmax/CS [%]	15.33 ± 0.47 [14.58,16.72]	14.34 ± 0.55 [13.26,15.70]	n.s.	14.44 ± 0.65 [13.17,15.83]	0.993	HTmax /CS_{COR} [%]	12.59 ± 0.48 [11.59,14.43]	7.27 0.008	12.81 ± 0.59 [11.33,14.06]	0.983
PeW/CS	0.385 ± 0.018 [0.356,0.431]	0.410 ± 0.027 [0.351,0.488]	n.s.	0.417 ± 0.024 [0.378,0.472]	0.983	PeW /CS_{COR}	0.307 ± 0.016 [0.276,0.373]	5.66 0.018	0.301 ± 0.012 [0.276,0.327]	1.020
PeCR/CS	0.199 ± 0.020 [0.151,0.231]	0.236 ± 0.026 [0.174,0.290]	n.s.	0.240 ± 0.027 [0.173,0.306]	0.983	PeCR/ CS_{COR}	0.109 ± 0.032 [0.000, 0.188]	9.25 0.003	0.124 ± 0.024 [0.071, 0.173]	0.879
PeH/CS	0.269 ± 0.017 [0.239,0.319]	0.271 ± 0.023 [0.223,0.331]	n.s.	0.275 ± 0.023 [0.215,0.319]	0.985	PeH /CS_{COR}	0.215 ±0.023 [0.138, 0.267]	8.94 0.003	0.225 ±0.018 [0.196, 0.266]	0.956
PeInc/CS [%]	1.37 ± 1.03 [0.00, 3.90]	3.93 ± 1.58 [0.66,8.52]	n.s.	3.41 ± 1.71 [0.21, 6.63]		PeInc /CS[%]	no data		no data	
MnHL/CS [%]	4.24 ± 0.56 [2.80,5.52]	5.71 ± 0.66 [4.39,7.83]	123.3 0.000	4.35 ± 0.63 [3.18,5.83]		MnHL /CS [%]	no data		no data	
GHL/CS [%]	4.50 ± 0.61 [3.49,5.79]	6.84 ± 0.90 [5.14, 9.57]	**111.3 0.000**	5.25 ± 0.64 [4.05,6.50]	1.303	GHL /CS_{COR} [%]	10.32 ± 0.56 [8.60, 11.98]	**37.49 0.000**	9.77 ± 0.56 [8.45, 10.97]	1.056
ScHD/CS [%]	1.57 ± 0.52 [1.47,1.58]	2.88 ± 0.46 [1.63,3.92]	207.5 0.000	1.53 ± 0.54 [0.68,2.98]		ScHD /CS [%]	no data		no data	
nGen	5.6 ±3.4 [0.5, 16.0]	19.3 ±5.7 [1.5, 32.0]	**312.3 0.000**	3.6 ±2.8 [0.0, 13.0]	5.361	$nGen_{COR}$	12.1 ±4.7 [2.7,30.2]	**224.8 0.000**	2.3 ± 2.3 [0.0, 11.8]	5.26
nHT	14.1 ± 5.0 [4.0,24.0]	31.9 ± 9.7 [10.8,60.0]	**188.5 0.000**	10.0 ± 6.4 [1.0,29.0]	3.190	nHT_{COR}	17.4 ± 7.8 [3.4, 42.9]	**142.5 0.000**	4.6 ± 3.6 [0.0, 16.0]	3.78
sqPDF	2.55 ± 0.18 [2.09,3.07]	2.64 ± 0.16 [2.17,3.00]	38.16 0.000	2.45 ± 0.17 [2.12,2.75]	1.078	$sqPDF_{COR}$	2.50 ± 0.18 [2.07, 3.13]	n.s.	2.50 ± 0.16 [2.14, 2.87]	1.000
sqPDG	2.08 ± 0.13 [1.87,2.52]	2.12 ± 0.16 [1.87,2.49]	21.79 0.000	1.98 ± 0.15 [1.73,2.55]	1.004	$sqPDG_{COR}$	2.35 ± 0.19 [1.95, 2.88]	n.s.	2.33 ± 0.17 [1.95, 2.87]	1.009

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770 Table 3: Composition of 667 nest samples of species containing populations of *Lasius castor* n.sp.
771 and *L. pollux.*The numbers in brackets also included samples in which the status "not mixed" was not
772 clear because of low sample size.

	not mixed	mixed with *castor*	mixed with *pollux*	mixed with *sabu-larum*	mixed with *umbratus* aggr.	mixed with *distinguen-dus* aggr.	mixed with *citrinus*
L. castor n.sp. (n=69)	5 (9)	–	0	9	50	0	1
L. pollux n.sp. (n=28)	1 (2)	0	–	1	4	21	0
L. sabularum n=71	61	9	1	–	0	0	0
L. umbratus aggr. n= 350	296	50	4	0	–	0	0
L. distinguendus aggr. n=127	106	0	21	0	0	–	0
L. citrinus n=22	21	1	0	0	0	0	–

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Table 4: RAV-corrected morphometric data of workers in the sequence arithmetic mean ± standard deviation [maximum, minimum]. The columns with data of a one-tailed ANOVA are placed between the compared entities.

	sabularum (n = 125)	ANOVA $F_{1,716}$, p	***umbratus* aggr.** (n=593)	***castor n.sp.*** with ***sabularum*** (n = 23)	ANOVA $F_{1,106}$, p	***castor n.sp.*** with ***umbratus* aggr.** (n = 85)
CS [µm]	1073 ± 59 [862,1175]	5.97 0.015	1052 ± 97 [780,1256]	1070 ± 67 [933,1161]	0.55 n.s.	1082 ± 74 [864,1250]
CL/CW_{COR}	1.070 ±0.013 [1.044,1.102]	**267.5** 0.000	1.045 ±0.016 [0.989,1.085]	1.023 ±0.011 [1.002,1.050]	2.78 n.s.	1.029 ±0.016 [0.991,1.065]
SL/CS_{COR}	0.870 ±0.016 [0.827,0.918]	42.3 0.000	0.880 ±0.016 [0.834,0.926]	0.856 ±0.017 [0.821,0.883]	1.94 n.s.	0.850 ±0.016 [0.815,0.904]
Smax / $Smin_{COR}$	1.239 ±0.048 [1.104,1.350]	**632.6** 0.000	1.435 ±0.084 [1.206,1.755]	1.262 ±0.057 [1.162,1.403]	13.7 0.000	1.207 ±0.065 [1.054,1.381]
$Smax/CS_{COR}$ [%]	9.22 ± 0.30 [8.57,10.01]	62.3 0.000	9.48 ± 0.34 [8.29,10.95]	8.87 ± 0.20 [8.57,9.28]	2.32 n.s.	8.75 ± 0.37 [8.29,10.95]
HTmax $/CS_{COR}$ [%]	13.37 ± 0.45 [12.67,14.85]	16.5 0.000	13.56 ± 0.51 [12.25,16.00]	12.43 ± 0.36 [11.93,13.57]	2.68 n.s.	12.59 ± 0.42 [11.59,13.54]
GHL/CS_{COR} [%]	7.56 ± 0.60 [6.03,9.21]	**817.2** 0.000	9.44 ± 0.68 [7.21,11.36]	10.49 ± 0.42 [9.74,11.15]	5.01 0.027	10.21 ± 0.59 [8.60,11.47]
nHT_{COR}	9.2 ± 4.4 [0.0,21.1]	183.8 0.000	17.6 ± 6.6 [1.2,37.7]	25.4 ± 6.6 [18.3,42.9]	36.10 0.000	15.8 ± 6.8 [3.4,33.5]
$sqPDF_{COR}$	2.56 ± 0.16 [2.26,3.25]	34.5 0.000	2.66 ± 0.17 [2.26,3.32]	2.54 ± 0.21 [2.11,3.13]	0.44 n.s.	2.51 ± 0.17 [2.07,3.09]
$sqPDG_{COR}$	2.34 ± 0.16 [2.03,2.83]	133.58 0.000	2.57 ± 0.21 [1.99,3.37]	2.31 ± 0.19 [1.95,2.62]	0.14 n.s.	2.32 ± 0.17 [1.99,2.85]
PeCr $/CS_{COR}$	0.093 ±0.018 [0.050,0.151]	42.2 0.000	0.081 ±0.018 [0.028,0.134]	0.108 ±0.029 [0.050,0.175]	0.32 n.s.	0.111 ±0.028 [0.046,0.188]
PeW $/CS_{COR}$	0.289 ±0.011 [0.266,0.326]	38.8 0.000	0.281 ±0.013 [0.238,0.323]	0.301 ±0.014 [0.276,0.328]	6.46 0.013	0.310 ±0.016 [0.238,0.323]
PeH $/CS_{COR}$	0.221 ±0.016 [0.171,0.259]	**419.8** 0.000	0.257 ±0.018 [0.186,0.326]	0.219 ±0.019 [0.184,0.263]	0.06 n.s.	0.218 ±0.017 [0.166,0.260]
PEAS $/CS_{COR}$ [%]	0.40 ± 0.45 [0.00,1.72]	10.4 0.001	0.60 ± 0.64 [0.00,4.17]	0.41 ± 0.50 [0.00,1.91]	0.01 n.s.	0.43 ± 0.56 [0.00,4.17]

Figure legends

Figs. 1 – 5: mode of measuring the characters PeCr, PeH, and PeAS. The arrow in Fig. 1 marks the proximal reference point to measure hind tibia length.

Fig. 6: Principal component analysis of worker nest sample means of *Lasius umbratus* aggr. (crosses*), L. distinguendus* aggr. (white dots) and of the cluster formed by the cryptic species *L. castor* n.sp. and *pollux* n.sp. (white rhombs).

Fig. 7: Principal component analysis of worker nest sample means of *Lasius umbratus* aggr. (crosses*), L. sabularum* (black dots) and of the cluster formed by the cryptic species *L. castor* n.sp. and *pollux* n.sp. (white rhombs).

Fig. 8: Principal component analysis of worker nest sample means of *Lasius umbratus* aggr. (crosses*), L. sabularum* (black dots) and of *L. distinguendus* aggr. (white dots).

Fig. 9: Linear discriminant analysis of worker invididuals of *Lasius umbratus* aggr. (crosses*), L. distinguendus* aggr. (white dots) and of the cluster formed by the cryptic species *L. castor* n.sp. and *pollux* n.sp. (white rhombs).

Fig. 10: Linear discriminant analysis of worker individuals of *Lasius umbratus* aggr. (crosses*), L. sabularum* (black dots) and of the cluster formed by the cryptic species *L. castor* n.sp. and *pollux* n.sp. (white rhombs).

Fig. 11: Linear discriminant analysis of worker individuals of *Lasius umbratus* aggr. (crosses*), L. sabularum* (black dots) and of *L. distinguendus* aggr. (white dots).

Fig. 12: Linear discriminant analysis of worker nest means of *Lasius umbratus* (black crosses*), L. jensi x umbratus* (white crosses) and of *L. jensi* (black rhombs).

Fig. 13: Linear discriminant analysis of worker nest means of *Lasius umbratus* (black crosses*), L. meridionalis x umbratus* (white crosses) and of *L. meridionalis* (black rhombs).

Fig. 14: Linear discriminant analysis and first principal component of worker nest means of *Lasius castor* n.sp. (crosses*)* and *L. pollux n.sp.* (dots). Red crosses and dots refer to populations mixed with

L. sabularum, white crosses and dots to pure samples, blue dots to populations mixed with *distinguendus* aggr. and the blue cross to a population mixed with *L. citrinus*.

Fig. 15: Principal component analysis of gynes considering 6 RAV-corrected characters. Crosses refer to gyne cluster A with red crosses to gynes supposed to represent *L. castor* n.sp. because of comming from a nest with *castor* and *sabularum* workers, black crosses to gynes comming from a nest with only *umbratus* workers and white crosses to single gynes in dispersal or comming from nests with both *castor* and *umbratus* workers. Dots refer to gyne cluster B with red dots supposed to represent *L. pollux* n.sp. because of comming from a nest with *pollux* and *umbratus* aggr. workers, white dots to single gynes in dispersal or comming from nests with both *pollux* and *distinguendus* workers. Black dots are *distinguendus* because of comming from a nest with only *distinguendus aggr.* workers.

Fig. 16: Linear discriminant analysis of gynes considering 9 primary characters. Crosses represent gyne cluster A with black crosses supposed to be *Lasius castor* n.sp. because of comming from a nest with *castor* n.sp. and *sabularum* workers and white crosses referring to single gynes in dispersal or comming from nests with only *umbratus* workers and from nests with *castor* and *umbratus* workers. Dots represent gyne cluster B with black dots supposed to be *Lasius pollux* n.sp. because of comming from a nest with *pollux* n.sp. and *umbratus aggr.* workers and white dots referring to single gynes in dispersal or comming from nests with only *distinguendus* aggr. workers and from nests with both *pollux* and *distinguendus* aggr. workers. Black rhombs represent *Lasius sabularum*.

Fig. 17: geographic distribution of *Lasius castor* n.sp. (black dots) and of *L. pollux* n.sp. (red triangles)

Fig. 18: Head of the holotype of *Lasius castor* n. sp.; Gantschenberg, Austria, 2021.

Fig. 19:Lateral aspect of the the holotype of *Lasius castor* n. sp.; gaster with petiole dissected; Gantschenberg, Austria, 2021.

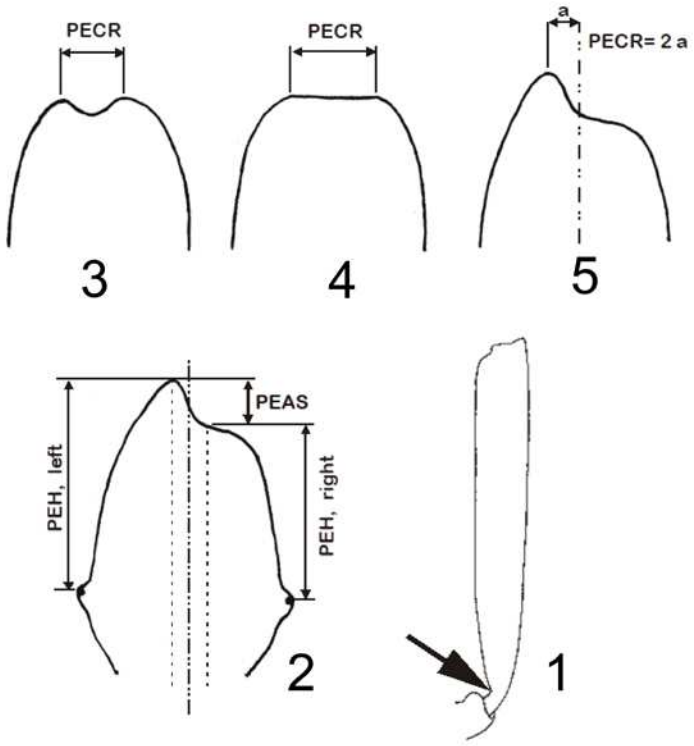
Fig. 20: Petiole in of the holotype of *Lasius castor* n. sp. in frontal view; Gantschenberg, Austria, 2021.

Fig. 21: Petiole of a *Lasius umbratus* worker from the holotype nest of *L. compactus* n. sp. in frontal view; Gantschenberg, Austria, 2021.

Fig. 22: Head of the holotype of *Lasius pollux* n. sp.; St. Pölten, Austria, 1995.

Fig. 23: Lateral aspect of the holotype of *Lasius pollux* n. sp.; St. Pölten, Austria, 1995.

Fig. 24: Petiole of a paratype of *Lasius pollux n.sp.* in frontal view; Hundsheimer Berge, Austria, 2000



Figs. 1 – 5: mode of measuring the characters PeCr, PeH, and PeAS. The arrow in Fig. 1 marks the proximal reference point to measure hind tibia length.

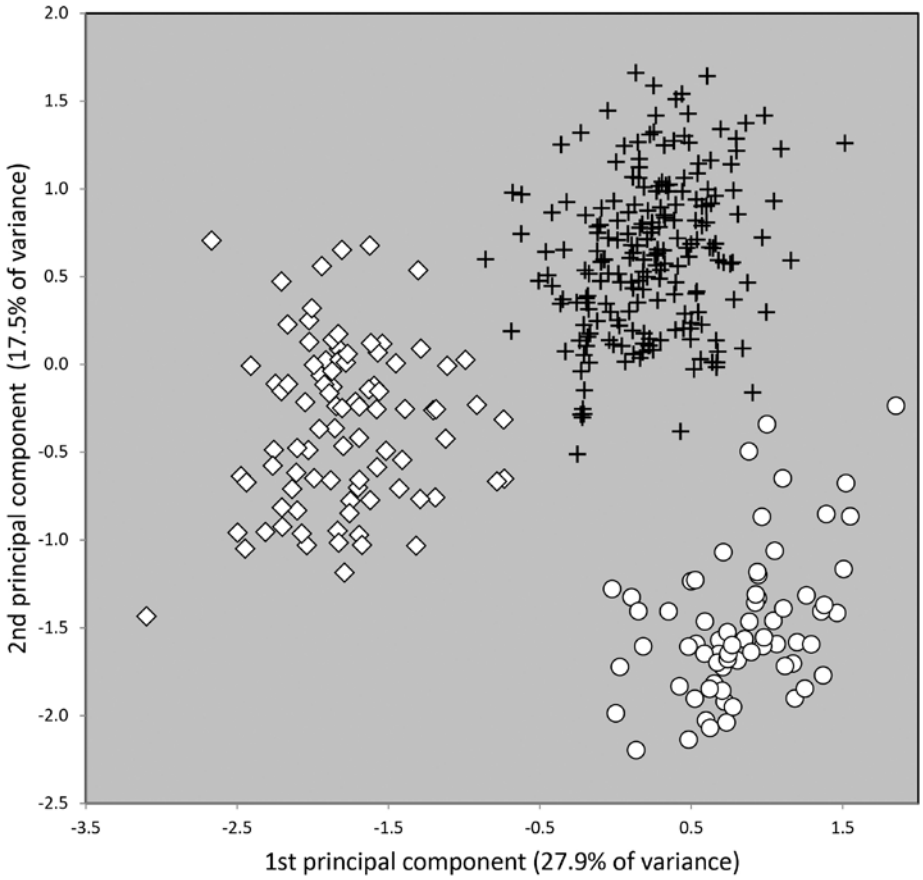


Fig. 6: Principal component analysis of worker nest sample means of *Lasius umbratus* aggr. (crosses*), L. distinguendus* aggr. (white dots) and of the cluster formed by the cryptic species *L. castor* n.sp. and *pollux* n.sp. (white rhombs).

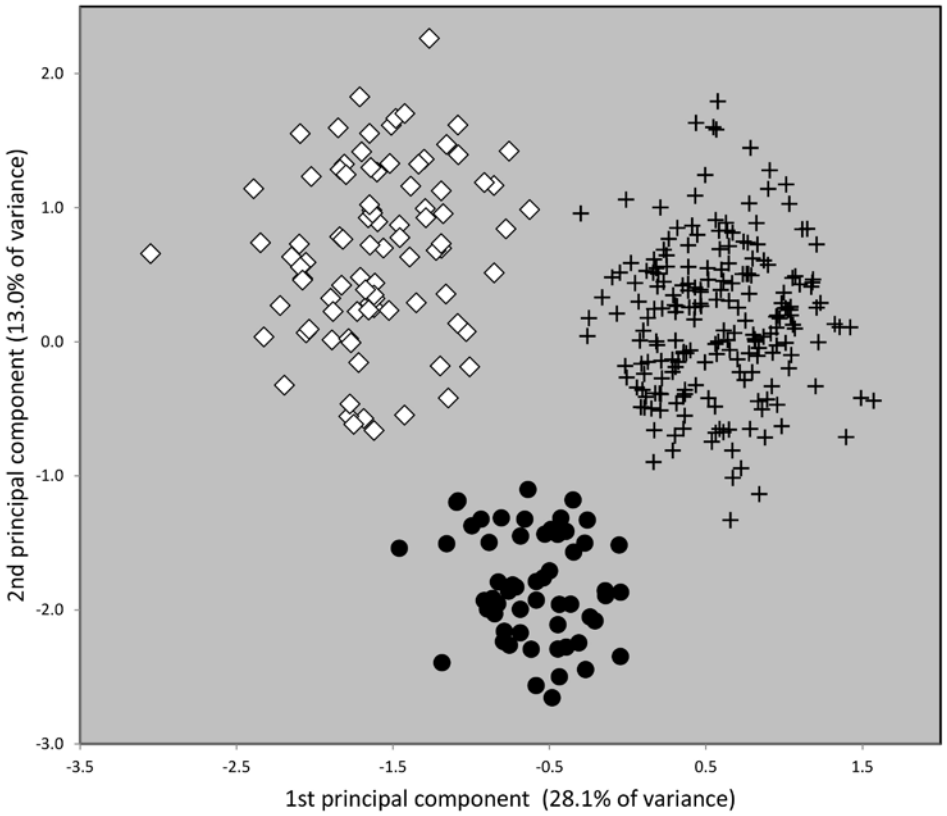


Fig. 7: Principal component analysis of worker nest sample means of *Lasius umbratus* aggr. (crosses*), L. sabularum* (black dots) and of the cluster formed by the cryptic species *L. castor* n.sp. and *pollux* n.sp. (white rhombs).

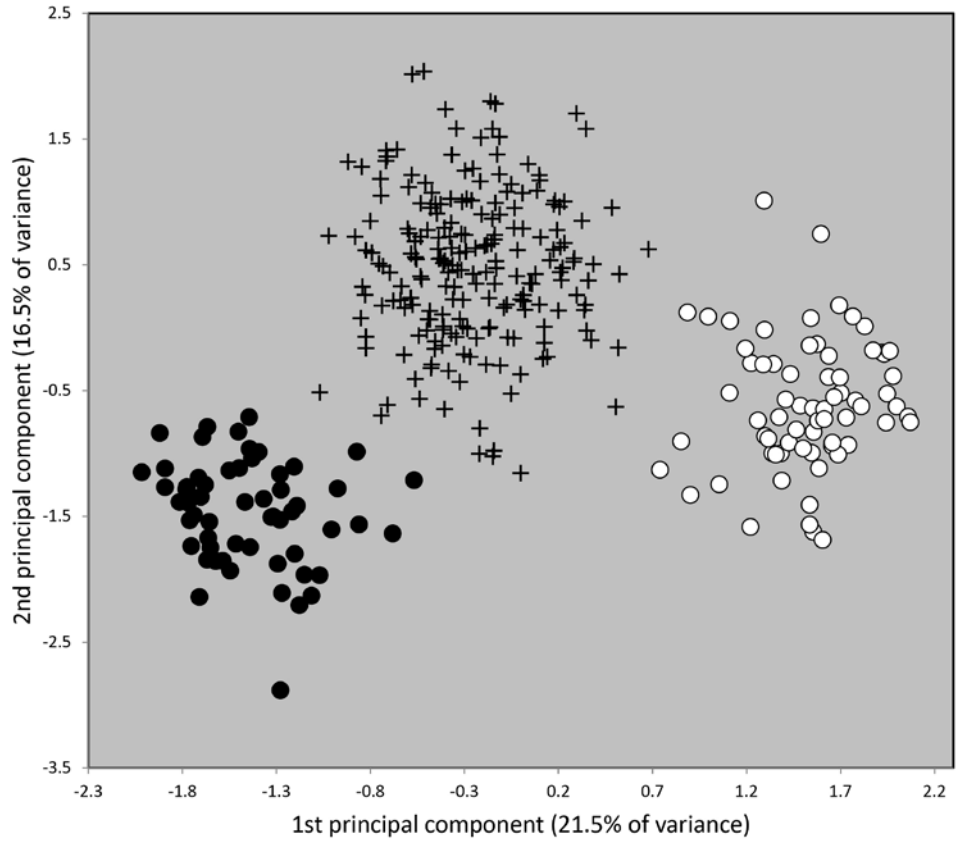


Fig. 8: Principal component analysis of worker nest sample means of *Lasius umbratus* aggr. (crosses*), L. sabularum* (black dots) and of *L. distinguendus* aggr. (white dots).

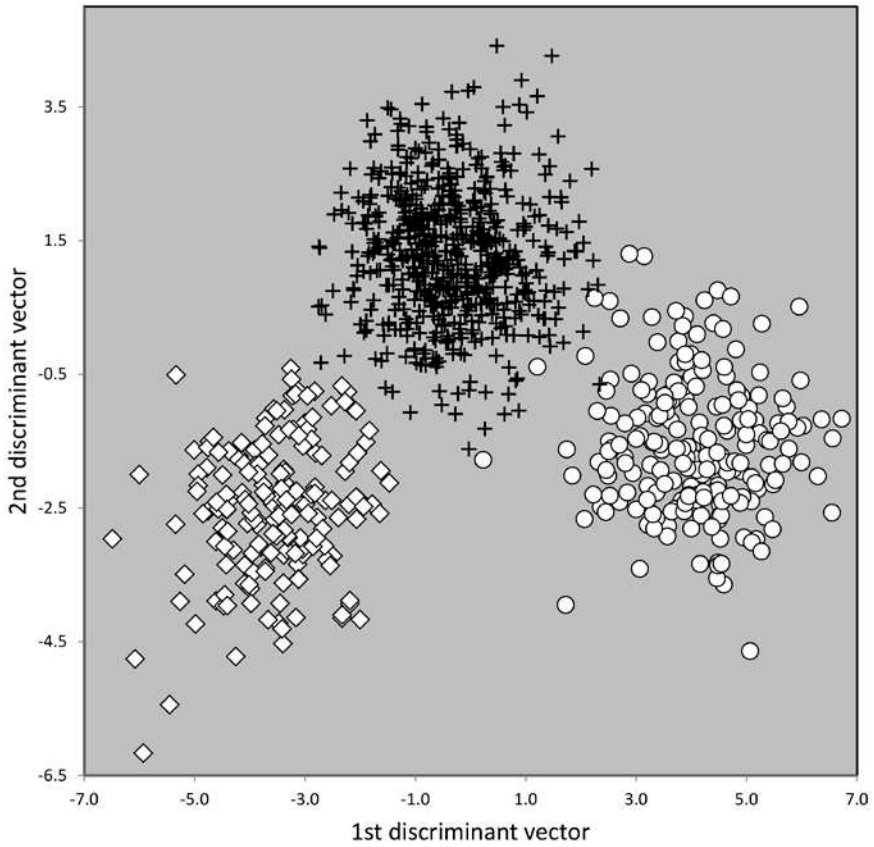


Fig. 9: Linear discriminant analysis of worker invidividuals of *Lasius umbratus* aggr. (crosses*), L. distinguendus* aggr. (white dots) and of the cluster formed by the cryptic species *L. castor* n.sp. and *pollux* n.sp. (white rhombs).

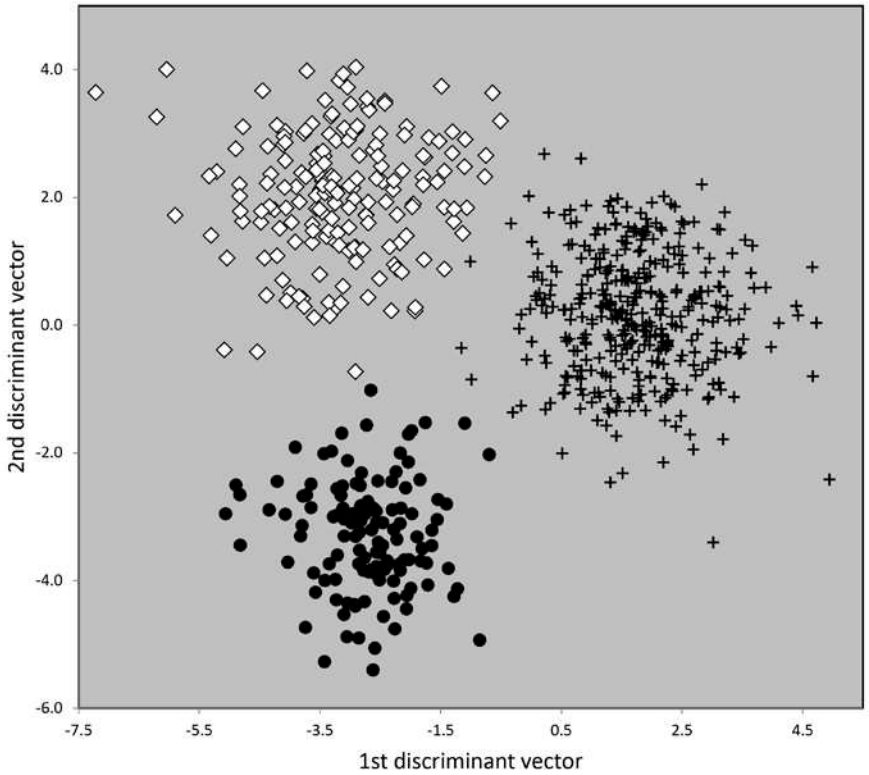


Fig. 10: Linear discriminant analysis of worker individuals of *Lasius umbratus* aggr. (crosses*), L. sabularum* (black dots) and of the cluster formed by the cryptic species *L. castor* n.sp. and *pollux* n.sp. (white rhombs).

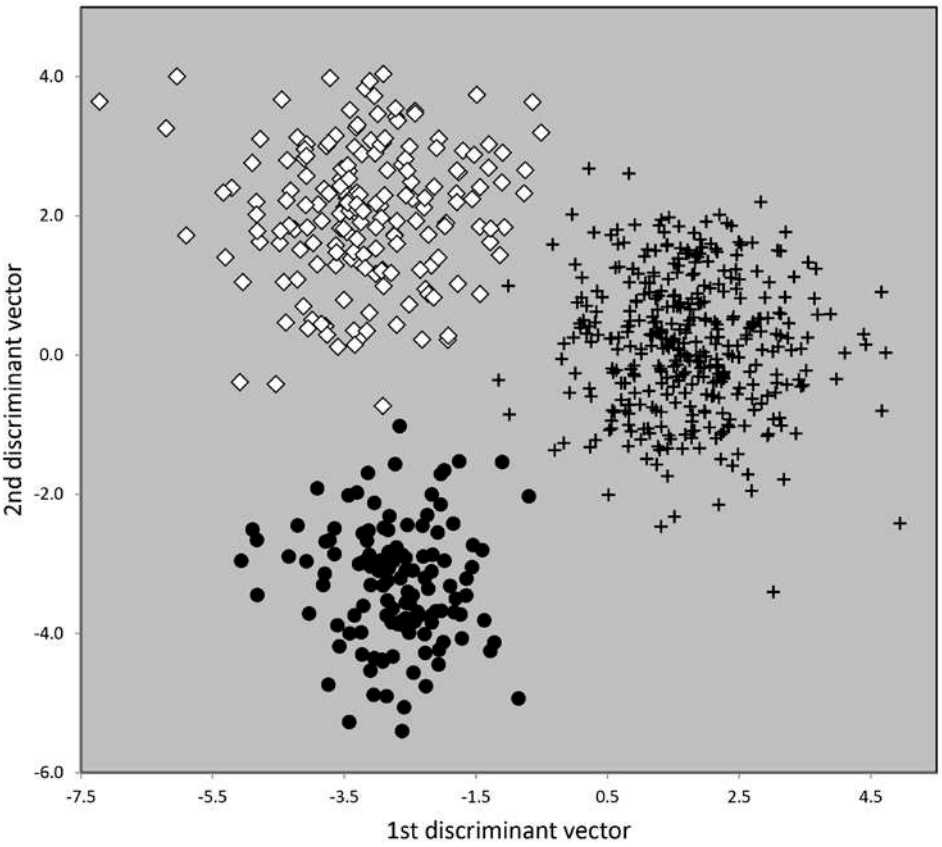


Fig. 11: Linear discriminant analysis of worker individuals of *Lasius umbratus* aggr. (crosses*), L. sabularum* (black dots) and of *L. distinguendus* aggr. (white dots).

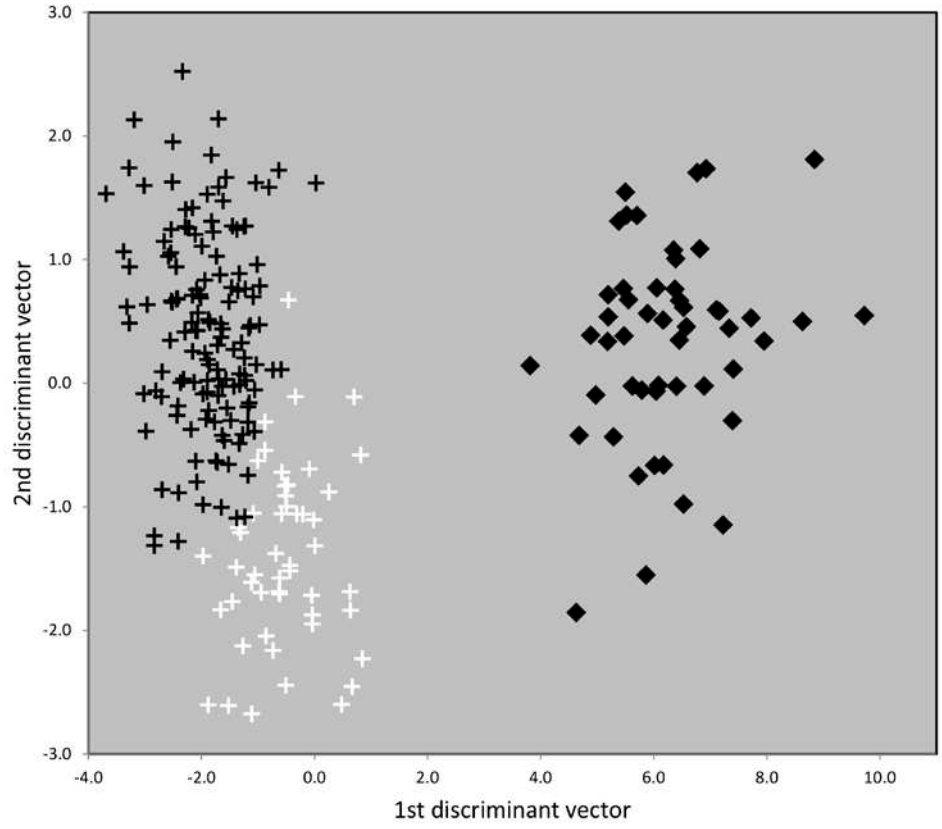


Fig. 12: Linear discriminant analysis of worker nest means of *Lasius umbratus* (black crosses*), L. jensi x umbratus* (white crosses) and of *L. jensi* (black rhombs).

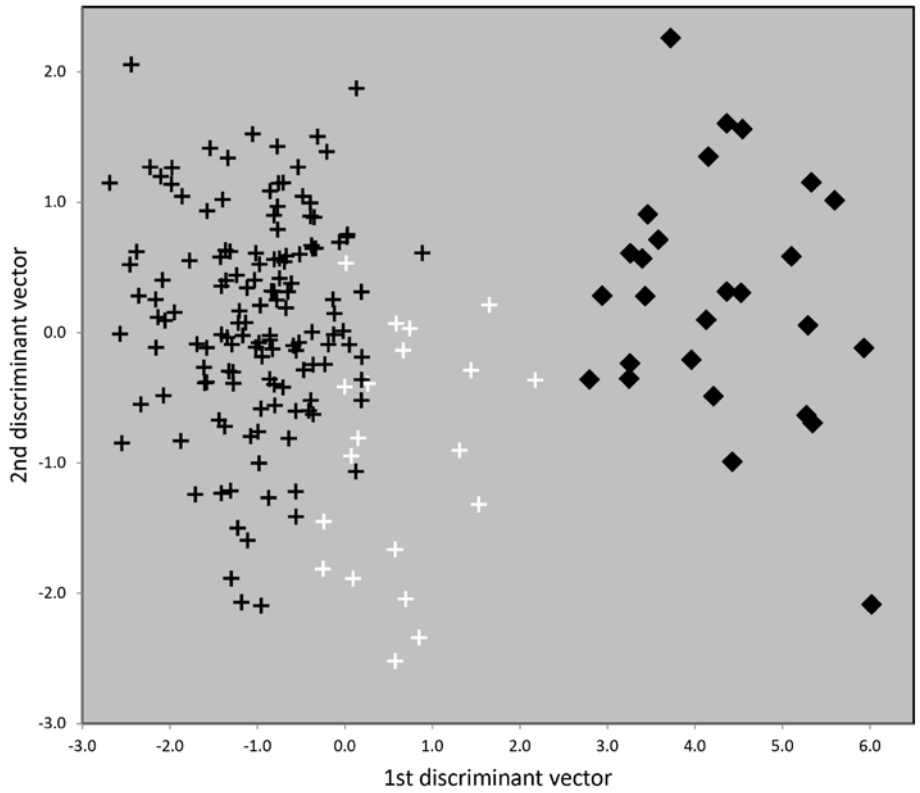


Fig. 13: Linear discriminant analysis of worker nest means of *Lasius umbratus* (black crosses*), L. meridionalis x umbratus* (white crosses) and of *L. meridionalis* (black rhombs).

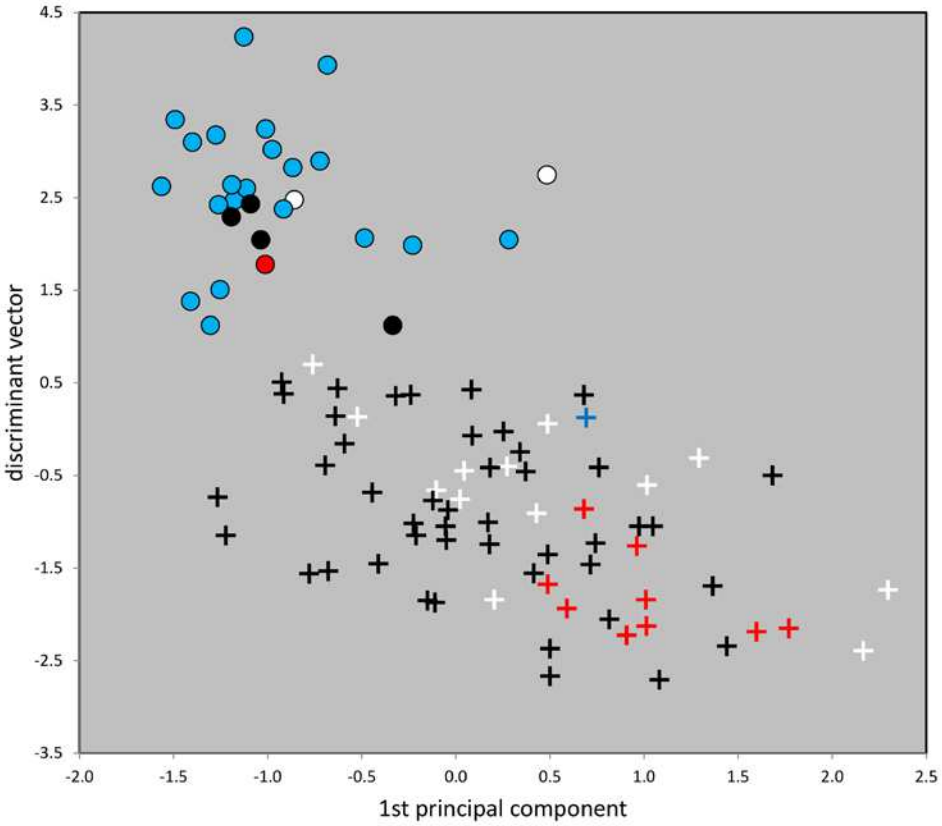


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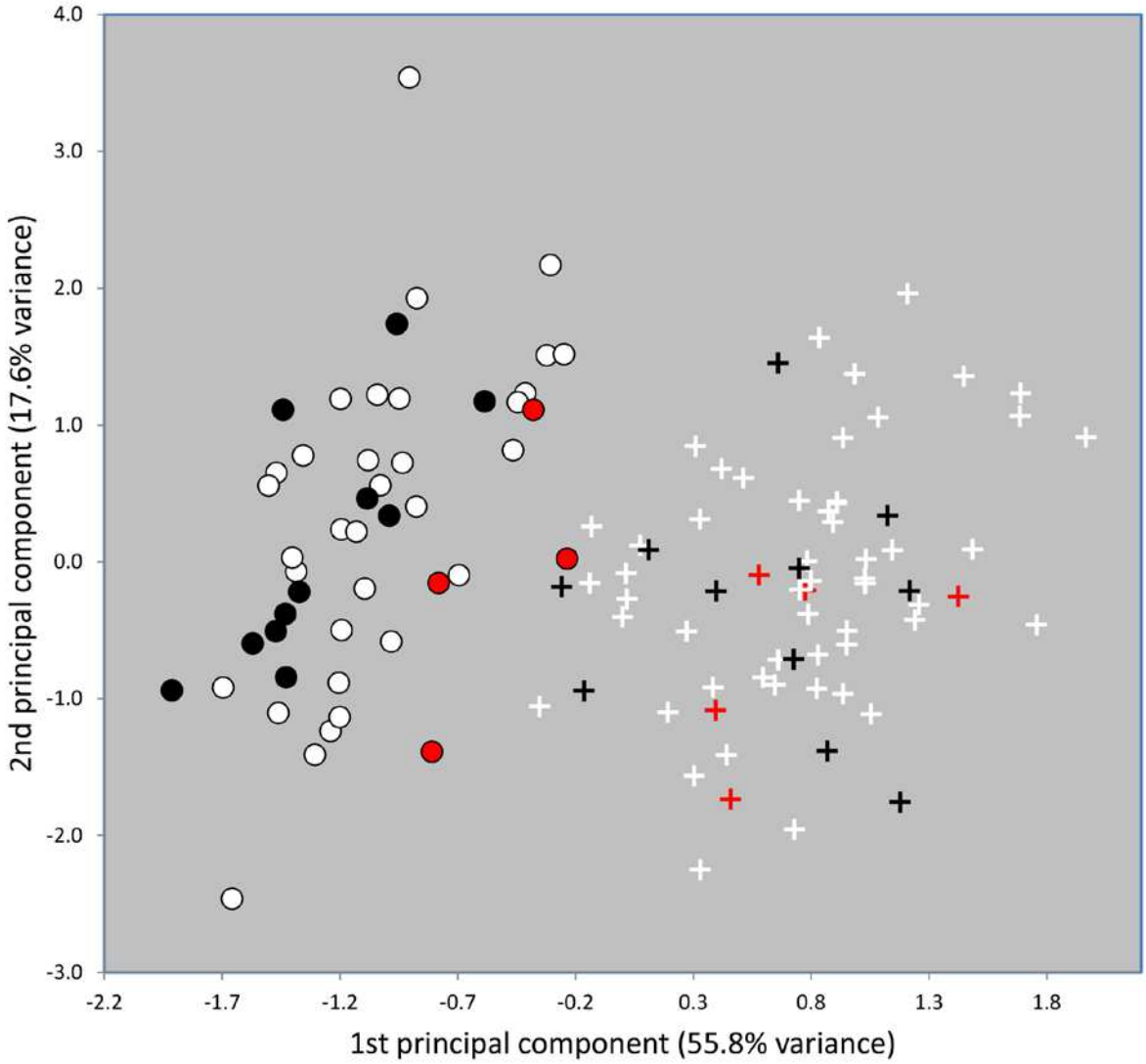


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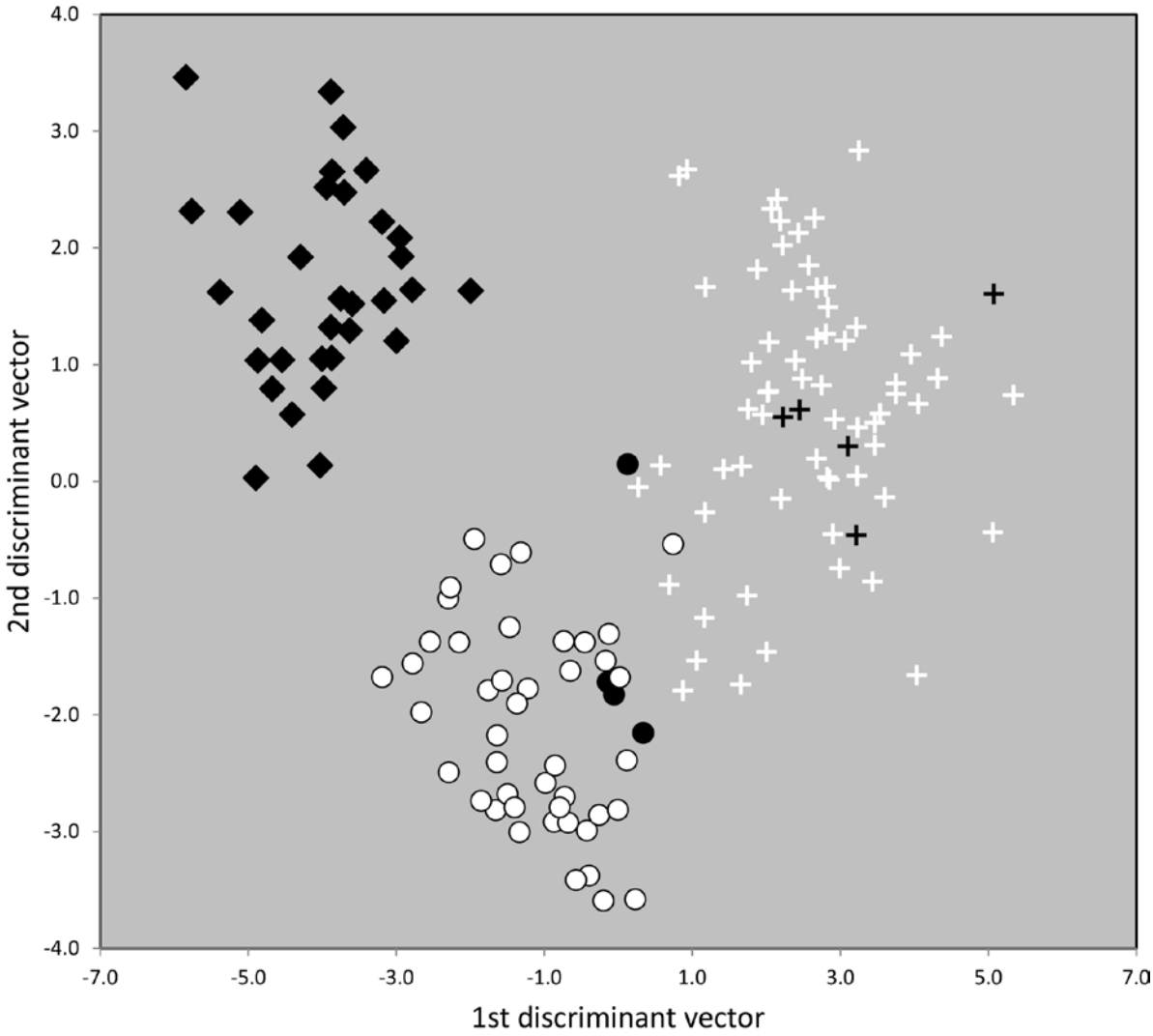


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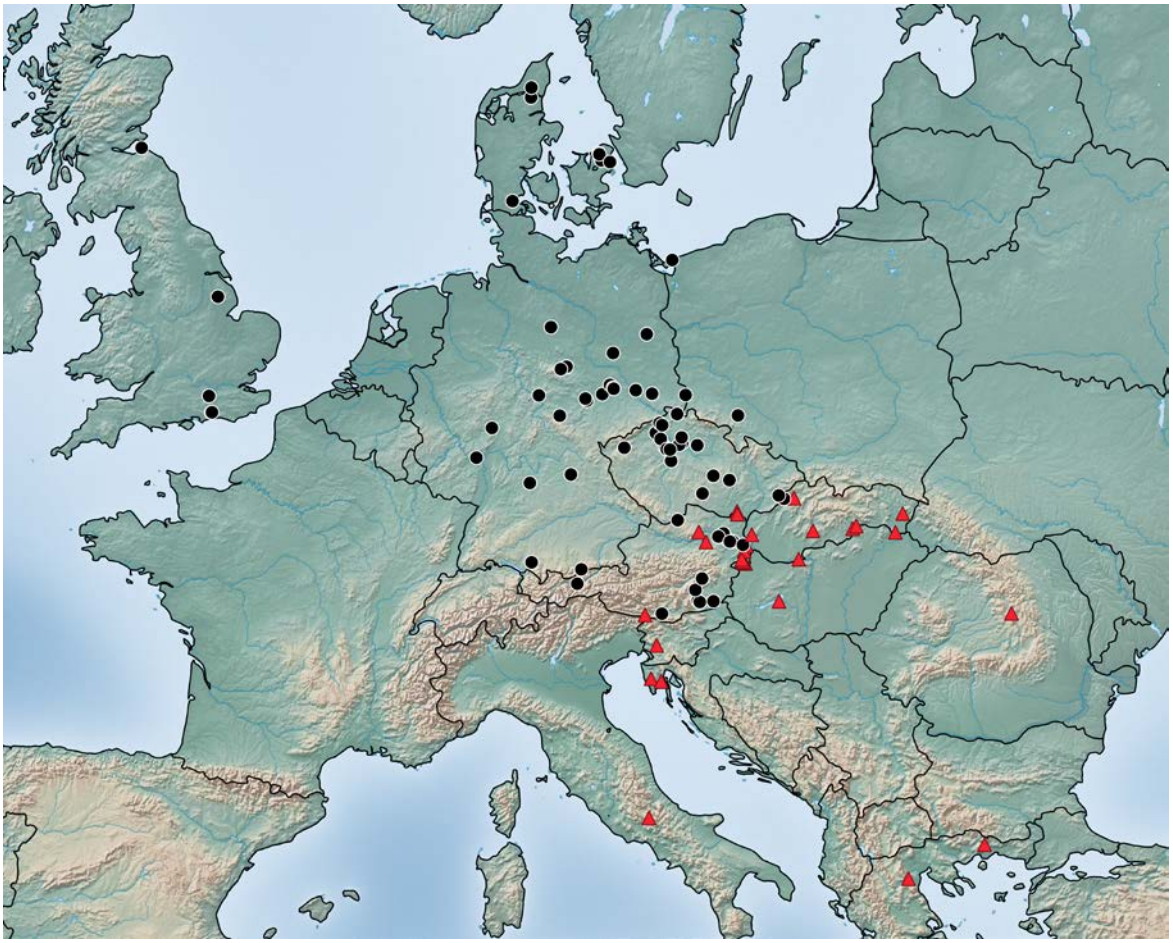


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Fig. 18: Head of the holotype of *Lasius castor* n. sp.; Gantschenberg, Austria, 2021.

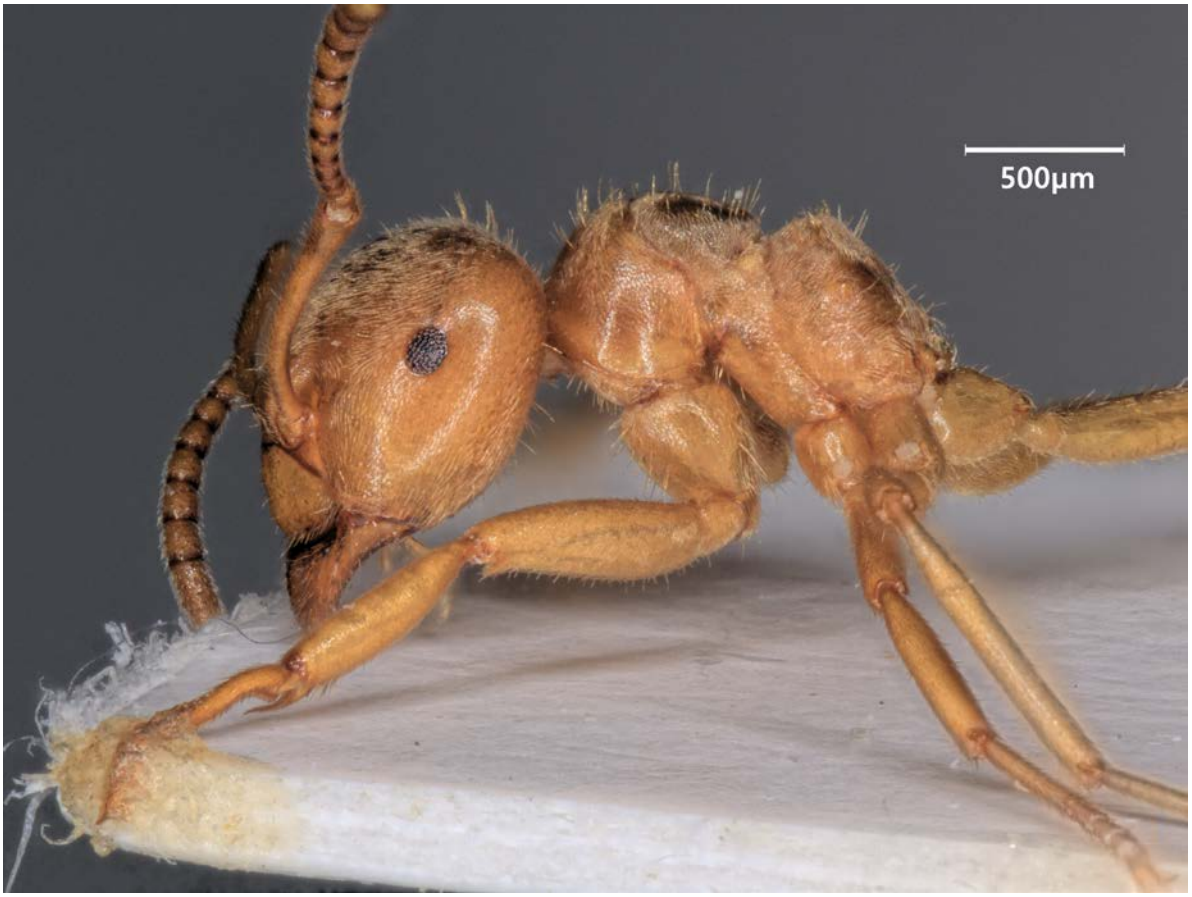


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Fig. 23: Lateral aspect of the holotype of *Lasius pollux* n. sp.; St. Pölten, Austria, 1995.



Fig. 24: Petiole of a paratype of *Lasius pollux n.sp.* in frontal view; Hundsheimer Berge, Austria, 2000

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- SI1relevantsamples.xlsx
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- SI3gynesprimarydata.xlsx
- SI4.workerratios.xlsx
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