The supercolonial European wood ant *Formica paralugubris* SEIFERT, 1996 (Hymenoptera: Formicidae) introduced to Canada and its predicted role in Nearctic forests[*]

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Abstract

A supercolonial mound-building wood ant was intentionally introduced from the Italian Alps to Quebec, Canada, in 1971. This species was believed so far to represent *Formica lugubris* ZETTERSTEDT, 1838. Yet, recent investigations on the distributions of *F. lugubris* and the closely related species *F. paralugubris* SEIFERT, 1996 in the Italian Alps showed presence of both species and also that the supercolonial social type is represented here mainly by the latter species. This raised doubts on the species identity of the Canadian ants and prompted a taxonomic re-investigation. Advanced exploratory and hypothesis-driven data analyses of worker phenotype of 152 nest samples of both species from the Alps and of two samples collected from the supercolony in Quebec convincingly confirmed the Canadian introduction to represent *F. paralugubris*. The Quebec samples were safely allocated to the *F. paralugubris* cluster in both Nest Centroid (NC)-Ward and NC-K-Means clustering, a nest-centroid based principal component analysis (PCA), and a linear discriminant analysis. The error of exploratory data analyses over all 154 samples varied between 0.6% (NC-K-Means) and 1.9% (NC-Ward, PCA). A new method calculating the size of nest populations of polygynous *Formica rufa* group ants is introduced, according to which the growth of the Valcartier introduction was estimated from about one million workers in 1971 to 19 million workers in 2005. Data on mating biology, strategy and speed of dispersal, colony structure, and ecological requirements indicate that active spreading of this ant to areas remote from the Valcartier beach-head is unlikely. There is also a low probability of passive dispersal by unintentional anthropogenic transfer of colony fragments. Although supercolonial, *F. paralugubris* lacks some of the essential properties of invasive tramp ants – its species-specific preadaptations are not comparable with the situation in the imported European Fire Ant *Myrmica rubra* (LINNAEUS, 1758). A prediction of the role of *F. paralugubris* in the Nearctic forest ecosystems is presented. The concluded low risk of it becoming a dangerous invasive species does not refute the importance of keeping the situation in Quebec under careful observation.

Key words: Neozoon, anthropogenous introduction, cryptic species, numeric morphology-based alpha-taxonomy, nest centroid clustering, population estimate.

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Introduction

The Palaearctic mound-building wood ants of the *Formica rufa* group are generally considered an important stabilizing element of temperate or boreo-montane woodland ecosystems. The idea of protecting forests from pest insects by wood ants led to extensive artificial introduction programs in Germany, Poland, European Russia, and Italy during the last century, particularly during the 1950s and 1960s (reviewed in OTTO 2005). Outstanding was a massive introduction program of a boreo-montane wood ant species to at least 42 localities over the whole of Italy south to Sicily and west to Sardinia. Big trucks delivered their load with ant-filled barrels all over the country. This enterprise was performed by the working group of Pavan & Ronchetti between 1959 and 1967 (sites listed by BARONI URBANI 1971). The transferred ants were named at that time *Formica lugubris* ZETTERSTEDT, 1838. These Italian introduction activities did not consider the species-specific climatic requirements, and the ants' survival in central and south Italy was not documented.

Highly polygynous colonies from the Italian Alps invariably served as source material for these actions. This was also the case in the introduction to the Nearctic region in 1971. Such an intentional transcontinental movement of a neozoon would certainly cause massive protest today. The introduction was executed by Finnegan in cooperation with the working group of Pavan and justified by FINNEGAN (1975) with the following words: "Owing to the absence in Canada of suitable, aggressive, predacious species of red wood ants, and the conviction that an efficient, facultative predator of this type would be beneficial to the forest ecosystem, it was decided to introduce *Formica lugubris* from northern Italy." Thirty-four years after introduction, Finnegan's colony near Valcartier, Quebec, had spread over a forested area of 3.8 ha and had

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[*] *This work is dedicated to Gennady Mikhailovich Dlussky (1937 - 2014) who impressed me with his productive and fresh attitude to ant research and holistic understanding of living organisms.*

grown to a supercolony of 95 living mounds with an estimated population of 8 million workers (STORER & al. 2008).

STORER & al. (2008) continued to name the supercolonial ants *Formica lugubris*. It had escaped their attention that, twelve years before, a cryptic sister species of *F. lugubris* had been described from the montane to subalpine zone of the Alps: *Formica paralugubris* SEIFERT, 1996. *Formica paralugubris* is a highly polygynous and supercolonial ant, with monodomous-monogynous nests being unknown. The high impact of this ant on forest ecosystems is illustrated by two figures: The largest supercolony in the Swiss Jura contained 1200 nests in a 70 ha forest area (GRIS & CHERIX 1977) giving a small-scale density of 17.1 nests / ha and the average large-scale density in the Swiss Jura mountains was determined by FREITAG (2002) as 80 nests / 1 km^2 forest area. In Europe, *F. paralugubris* has been the focus of about three dozen investigations on ethology, genetics, reproductive biology, phylogeography, ecology, taxonomy, and faunistics, making it one of the best studied wood ant species of the Palaearctic.

Morphology-based separation of *Formica paralugubris* from the sympatric *F. lugubris* is extremely difficult in worker ants (SEIFERT 1996). The interspecific overlap of the 95% confidence intervals of the most diagnostic morphological character – mean pronotal hair length – exceeds 60% and other characters overlap much more. Nest series of gynes are easier to separate. A recent re-examination of gyne material of the Pavan & Ronchetti wood ant collection stored in the University of Pavia (BERNASCONI & al. 2006) showed that the majority of ants determined as "*Formica lugubris*" at that time (PAVAN & al. 1971) really belonged to *F. paralugubris*. Extending their view by recent sampling in the Italian Alps, BERNASCONI & al. (2006) showed both cryptic species to occur sympatrically and syntopically in that area in the 1960s and today. This was also confirmed by collection material stored in the Senckenberg Museum of Natural History Görlitz. Accordingly, the species identity of the ants introduced to Canada cannot be concluded from geographic data. However, the highly polygynous and supercolonial life form is a constant feature in *F. paralugubris* whereas it is only regionally observed in *F. lugubris* (SEIFERT 2007). This suggested that the source material of the Canadian introduction was probably *F. paralugubris*.

By courtesy of Johanne Delisle from Laurentian Forestry Centre Quebec, who kindly collected fresh material from the colony in 2014, I was able to confirm this assumption by Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT). NUMOBAT designates no particular analytical method but rather the working philosophy of applying any form of reproducible numeric description of phenotypical characters from very simple to very complex procedures. The term was created to signal the difference from mainstream taxonomy that is based on simple subjective eye inspection and does not produce analyzable or testable data sets. The NUMOBAT method proposed by SEIFERT (1996) to separate workers of *Formica paralugubris* and *F. lugubris* by only four selected characters was developed on the basis of only 79 samples from mainly the Western Alps. It separated sympatric populations of both species in the Swiss and French Jura Mountains very well. Yet, later investigations of *F. lugubris* from the whole of

the Alps showed enormous intraspecific polymorphism with several aberrant local populations. Applying the method of 1996 on this extended sample resulted in an unacceptably high error rate. In order to solve this problem, I will present here a new, highly discriminative species delimitation system for *F. paralugubris* and *F. lugubris* workers that is based on explorative data analyses and is applicable all over the Alps. The discrimination from *F. paralugubris* is also given for a local population of *F. lugubris* from Eastern Switzerland possibly having taxonomic significance (BERNASCONI & al. 2011). Furthermore I present a simple system for a population estimate in polygynous nests of mound building wood ants. Finally, I address whether the introduced neozoon might be a real danger for Nearctic forest ecosystems.

I conclude this introduction with a comment on terminology. The understanding of the term "supercolony" has recently raised discussions (e.g., MOFFET 2012, LESTER & GRUBER 2012). As ant supercolonies have attracted much interest among biologists due to their impressive power and organization, and the fact that some of the most dangerous invasive ant species show a supercolonial organization, a semantic clarification seems necessary. Discussing at which point of growth or extension a large polydomous-polygynous colony has to be termed a supercolony makes little sense for adaptive, transitionary systems of extremely variable size. A supercolony (adj. supercolonial) is understood here simply as a very large polydomous-polygynous colony with permanent connections between the nests and absence of mutual aggression between workers originating from any nest of the system independent of the distance separating them. If members of two geographically isolated supercolonies show no mutual aggression after mixing, I would not speak of a single colony. Functional unity or connectivity is more important than any global loss of aggression (see also SEIFERT 2007) but this is a matter of taste. I would put more emphasis on which word is used for a supercolonial system. For semantic reasons (or self-explanatory wording), I generally advocate replacing the synonymous term "unicolonial" by "supercolonial". Both a small monogynous-monodomous nest of a *Temnothorax* ant and a huge supercolony of an Argentine Ant can each be considered as "one" colony. The term "uni" provides no meaning in this word connection whereas "super" indicates something very large or a combination of elements in a system of higher level of order.

Material and methods

Material

Evaluation was restricted to material from Alps and adjacent regions of Czechia. 93 nest samples of *Formica lugubris* with 389 worker individuals originated from Austria (5 samples), Czechia (4), France (8), Germany (17), Italy (12) and Switzerland (47).

Sixty-one nest samples of *Formica paralugubris* with 287 worker individuals originated from Austria (6), Canada (2), France (2), Italy (2) and Switzerland (49). The two samples, No. 3 and No. 9, of *F. paralugubris* from Canada (Quebec, Valcartier) were collected by J. Delisle in a supercolony in July 2014. This colony is situated at 46.972° N, 71.503° W and 288 m above sea level. Its structure and dimensions were described by STORER & al. (2008).

Character recording

An average of 4.4 dried and mounted workers per nest (a total of 676 specimens) was evaluated for 11 numerically recorded phenotypic characters. Sample size was occasionally reduced to three in monomorphic nests and extended to six in polymorphic nests. All measurements were made on mounted and dried specimens using a pin-holding stage, permitting full rotations around X, Y and Z axes. Two high-performance stereomicroscopes, a Leica Wild M10 equipped with a 1.6× planapochromatic objective (resolution 650 lines / mm) and Leica M165C equipped with a 2.0 planapochromatic objective (resolution 1050 lines / mm) were used at magnifications of 120 - 384×. A Schott KL 1500 LCD 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 optimum 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 the voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks was used. To avoid the parallax error, its measuring line was constantly kept vertical within the visual field. The mean relative measuring error over all magnifications and systems was 0.4%. To avoid rounding errors, all measurements were recorded in µm even for characters for which this precision is impossible.

Setae, also called pilosity or simply "hairs", are differentiated from pubescence hairs in having at least twice the basal diameter of neighbouring pubescence hairs. All setae counts (acronyms beginning with "n") are restricted to standing setae projecting > 10 µm from cuticular surface. Setae counts and scape measurements are, if not otherwise stated, averages of both body halves with exception of strongly and symmetrically haired specimens. The description of the evaluated numeric characters is given in the following.

CL   Maximum cephalic length in median line. The head must be carefully tilted to the position with the true maximum. Excavations of occiput and / or clypeus reduce CL.

CS   Cephalic size. Arithmetic mean of CL and CW, used as a less variable indicator of body size.

CW   Maximum cephalic width.

METHL   Length of the longest seta on a metapleuro-propodeal area below a straight reference line that is directed parallel to the straight section of lower metapleural margin and touches the lower margin of propodeal spiracle. The same-level area of caudal propodeal slope is included, weir hairs protecting the orifice of metapleural gland and proprioreceptive setae immediately at the caudal margin near petiole junction are excluded. Care is taken to visualize the lateral suture between meso- and metapleuron.

mPNHL   Mean pronotal hair length. Applied measuring schedule: select one of the longest hairs on dorsal pronotum and calculate the arithmetic mean length of this hair and of its seven nearest neighbours. Pro-prioreceptive setae on anterior pronotal shield are excluded.

nMET   Bilateral mean of the number of standing setae on a metapleuro-propodeal area below a straight reference line that is directed parallel to the straight section of lower metapleural margin and that touches the lower margin of propodeal spiracle. Include also the infraspiracular area of caudal propodeal slope and take care to visualize the antero-lateral suture between meso- and metapleuron. Setae positioned directly on the suture are counted as 0.5. Definitely excluded from count are weir hairs protecting the orifice of the metapleural gland, proprioreceptive setae immediately at the caudal margin near petiole junction and hairs standing on the ventrolateral edge of metapleuron (hatched area in Fig. A 282 in Seifert 2007: 175).

nOCC   Bilateral mean of the number of standing hairs protruding more than 10 µm from head silhouette as seen in full face view. Counting begins at the level of anterior eye margin and ends at median occiput. The full depth of focus is used for counting and the parallax error is considered.

nSC   Bilateral mean of the number of setae on the dorsal plane of scape.

OCCHL   Apparent protrusion length of the longest standing seta over the postocular head contour in full face view. This value does not indicate the real setae length because setae bases are often concealed and setae axes are inclined compared to visual plane. This measuring mode was selected to save time.

PEW   Maximum width of petiole scale measured in dorsal, frontodorsal or caudodorsal view.

SL   Maximum scape length excluding articular condyle and its neck.

SMAX   Maximum scape width at midpoint; data of both scapes are averaged.

Removal of allometric variance

In most species groups of *Formica*, morphological characters are strongly influenced by allometric growth. In order to make characters directly comparable in synoptic tables (side-by-side comparisons), a removal of allometric variance (RAV) was performed with the procedure described by Seifert (2008). As standard for all members of the *Formica rufa* group, RAV was calculated assuming that all individuals have an identical cephalic size of 1.75 mm. RAV functions used collective parameters calculated as the arithmetic mean of the species-specific functions of twelve Palaearctic *Formica rufa* group species. Inspection of scatter plots indicated the use of monophasic linear RAV functions. The RAV functions of four shape and six pilosity characters were

$$CL / CW_{1.75} = CL / CW / (-0.1085 * CS + 1.2922) * 1.1023$$
$$SL / CS_{1.75} = SL / CS / (-0.0762 * CS + 1.0556) * 0.9222$$
$$SL / SMAX_{1.75} = SL / SMAX / (-0.1561 * CS + 9.995) * 9.722$$
$$PEW / CS_{1.75} = PEW / CS / (0.0227 * CS + 0.4395) * 0.4792$$
$$nOCC_{1.75} = nOCC / (7.20 * CS + 2.28) * 14.87$$
$$nSC_{1.75} = nSC / (0.59 * CS + 1.88) * 2.90$$
$$OCCHL_{1.75} = OCCHL / (41.23 * CS + 17.0) * 89.1$$
$$mPNHL_{1.75} = mPNHL / (27.07 * CS + 34.12) * 81.5$$
$$nMET_{1.75} = nMET / (5.19 * CS - 2.16) * 6.93$$
$$METHL_{1.75} = METHL / (44.01 * CS + 65.69) * 142.7$$

Tab. 1: Morphometric data of one size, four shape, and six pilosity characters in worker individuals of the cryptic wood ant species *Formica lugubris* and *F. paralugubris* given in the sequence arithmetic mean ± standard deviation [minimum, maximum]. Removal of allometric variance was performed with the assumption of each individual CS = 1750 µm. F and p values of a univariate ANOVA are given. i = number of workers, n.s. = not significant.

	Primary data without removal of allometric variance				Data with removal of allometric variance		
	F. lugubris (i = 389)	ANOVA F; p	*F. paralugubris* (i = 287)		*F. lugubris* (i = 389)	ANOVA F; p	*F. paralugubris* (i = 287)
CS [µm]	1820 ± 188 [1272, 2164]	**99.76** 0.000	1689 ± 136 [1270, 2020]	CS [µm]	1820 ± 188 [1272, 2164]	**99.76** 0.000	1689 ± 136 [1270, 2020]
CL / CW	1.094 ± 0.028 [1.023, 1.167]	2.45 n.s.	1.097 ± 0.023 [1.029, 1.166]	CL / CW$_{1.75}$	1.102 ± 0.020 [1.049, 1.148]	54.18 0.000	1.091 ± 0.018 [1.044, 1.138]
SL / CS	0.887 ± 0.029 [0.817, 0.977]	77.83 0.000	0.905 ± 0.023 [0.849, 0.981]	SL / CS$_{1.75}$	0.892 ± 0.023 [0.804, 0.960]	24.90 0.000	0.901 ± 0.020 [0.845, 0.983]
SL / Smax	9.22 ± 0.41 [8.12, 10.65]	0.35 n.s.	9.20 ± 0.37 [8.41, 10.35]	SL / SMAX$_{1.75}$	9.23 ± 0.41 [8.15, 10.64]	1.504 n.s.	9.19 ± 0.37 [8.41, 10.35]
PEW / CS	0.498 ± 0.030 [0.427, 0.580]	1.88 n.s.	0.495 ± 0.025 [0.402, 0.555]	PEW / CS$_{1.75}$	0.500 ± 0.030 [0.426, 0.571]	0.00 n.s.	0.497 ± 0.025 [0.411, 0.554]
nSc	1.44 ± 2.68 [0.0, 18.0]	**173.00** 0.000	5.80 ± 5.74 [0.0, 24.0]	nSC$_{1.75}$	1.43 ± 2.65 [0.0, 17.9]	**176.35** 0.000	5.86 ± 5.82 [0.0, 24.2]
nOcc	21.79 ± 5.77 [1.5, 38.0]	37.38 0.000	24.56 ± 5.88 [6.0, 39.0]	nOCC$_{1.75}$	21.13 ± 5.45 [1.3, 35.3]	**91.60** 0.000	25.25 ± 5.64 [6.1, 39.1]
OccHL	120.3 ± 23.8 [63, 192]	73.27 0.000	105.6 ± 19.7 [58, 162]	OccHL$_{1.75}$	116.8 ± 22.3 [55, 193]	25.99 0.000	108.5 ± 18.6 [60, 160]
mPNHL	102.60 ± 15.9 [47.9, 152.8]	**508.92** 00.000	76.8 ± 12.8 [44.6, 127.0]	mPNHL$_{1.75}$	100.4 ± 14.8 [44.0, 143.9]	**423.28** 0.000	78.4 ± 12.2 [46.3, 127.5]
nMET	10.23 ± 2.95 [2.5, 21.0]	**178.77** 0.000	7.31 ± 2.62 [2.5, 15.3]	nMET$_{1.75}$	9.72 ± 2.45 [3.1, 16.8]	**124.48** 0.000	7.61 ± 2.42 [3.0, 14.8]
METHL	183.2 ± 22.8 [121, 261]	**348.48** 0.000	152.2 ± 19.1 [98, 200]	METHL$_{1.75}$	179.3 ± 20.4 [130, 248]	**272.95** 0.000	155.0 ± 17.4 [98, 200]

Explorative and supervised data analyses and classification methods

The delimitation of the cryptic species was done by an interaction of Nest-Centroid Clustering (NC clustering) and a confirmative linear discriminant analysis (LDA). NC Clustering was run both as hierarchical NC-Ward clustering and non-hierarchical NC-K-means clustering. These methods were described in more detail by SEIFERT & al. (2014c) who also provided a script written in R and freely available under the GNU / GPL license from the website: http//sourceforge.net/projects/agnesclustering/.

NC-Ward clustering was run first to indicate the putative number of K main clusters. In the second step NC-K-Means was performed with the setting of K classes suggested by NC-Ward. Classifications being coincident between the hierarchical and non-hierarchical clustering formed the hypothesis for the controlling LDA that was subsequently run. Samples with classifications disagreeing between NC-Ward and NC-K-means were run in this LDA as wildcards. The final classification ("final species hypothesis") was established by the LDA in the iterative procedure described by SEIFERT & al. (2014c). There remained no undecided cases, regardless of which posterior probabilities they had. LDA and ANOVA tests and principal component analyses (PCA) were performed with the software package SPSS 15.0.

Results and discussion

The NUMOBAT analysis

The NUMOBAT analysis of the 154 nest samples yielded a most effective species delimitation of *Formica paralugubris* from *F. lugubris* in the Alps. The analysis was started unselectively using all eleven available characters. These were the absolute size indicator CS and the shape and pilosity characters CL / CW$_{1.75}$, SL / CS$_{1.75}$, SL / SMAX$_{1.75}$, PEW / CS$_{1.75}$, nOCC$_{1.75}$, nSC$_{1.75}$, OCCHL$_{1.75}$, mPNHL$_{1.75}$, nMET$_{1.75}$, and METHL$_{1.75}$. Table 1 presents a comparison of these data for the two species both as primary and RAV-corrected data. Removal of allometric variance reduced within-species variance in the characters CL / CW by 44%, in SL / CS by 31%, in nMET by 23%, in METHL by 19%, in mPNHL by 11%, in OCCHL by 11% and in nOCC by 10%. No reduction was achieved in PEW / CS, SL / SMAX, and nSC. In the most discriminative character, mPNHL$_{1.75}$, only 37% of all individuals were outside the interspecific overlap range described by the 95% confidence interval. This illustrates the enormous phenotypic similarity.

Hierarchical NC-Ward clustering indicated the presence of two strongly separated major branches. Nonhierarchical NC-K-Means clustering with a setting of K = 2 confirmed this classification in 94.8% of samples. The
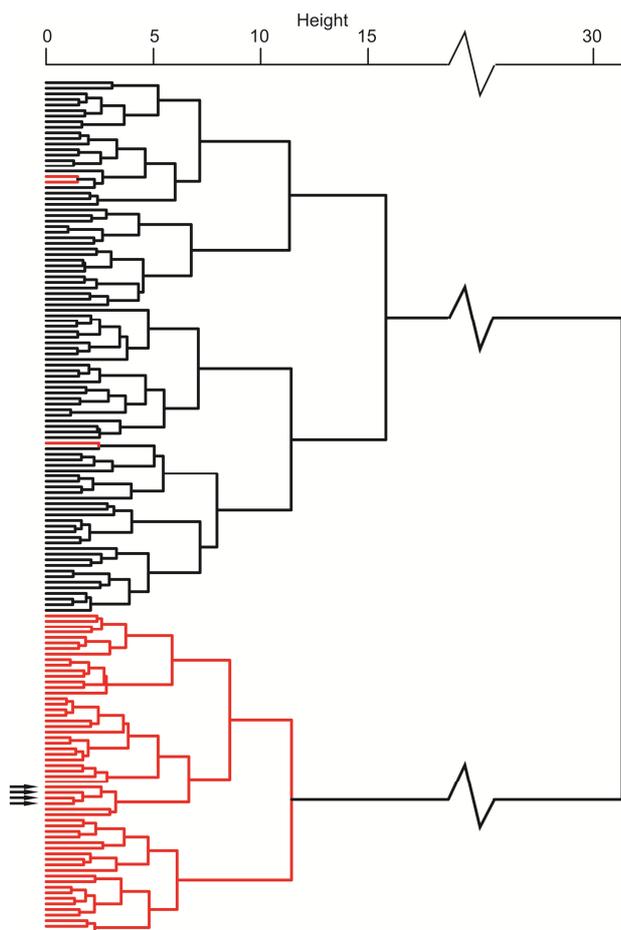
Fig. 1: NC-Ward clustering of 154 samples of the cryptic wood ant species *Formica lugubris* (black branches) and *F. paralugubris* (red branches) considering nine morphological characters. The upper two arrows point to the samples from Canada, the lower two to those from the Italian Alps. Classification error over all samples 1.9%. The same dendrogram is presented in the electronic supplement, Fig. S1, with sample labels given.

5.2% of disagreeing samples were then run as wildcards in the iterative LDA. With the new classification obtained, a stepwise LDA was performed that removed the characters PEW / CS $_{1.75}$ and OccHL$_{1.75}$ from the data set and fixed the final species hypotheses. Using the nine remaining characters, the disagreement with the final species hypothesis achieved 1.9% in NC-Ward (Fig. 1) and 0.6% in NC-K-Means. These data are well below the 4% threshold proposed by SEIFERT & CSŐSZ (2015) within the context of the Pragmatic Species Concept (SEIFERT 2014). A principal component analysis (PCA), also run with these nine characters, resulted in 1.9% misclassification. Figure 2 shows the LDA score plotted against the 1st factor of the PCA.

The samples "CAN: Quebec: Valcartier, 2014.07-3", "CAN: Quebec: Valcartier, 2014.07-9", and the holotype nest sample of *Formica paralugubris* "SWI: Jura (Cherix) 1993.05.05-G5" were allocated to the *F. paralugubris* cluster by each of the three exploratory data analyses and they were classified accordingly by the LDA with posterior probabilities of p = 0.9541, 0.9466 and 0.9927 respectively. These data provide convincing evidence that the ants intro-
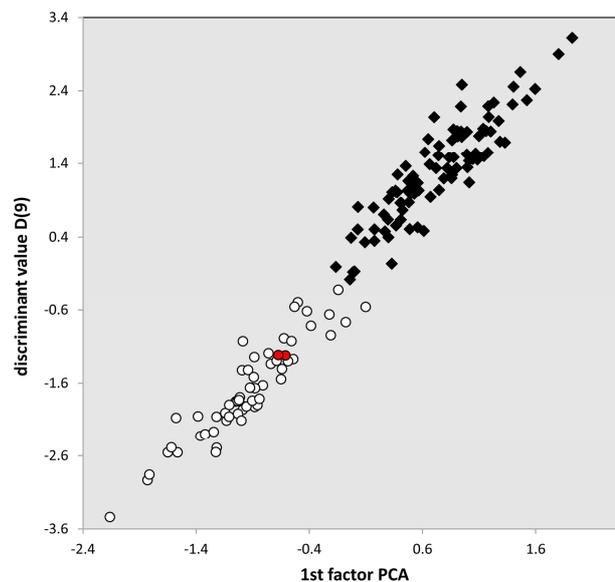
Fig. 2: Plotting of linear discriminant scores D(9) against the 1st factor of principal component analysis of 154 samples of the cryptic wood ant species *Formica lugubris* (black rhombs) and *F. paralugubris* (white discs) from the European Alps plus two samples of *F. paralugubris* from Quebec / Canada (red discs).

duced to Canada in 1971 must be named *Formica paralugubris*. Figure 1 provides an interesting detail: Both samples from Canada form a "monophyletic" branch together with just the two available samples from the Italian Alps – the region from which the source material of the Valcartier foundation came (Fig. S1 of the Appendix, as digital supplementary material to this article, at the journal's web pages, provides the dendrogram with the labels of the samples). This illustrates that NC-Ward clustering is not only a powerful tool of delimiting cryptic species – the method reflects the intraspecific structure expected from the perspective of population genetics, at least roughly. A significant correlation of the intraspecific structuring of any NC-Ward dendrogram with regions of geographic origin was evident in over 100 cases of eight ant genera (CSŐSZ & al. 2013, SEIFERT & al. 2014a, b, c, SEIFERT & CSŐSZ 2015; B. Seifert, unpubl.).

A population estimate of the introduced *Formica paralugubris* population

Formica paralugubris was introduced to eastern Canada in 1971 into a 35-year-old plantation of *Pinus resinosa*, *Pinus strobus*, *Picea glauca*, *Abies balsamea*, and *Betula papyrifera* (STORER & al. 2008). The number of initially released ants was estimated by FINNEGAN (1975) to be up to 1.3 million worker ants. Assuming an average worker queen ratio of 400 : 1 in polygynous wood ant nests (GÖSSWALD 1951, ITO 1973), it may have had some 3000 queens. By the end of 1973, the ants had formed five large nests which were over 125 cm tall and were estimated to contain three million ants (FINNEGAN 1975). No figures are available after this until the census of STORER & al. (2008). The study of these authors was conducted in 2005. They recorded 95 living nests distributed over 3.8 ha forest area, summing up to 41.6 m³ above-ground mound volume and 33.9 m² basal area. They estimated the whole colony to

Basal nest area [m²]	Worker population [10⁶]	Species	Source
0.340	0.344	*F. polyctena*	HORSTMANN (1982)
0.665	0.428	*F. aquilonia*	ZACHAROV (1978)
0.870	0.558	*F. pratensis*	SÖRENSEN & SCHMIDT (1987)
1.130	0.515	*F. polyctena*	HORSTMANN (1982)
1.650	0.770	*F. polyctena*	ROSENGREN & SUNDSTRÖM (1987)
4.910	1.590	*F. rufa*	SÖRENSEN & SCHMIDT (1987)
7.070	3.160	*F. pratensis*	SÖRENSEN & SCHMIDT (1987)
10.180	4.800	*F. polyctena*	SÖRENSEN & SCHMIDT (1987)
38.500	15.000	*F. polyctena*	G. Dlussky (pers. comm.); method in ZACHAROV (1976)

Tab. 2: Relation of basal nest area in square meters and total population in million workers in polygynous societies of mound-building wood ants of the *Formica rufa* group. Eight records from the literature and an unpublished study by Dlussky are shown.

contain 8.1 million ants based on the assumption that one cubic meter mound volume corresponded to 195,000 ants.

This population estimate based on mound volume is probably too low. It is common sense among myrmecologists that mound volume is strongly influenced by population-independent factors such as sun exposure, soil moisture and availability of nest material (e.g., DLUSSKY 1967, ITO 1973, MABELIS 1977, SEIFERT 2007). In the extreme, a huge population may be associated with a huge concave nest surface of mineral soil material but only rudimentary above-ground vegetable mound material. This is exemplified by the nest type "Hühnengrabnest" (GÖSSWALD 1951) of *Formica polyctena* FÖRSTER, 1850. Accordingly, population numbers are better correlated with basal nest area (GÖSSWALD 1951, ITO 1973, SAVOLAINEN & al. 1996). Basal nest area is most adequately measured within the perimeter defined by the most peripheral nest exits which are frequently found within a ring of soil ejections. These exits are more or less outside the basal margin of the true vegetable mound.

In order to find a rule to estimate population numbers in polygynous wood ant nests, I made a literature survey for data sets in which both worker population and basal nest area were recorded. Excluding nests reported to be dying out or being just founded, only eight data sets from the studies of ZACHAROV (1978), HORSTMANN (1982), ROSENGREN & SUNDSTRÖM (1987) and SÖRENSEN & SCHMIDT (1987) remained. These authors applied differing methods aiming at a census of the forager population – basically mark-recapture methods or traffic counts related to mean forager circulation time. The total nest population was then calculated by division of the estimated forager population by their typical month-specific ratio of the total nest population. Integrating the data of KRUK DE BRUYN & al. (1977), HORSTMANN (1982), ROSENGREN & SUNDSTRÖM (1987) and GOEBEL (1988) the month-specific percentage of foragers in the temperate zone of Europe may be estimated as 26% in April, 40% in May, 43% in June, 45% in July, 37% in August, 32% in September and 26% in October. The eight evaluated censuses were carried out from June to early September, meaning that forager number had to be divided by ratios between 0.35 and 0.45 to estimate the total population. Based on the data in Table 2, total worker population N in million workers is described as a function of basal nest area A by the following highly significant linear function

$N = 0.4399 A + 0.0463$ (r = 0.9863, p < 0.001, n = 8).

Applying the function to 95 nests with a mean basal area of 0.357 m² results in a total of 19.3 million workers for the Valcartier supercolony. The figure could be even larger considering that STORER & al. (2008) measured the diameter of the vegetable mound only.

Is the method applicable to exceptionally large nests? Zacharov and Dlussky made an unpublished census of a huge nest in a shady spruce forest near Moscow by the method of exhaustive sampling (ZACHAROV 1976). Exhaustive sampling is probably the most accurate method to estimate forager populations but it causes damage to the nest. According to ZACHAROV (1973, 1979), large polygynous wood ant nests are organized in sectors. Foragers going out from a certain sector use the same trunk trail and return always to their home sector. The largest nests may have dozens of sectors. During exhaustive sampling, closure of all exits in a sector by the ants signals the investigator to have captured the whole forager population of a sector. Because innendienst (= indoor service) workers cannot immediately shift to aussendienst (= field service) tasks, it takes several days before foraging activity is resumed. Zacharov & Dlussky censused a number of sectors of that huge nest and extrapolated the total population size to be 15 million (G. Dlussky, pers. comm.). This result, to my knowledge, has never been published – possibly because they did not trust their own data. Gennady Dlussky showed me this nest during a visit in 1985. The traffic noise of this "Metropolis" could be heard before the mound became visible in the twilight of the fog-draped spruce stand. My own estimate of the basal area of this nest was 38.5 m² which would result in 17 million workers according to the regression function above. These data suggest the area-based population estimate method is probably realistic. Yet, the number of error factors in such population estimates is large. We cannot exclude that each of the four investigation teams made errors causing a bias in the same direction. Complete nest excavations before hibernation are needed to finally check the reliability of the method.

It must be emphasized that the regression function proposed above refers to a selection of well-populated nests of the highly polygynous social form as is typical for polydomous colonies or supercolonies. These relations do not apply to monogynous to weakly polygynous nests. This is exemplified by data on Irish and Scottish *Formica lugubris*. I evaluated five censuses of forager populations from Ireland performed in early July (BREEN 1979) and divided these data by the July-typical forager ratio of 0.45.
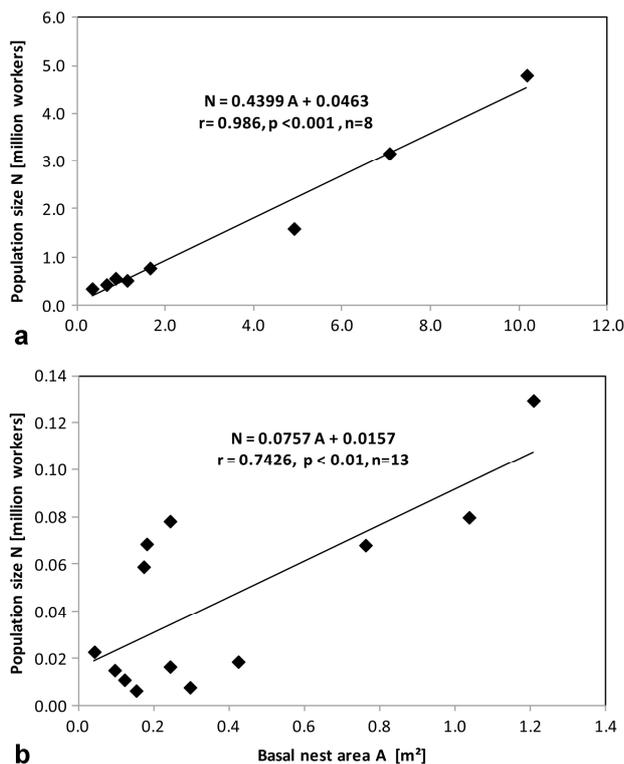
Fig. 3: Population size N in million workers plotted against basal nest area A in square meters. (a) Data of strongly polygynous nests of *Formica polyctena*, *F. aquilonia*, *F. lugubris*, and *F. pratensis* from Europe. (b) Data of monogynous to weakly polygynous nests of *F. lugubris* from Ireland and Scotland.

Additionally, I considered data of eight nests from Scotland completely excavated after hibernation in April (CHEN & ROBINSON 2013). The found worker number was multiplied by the factor 1.2 to correct for moderate winter losses. The resulting regression function was

$N = 0.0757 \, A + 0.0157$ (r = 0.7426, p < 0.01, n = 13).

One square meter of nest area seems to correspond here to 91,000 workers – instead of 445,000 in strongly polygynous nests of other species (Fig. 3). According to my own subjective impressions with British *Formica lugubris* in North Yorkshire in 1990, it seems that basal nest area and mean worker number of this population is comparable to the situation in monogynous or weakly polygynous nests of *F. rufa* LINNAEUS, 1761 from Germany. The regression function also reflects the subjective impression of wood ant students that monogynous to weakly polygynous nests have larger mound dimensions relative to worker number than strongly polygynous nests. Figure 3 shows that there is a large prediction error in monogynous to weakly polygynous nests with a basal area < 0.5 m². It seems that environmental influences such as ground properties, exposition or predation cause a much stronger noise of data in small nests than in the populous nests of strongly polygynous societies.

Does this introduced species represent a real danger for North American forest ecosystems?

With a population growth from 1.1 million in 1971 to 19 million workers in 2005 and development into a true supercolony spread with 95 living mounds over an area of 4 ha (STORER & al. 2008 and the calculations presented in the previous section), the introduced *Formica paralugubris* ants undoubtedly show a high competitive power and exhibit a strong impact on that particular forest area. The population estimate corresponds to 53 kg worker fresh weight per ha if the average weight of workers of all age classes is 11.4 mg [data adopted from OTTO (1960) referring to *F. polyctena*; mean body size is nearly equal: CS was 1.674 ± 184 µm in 305 workers *F. polyctena* from all of Europe (B. Seifert, unpubl.) and 1.689 ± 136 µm in 287 *F. paralugubris* workers of this study]. Size and spatial distribution of nests are fully comparable to the most densely populated parts of supercolonies in the Swiss and Italian Alps. This raises the question if this neozoon might become a danger for Nearctic forest ecosystems.

The supercoloniality of *Formica paralugubris* is connected with a strong tendency for intranidal mating, and the small fraction of sexuals performing a nuptial flight will fly to mating places usually placed within or very near to the boundaries of the supercolony. There is a strong genetic viscosity of the particular nests indicating that new queens are largely recruited from their own nest (CHAPUISAT & al. 1997, CHAPUISAT & KELLER 1999). Genetic data from a Swiss supercolony indicate that 99.8% of queens have mated in or very near to the nest and did not disperse. Isolated nests outside the supercolony show a lower genetic divergence than predicted by distance and are suspected to represent foundations by flying gynes (CHAPUISAT 1998). However, there is no direct observation over several decades of studies in the Swiss Jura confirming that single-queen or pleometrotic colony foundation of disperser gynes is possible. If occurring at all, it seems that such events are rare and effective only over a rather short distance.

Evidence against a potency for long-range single-gyne dispersal is also provided by zoogeography. The natural geographic range of *Formica paralugubris* is the smallest known for any described species of the *F. rufa* group in the Palaearctic. It extends over the montane-subalpine zone in the western Alps between 6° E and 11.5° E. This is an area of only 80,000 km² – for comparison: the range of the long-range disperser *F. lugubris* extends over 11,000 kilometers from Ireland to Kamchatka and over an estimated area of 14 million square kilometers (GOROPASHNAYA & al. 2004). Reports of *F. paralugubris* from the Pyrenees clearly refer to *F. lugubris* (see SEIFERT & GOROPASHNAYA 2004; plus NUMOBAT analyses of the author in 23 Pyrenean samples, B. Seifert, unpubl.). Postglacial spreading of *F. paralugubris* must have occurred from a refuge in the Italian Prealps or the plain of the river Po. Migration northwest to the French Alps and Jura and northeast to approximately Innsbruck was very slow with an average progress of 20 m per year. Long-lasting glaciation of the high passes in combination with the weak or missing potency for long-range flight dispersal and socially parasitic colony foundation and, finally, competition with the ecologically most similar *F. aquilonia* YARROW, 1955 approaching from the east can explain the small range of *F. paralugubris* in the Alps. Despite several thousand years of confrontation, the range overlap of both sister species is currently not wider than 130 km (SEIFERT 2007).

This knowledge about the life history of *Formica paralugubris* suggests that rapid long-range spread of this ant

from its beachhead at Valcartier is unlikely. If a mated gyne should perform a dispersal flight, it faces a major problem: It cannot found independently on the basis of its own body reserves. Instead it has to be adopted by a colony of the subgenus *Serviformica* for socially parasitic colony foundation. This form of colony foundation implies a high risk for the wood ant gyne of being killed by the *Serviformica* workers. Even in the best adapted monogynous life forms of *F. rufa*, *F. lugubris*, and *F. pratensis* RETZIUS, 1783, there is an enormous mortality during penetration of host colonies (SEIFERT 2007). In *F. rufa*, gynes of monogynous nests are significantly larger and physically stronger than those of polygynous conspecific societies (SEIFERT 1991) and they are supposed to have a stronger influence on workers in having a stronger attracting or appeasing pheromonal system = "grössere duftliche Dominanz" (GÖSSWALD 1982). Both factors give them a higher fitness during socially parasitic colony foundation in *Serviformica*. It appears probable that the less adapted smaller gynes of *F. paralugubris* would have very little success if trying to found in a socially parasitic way. The situation in Canada is perhaps even more difficult as they would have to adapt to a completely different set of host species.

Given this low probability for active long-range dispersal, could these ants colonize new areas passively by unintentional human transport? This is also unlikely because some of the essential properties of classical tramp or invasive ant species are missing in *Formica paralugubris*. Firstly, it is impossible for them to colonize new sites from a handful of workers with some brood. Larger colony fragments with queens would need too much incompressible space during hitchhiking and the ants are sensitive to changing moisture and temperature conditions. This implies a high mortality risk during unintentional human transport. Even if exceptionally surviving such a transfer, the material with the colony fragment needs to be discarded in just an appropriate forest ecosystem that has no competitive and hostile ants of the upper dominance hierarchy. On the contrary, releasing or discarding the material in an anthropogenous environment where these competitors and enemies are absent would mean the death of the ants because the resources are missing. Considering the low long-term success of the well-organized and careful efforts of German wood ant conservationists during 5600 rescue relocations ("Rettungsumsiedlungen") of *F. rufa* group nests in appropriate environments (FLEISCHMANN 2014), it appears very unlikely that unintentional human transport and random deposition of some colony fragments might represent a way to disperse *F. paralugubris* over North America.

To conclude, the biological properties of *Formica paralugubris* radically differ from those of the imported European Fire Ant *Myrmica rubra* (LINNAEUS, 1758) which has developed into an important pest in North America and is easily spread by dumping of some soil material (WETTERER & RADCHENKO 2011, DE FISHER & BONTER 2013). Due to its very slow spreading and high requirements for a set of complex environmental variables, *F. paralugubris* should not represent a danger for Nearctic forest ecosystems on a broader geographic scale. The local impact in the Valcartier site can be predicted from broad knowledge of *F. rufa* group ants in Europe derived from hundreds of published studies. Only few, subjectively selected papers are cited here. OTTO (1967) presented a synopsis of 154 papers considering predatory effects on arthropods and many studies followed during the next 50 years. Among some thirty papers on mutualistic relations to trophobiotic insects, to Lachnidae in particular, those of KLOFT (1965), ECKLOFF (1976, 1978), KLOFT & al. (1985), and GOEBEL (1988) may be mentioned here. The effect on seed dispersal was studied in the most comprehensive way by GORB & GORB (2003). The relations to other ant species were in the focus of several studies (e.g., MABELIS 1979, 1984, BEZDECKA 1999). Similar aspects of biology were studied in a *F. paralugubris* supercolony in the Swiss Jura (e.g., CHERIX 1980, 1981, CHERIX & BOURNE 1980, CHERIX & ROSENGREN 1980). According to these data, the following predictions for Valcartier appear reasonable: There will be displacement of other ant species with the exception of those with cryptic behaviour and belonging to the smallest size class (e.g., *Temnothorax*) or of species living and foraging in litter. The very broad nutrition spectrum and missing specialization of *F. paralugubris* should not result in strong changes of ecosystem function – *F. paralugubris* will more or less take over the functions of the native ants they have displaced. It will certainly raise population densities of plant sap suckers, in particular of Lachnidae. It will distribute seeds of myrmecochorous plants in a way similar to native ants of its body-size class and it will exert some regulating effect on outbreaks of pest insects on smaller forest patches. These sentences should not be understood as an invitation for carelessness. I recommend constant observation of the Valcartier supercolony and its effects on the habitat – some of these may differ from the situation in Europe. If an administration decides to eradicate the introduced species, this can be executed with a good chance of success in a reasonably short time.

Acknowledgements

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