

Ants of Kamchatka: checklist, DNA-barcoding and key (Hymenoptera, Formicidae)

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Abstract

Kamchatka is a remote volcanic peninsula in the sub-arctic Far East of Russia. Its myrmecofauna has been scientifically addressed several times. However, previous species lists are contradictory in part and a modern study seems to be lacking. Based on literature review, I conclude that 12 species and 4 genera (*Camponotus*, *Formica*, *Leptothorax* and *Myrmica*) of ants may be native to Kamchatka. Of those, 9 were found in the field, 3 having a Holarctic, 3 a trans-Palearctic and 3 an East Palearctic distribution. Most species diversity was found at mid-elevations, in pine shrubs and mixed forests, while deciduous forests and open habitats at lower altitudes were surprisingly species poor. DNA-barcoding was performed for 57 specimens/9 species. Genetic diversity (COI) was low for 8/9 species, with the exception of *Leptothorax acervorum*, for which all samples were of a different haplotype and 2 haplogroups were identified. For the encountered Holarctic and trans-Palearctic species, closely related haplotypes (<0.7% raw distance) occur in Europe and/or North America. Some *Formica* ants were morphologically atypical, with workers partially resembling those of *F. fusca*, while gynes identified as *F. lemani*. Morphometric analysis and DNA-barcoding suggested that all these specimens belong to a single species, *F. lemani*. Standard images for all specimens and an illustrated key to the worker caste are provided.

Key Words

Subarctic, boreal zone, COI, mtDNA, Russian Far East, Siberia

Introduction

The entomofauna of the Russian Far East is still relatively understudied (Kirichenko et al. 2019). This region contains some diverse and unique insect communities (Teslenko 2009). High diversity is found in broad-leaf forest of its southern regions, while diversity in the northern tundra is much lower (Lelej and Storozhenko 2010). Kamchatka is a large mountainous peninsula in northeastern Russia, located between approximately 51°N–60°N and 156°E–164°E (Fig. 1A). Besides being one of the world's most volcanically active areas, it is also known for its epic landscapes and large populations of wild life. The Kamchatka-peninsula is connected to the Siberian mainland only by a narrow stripe of mountainous land in the northern tundra zone (Fig. 1A). To the West lies the Sea of Okhotsk and the Pacific to the East. Large chains

of volcanic mountains shape the centre, North (Sredinny Mountains) and South-East (Vostocny Mountains), while the West, North-East and South are relatively flat (Fig. 1A). The highest volcano (Klyuchevskoy) reaches 4,754 m altitude. The climate is considered sub-arctic and milder than in mainland Siberia. Kamchatka's fauna has relatively low diversity and there is not much of an endemic flora (Storozhenko et al. 2002; Nazarova et al. 2013; Jones and Solomina 2015). The vegetation of central Kamchatka consists of boreal forests dominated by larch (*Larix cajanderi*) and birch (*Betula platyphylla*) (Eichhorn 2010) (Fig. 1B) up to elevations around 800 m a. s. l., and Siberian dwarf pine (*Pinus pumila*) shrubs are found at elevations between ca. 800–1500 m (Fig. 1C). In southern and peripheral parts of the peninsula, there are open landscapes such as meadows (Fig. 1D) and wetlands, but most widespread are boreal deciduous forests

dominated by stone birch (*Betula ermanii*, Fig. 1E), extending up to around 800 m a. s. l. Between 800–1000 m, these humid “snow forests” are replaced by dwarf pine shrubs. Above the dwarf pine zone, only alpine tundra and plantless landscapes remain (Krestov et al. 2008).

The ant fauna of Kamchatka has been described by several authors during the past century. Different sources in sum reported at least 25 different ant taxa from Kamchatka. However, these publications are contradicting each other regarding the presence and absence of species (Table 1). Some of this confusion may have been introduced by the first species list (Ruzsky 1920). This list deviates quite strongly from later studies on ants from Kamchatka (Table 1), casting doubt whether the underlying material truly originated (only) from Kamchatka. Additionally, distributions of species reported from Kamchatka earlier are now better understood due to recent taxonomic revisions. The status of these taxa may therefore require verification. Recent work has also raised the possibility of cryptic diversity in two relevant species (*Formica candida* and *F. gagatoides*) (Zhigul'skaya et al. 2022; Chesnokova et al. 2023).

DNA-barcoding has become an important tool for biologists. Its various applications include the identification, molecular taxonomy, classification of poorly known biota, phylogeography and studies of ecological and evolutionary patterns (Hebert et al. 2016). The main repository for DNA-barcodes, the public “Barcoding Of Life Data System” (BOLD) database (Ratnasingham and Hebert 2007) now contains more than 13 million specimens with DNA-barcodes, representing nearly 345,000 species. This resource allows comparison of new sequences to a large amount of reference material.

The aim of this study was to compile a reliable, up to date checklist of ants occurring in Kamchatka, to study their distribution, ecology and genetic relationships as inferred by mtDNA sequences. Additionally, it was the aim to clarify the taxonomic identity of ants from Kamchatka morphologically resembling *Formica fusca* (setae on pronotum absent) and *F. lemani* (setae on pronotum present). Providing high resolution specimen images and an illustrated identification key to the species level was another aim.

Methods

Checklist and field observations

A total of 14 literature sources containing substantial information about ants in Kamchatka, were reviewed. Species records were classified by reliability according to two categories: “reliable” (supported by multiple, including recent sources) and “questionable” (supported by single and/or dated sources, but contradicted by more recent work). Ants were collected manually in >95% ethanol in 8 localities in Kamchatka (Fig. 1A). Geographic coordinates and elevations were recorded for each sample using a Garmin etrex 10 GPS device. Habitats were photographed during field observations. Specimens were

pinned for morphological study or kept in >95% ethanol at -20 °C. All specimens are stored in the private collection of the author (Dietikon, Switzerland). A key to the worker caste was developed based on comparative morphology of material in the author’s collection and inspired by literature (Radchenko and Elmes 2010; Seifert 2018). Pictures of specimens were taken using a Samsung Galaxy Xcover 5 cell phone in combination with a Leica EZ4 binocular at 16–70 × magnification. Focus stacking and image processing was performed using the programs `align_image_stack` v. 2019.0.0, `Enfuse` 4.2 and `GIMP` v. 2.10.30.

DNA-barcoding

DNA-barcoding was performed performed *de novo* for 47 ant specimens by the company Sinsoma GmbH (Völs, Austria). For COI, the primer pair LCO1419 and HCO2198 (Folmer et al. 1994) were used, yielding 658 bp of the barcode region of the COI gene. The resulting chromatograms were edited in UGENE 46.0 (Okonechnikov et al. 2012). Sequences were aligned using MAFFT v7.490 (Katoh and Standley 2013). A total of 10 sequences published earlier (Schär et al. 2018) were added, yielding a data set of 57 sequenced specimens in total. A maximum likelihood tree was calculated in IQ-TREE v1.6.12 (Nguyen et al. 2015), selecting the TIM2+F+I model with ModelFinder (Kalyaanamoorthy et al. 2017). Monophyly of the subfamilies was enforced by constraint. Sequences were collapsed into haplotypes and a haplotype network was visualized using the R package “pegas” (Paradis 2010) in R v4.2.3 (R Core Team 2023). Within species raw genetic distances were calculated using the R package “ape” (Paradis and Schliep 2019). All retrieved unique COI sequences were compared to sequences on BOLD.

Formica lemani or *F. fusca*?

Worker specimens of suspected *F. lemani* from Kamchatka displayed some morphological traits intermediate between *F. lemani* and *F. fusca*. Therefore, a morphometric analysis was performed to clarify their identity. Measurements were taken using a Leica EZ4 binocular with graticule at 70× magnification. A total of 110 specimens from the author’s private collection were examined: 67 specimens of *Formica lemani*, 31 workers and 3 gynes from Kamchatka and 28 workers and 5 gynes from Europe. For comparison, 43 specimens (39 workers and 4 gynes) of *F. fusca* from Europe were measured as well. The following measurements were taken as suggested and defined by Seifert (2018): SL, EYE, OceD, nPn and nHFfl (workers) and SL, nPn and nMFfl (gynes). The discriminant given by Seifert (2018) to separate *F. lemani* (positive values) from *F. fusca* (negative values) was calculated for workers ($D = 0.385 * nPn + 0.51 * nHFfl - 6.546 * SL - 27.22 * EYE + 38.46 * OceD + 10.578$) and gynes ($-D = 0.215 * nPn + 0.666 * nMFfl - 7.179 * SL + 8.657$).

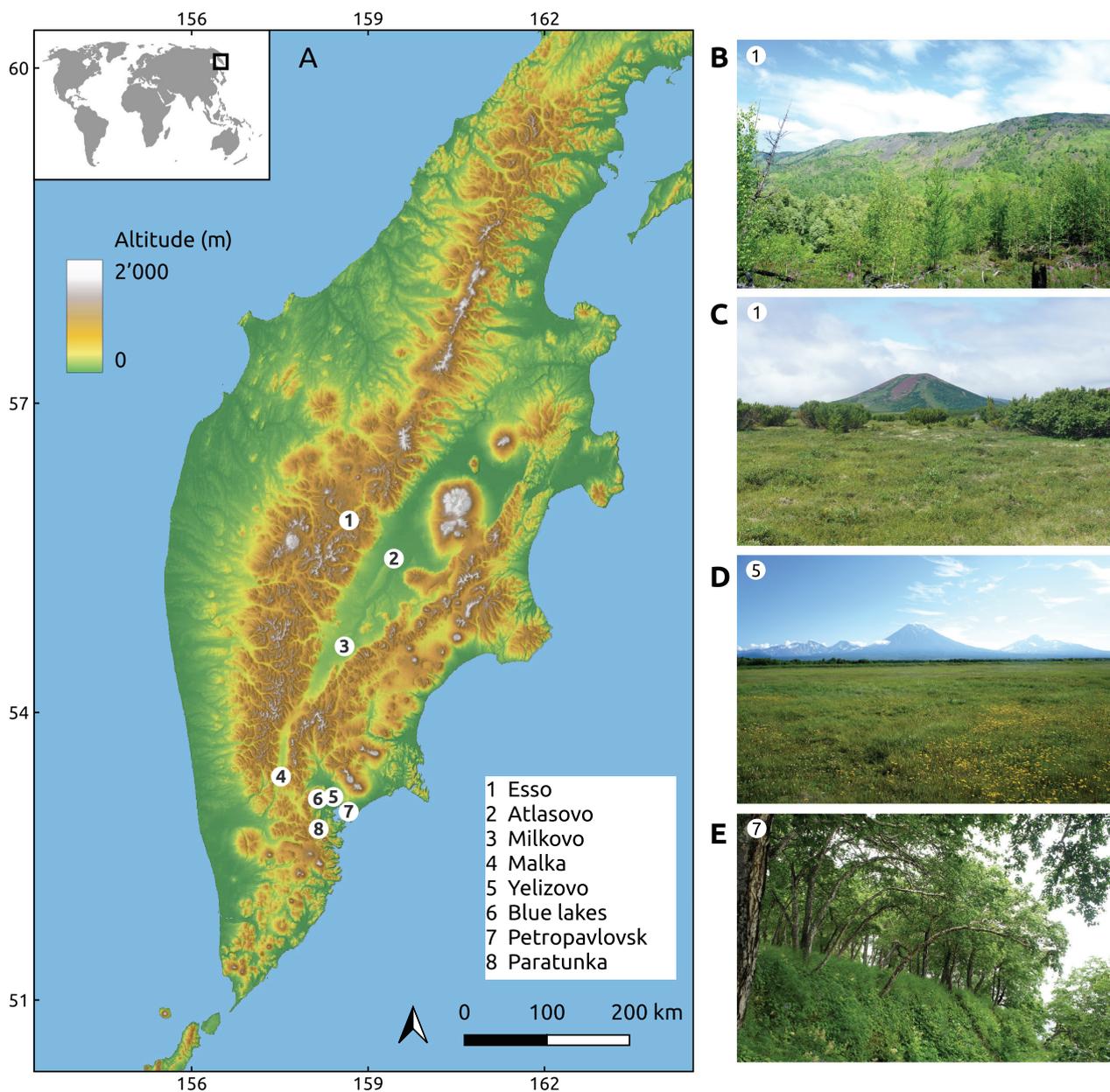


Figure 1. A. Map of Kamchatka and the study sites (numbered 1–8). The main types of habitats along with their location on the map (numbers) are given as well: B. Larch/birch forest near Esso; C. Dwarf pine zone near Esso; D. Open lowland meadow near Yelizovo; E. Stone birch forest near Petropavlovsk. Pictures: S. Schär.

Results

Checklist and field observations

Literature review suggested a relatively clear distinction between reliable and questionable records among the 25 species of ants reported from Kamchatka (Table 1). For a total of 12 reported species, presence in Kamchatka seems reliable. These taxa were mentioned by at least 2 independent sources and including sources published within the last two decades. They belong to 4 genera (*Camponotus* Mayr, 1861, *Formica* Linnaeus, 1758, *Leptothorax* Mayr, 1855, and *Myrmica* Latreille, 1804). For the other 13 species and 1 genus (*Lasius* Fabricius, 1804), reports from Kamchatka appear questionable and

these taxa are considered absent from Kamchatka for the remaining manuscript. These questionable records were reported by a single ($n = 11$) or multiple ($n = 2$) dated sources, but have all been contradicted by more recent work (Table 1).

A total of 9 species of ants were found across 8 localities in Kamchatka (Fig. 1A) during a search of the author in the year 2016 (Table 1). Of those, 3 species are restricted to the East Palearctic, 3 have a (trans-) Palearctic and 3 a Holarctic distribution range (Table 2). Ants were found from sea level up to an altitude of approximately 1000 m. At low and intermediate elevations, they inhabited meadows (Fig. 1D), stone birch- (Fig. 1E) and larch/birch forests (Fig. 1B). At higher altitudes they were found in the dwarf pine zone (Fig. 1C). Ant species compositions

Table 1. Checklist of ants reported from Kamchatka grouped by reliability, ordered alphabetically. Meaning of symbols: + present; (+) presence indicated by a different name; - absent/out of range.

Count	Species	Source											
		(Ruzsky 1920)	(Collingwood 1962)	(Dlussky 1965)	(Kupianskaya 1990)	(Radchenko 1997)	(Terayama et al. 2000)	(Czechowski et al. 2002)	(Goropashnaya et al. 2004, 2007)	(Radchenko and Elnes 2010)	(Antropov et al. 2017)	(Seifert 2007, 2018)	(Schär et al. 2018); this study
reliable													
1	<i>Camponotus herculeanus</i> (Linnaeus, 1758)		+		+	+		+			(+)	+	
2	<i>Formica candida</i> Smith, 1878											+	+
3	<i>Formica exsecta</i> Nylander, 1846				-	+				-	+	+	+
4	<i>Formica gagatoides</i> Ruzsky, 1904				+	+						+	+
5	<i>Formica lemani</i> Bondroit, 1917					+		+	+			+	+
6	<i>Formica lugubris</i> Zetterstedt, 1838		+		+			+	+	+		+	+
7	<i>Leptothorax acervorum</i> (Fabricius, 1793)	+			+			+	+			+	+
8	<i>Leptothorax muscorum</i> (Nylander, 1846)	+			+				+			+	
9	<i>Myrmica displicentia</i> Bolton, 1995											+	+
10	<i>Myrmica kamschatica</i> Kupyanskaya, 1986				+			+				+	+
11	<i>Myrmica ruginodis</i> Nylander, 1846											+	
12	<i>Myrmica sulcinodis</i> Nylander, 1846	+			+							+	+
questionable													
13	<i>Camponotus japonicus</i> Mayr, 1866	+						-					
14	<i>Camponotus saxatilis</i> Ruzsky, 1895	+						-					
15	<i>Formica aquilonia</i> Yarrow, 1955									+			-
16	<i>Formica fusca</i> Linnaeus, 1758	+				+				+			-
17	<i>Formica picea</i> Nylander, 1846	+											-
18	<i>Formica rufa</i> Linnaeus, 1761	+											-
19	<i>Formica sanguinea</i> Latreille, 1798					+				-			-
20	<i>Lasius flavus</i> (Fabricius, 1782)	+								+			-
21	<i>Lasius myops</i> Forel, 1894	+											-
22	<i>Lasius niger</i> (Linnaeus, 1758)	+											-
23	<i>Myrmica angulinodis</i> Ruzsky, 1905		+										-
24	<i>Myrmica kasczenkoi</i> Ruzsky, 1905					+							-
25	<i>Myrmica rubra</i> (Linnaeus, 1758)	+								-			-

Table 2. Biogeography, ecology and genetics of 9 common species of ants from Kamchatka. Abbreviations of biogeographic regions: H: Holarctic; P: Palearctic; EP: East-Palearctic.

Species	N	Distribution	Ecology				Altitude (m)	No. of haplotypes	Genetic raw distance (%)		
			Open (lowland)	Stone birch forest	Larch/birch forest	Dwarf pine zone			Within Kamchatka	vs Europe	vs North America
<i>Camponotus herculeanus</i>	3	H			+		503–599	1	0	≥1.67	≥0.69
<i>Formica lemani</i>	12	P	+	+	+	+	18–841	2	0–0.15	≥0.64	
<i>Formica lugubris</i>	6	P	+	+	+	+	48–841	1	0	≥0	
<i>Formica candida</i>	5	EP	+				34–503	1	0		
<i>Formica exsecta</i>	4	P			+	+	526–951	1	0	≥0.15	
<i>Formica gagatoides</i>	7	H			+	+	575–951	1	0	≥1.37	≥0.52
<i>Leptothorax acervorum</i>	7	H			+	+	28–833	7	0.15–2.13	≥0.15	≥0.15
<i>Myrmica displicentia</i>	5	EP	+				25–148	2	0–0.31		
<i>Myrmica kamschatica</i>	8	EP		+	+	+	14–834	1	0		

in these habitat types are shown in Table 2. Larch/birch forests at mid-elevations harbored the highest number of species, while the widespread stone birch communities at

low elevations were species poor, with only 3 ant species observed (Table 2). No ants were observed in alpine tundra and plant-less habitats above 1200 m a. s. l.

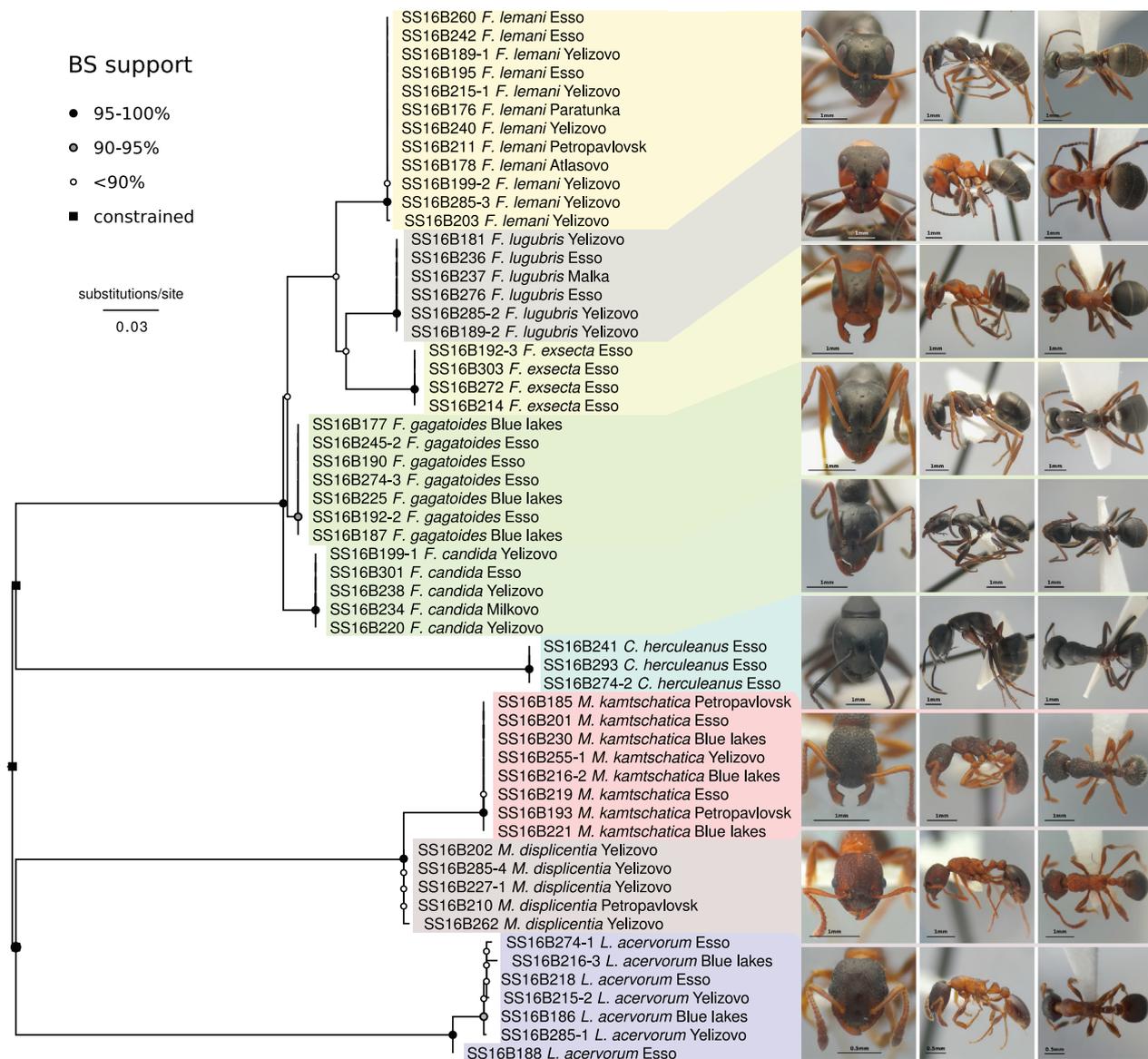


Figure 2. Maximum-likelihood tree of 57 COI DNA-barcode sequences representing 9 species of ants from Kamchatka. Pictures: S. Schär.

DNA-barcoding

DNA-barcoding succeeded in high quality for all 9 species and 57 specimens examined. For all but 2 specimens, the full length DNA-barcode (658 bp) could be retrieved. The exceptions were a specimen of *M. kamtschatica* and one of *M. displicentia*, for which sequences of 651 and 647 bp length were obtained. All 9 studied species were separated by a clear DNA-barcoding gap (Fig. 2). Interspecific genetic raw distance ranged from 2.3% (*F. candida* vs. *F. gagatoides*) to 22.5% (*C. herculeanus* vs. *L. acervorum*). Genetic diversity was low and for 6 of the 9 investigated species, only a single COI haplotype was found. Another 2 species (*Formica lemani* and *Myrmica displicentia*) were represented by 2 haplotypes each. The exception was *Leptothorax acervorum*, for which all 7 sequenced specimens displayed a different haplotype, and two haplogroups were detected (Fig. 3). Genetic raw distances within *L. acervorum* ranged between 0.15%–2.13% (Table 2). Genetic

comparison to sequences on BOLD revealed that all except one encountered haplotypes of ants from Kamchatka were private, not found anywhere else so far. The exception is the only haplotype found for *F. lugubris*, which also occurs in Finland (Roslin et al. 2022). Closely related sequences were found for all observed haplotypes (Table 2), with the exception of the two *Myrmica* species endemic to East Asia. For those, the most closely related sequences were from unidentified *Myrmica* from the Primorje region of Far Eastern Russia (*M. displicentia*: 97.22%; *M. kamtschatica*: 97.41% similarity). Sequences were made accessible via the European Nucleotide Archive (see ESM for accession numbers).

Formica lemani or *F. fusca*?

The discriminant D suggested by Seifert (2018) to distinguish *F. lemani* from *F. fusca* in Europe allowed almost complete separation between the two species for samples

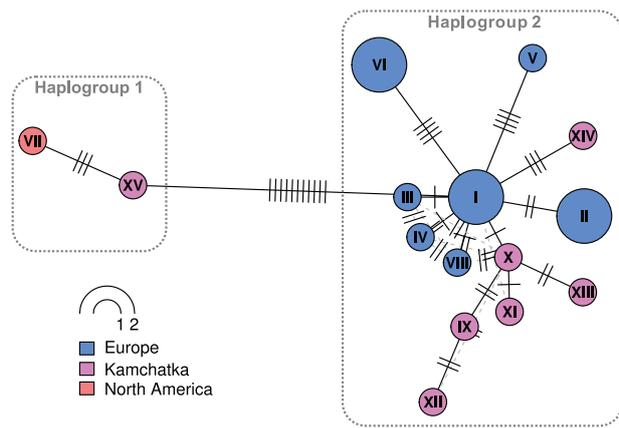


Figure 3. Haplotype network for 18 COI DNA-barcode sequences of *Leptothorax acervorum*. Sequences not from Kamchatka are from Schär et al. (2018).

from Europe. Only a single *F. fusca* worker had a value for D slightly surpassing 0 (Fig. 4). For the investigated workers from Kamchatka, values of D were intermediate between European *F. lemani* and *F. fusca* (Fig. 4). About two-thirds (68%) of these workers had negative values of D, as European *F. fusca*, while the remaining third (32%) had positive values of D, as European *F. lemani*. None of the colonies from Kamchatka contained only workers with positive values of D, unlike European *F. lemani*. A different result was found for the 3 available gynes from

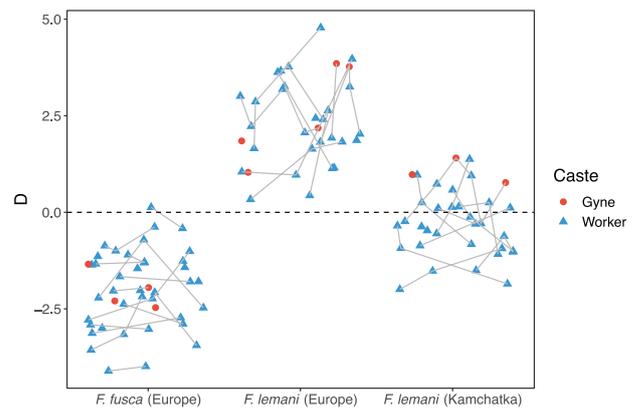


Figure 4. Scatterplot showing a morphometrical comparison of 34 *Formica lemani* ants from Kamchatka with 33 *F. lemani* specimens from Europe and 43 *F. fusca* specimens from Europe. The discriminant D has been calculated according to Seifert (2018). For gynes, D was multiplied by -1 in order to match directionality of the discriminant used for workers. Symbols connected by lines are specimens from the same nests.

Kamchatka, all of which yielded discriminant values suggestive of *F. lemani*. Additionally, they also showed a trait of *F. lemani*-gynes not included in the discriminant calculation, namely a shiny tergite I (covered by dense pubescence in *F. fusca*). Two of these gynes were found in the same nest with workers with partially negative values of D, as in European *F. fusca* (Fig. 4).

Key to the worker caste for the ants of Kamchatka

- 1 Waist 2 segmented..... 2
- Waist 1 segmented..... 7
- 2 Antenna 11 segmented 3
- Antenna 12 segmented 4
- 3 Tibiae with standing setae..... *Leptothorax acervorum*
- Tibiae with adjacent hairs only *Leptothorax muscorum*
- 4 In face view, frontal carinae curved around antennal sockets (Fig. 5A). In caudal view, antennal scape gently curved, long and slender proximally, continuously increasing in width distally (Fig. 5C)..... *Myrmica ruginodis*
- In face view, frontal carinae extend along head (curved rugae may be present underneath, Fig. 5B). In caudal view, antennal scape strongly curved or angled, robust, more or less equally wide along its length (Fig. 5D–F)..... 5
- 5 In caudal view, antennal scape sharply angled near insertion, its foot with a narrow, vertical fold (Fig. 5D). Mesosoma usually dark brown, propodeal spines short (Fig. 5G)..... *Myrmica kamtschatica*
- In caudal view, antennal scape curved near insertion, its foot without a vertical fold (a dark vertical line may be present) (Fig. 5E, F). Mesosoma yellowish to reddish brown, propodeal spines may be short or long (Fig. 5H, I)..... 6
- 6 In lateral view, propodeal spines long and slender. Sides of petiole with distinct horizontal rugae (Fig. 5H)..... *Myrmica sulcinodis*
- In lateral view, propodeal spines short. Sides of petiole smooth or with weak, irregular sculpture (Fig. 5I)..... *Myrmica displicentia*
- 7 In lateral view, dorsal outline of mesosoma not interrupted, convex or straight. Large, massive species, total length 7–10 mm..... *Camponotus herculeanus*
- In lateral view, dorsal outline of mesosoma interrupted, promesonotum bulged above level of propodeum. Smaller species, total length 4–8 mm..... 8
- 8 In full face view, posterior margin of head distinctly concave..... *Formica exsecta*
- In full face view, posterior margin of head straight or convex..... 9
- 9 In full face view, posterior margin of head with abundant setae. Body bicolored (black/reddish)..... *Formica lugubris*
- In full face view, posterior margin of head without or only scattered setae. Body concolorous dark..... 10

- 10 In dorsal view, entire gaster smooth and glossy. All tergites only with single, scattered microscopic pubescence hairs (Fig. 5J). Pronotum with setae *Formica candida*
- In dorsal view, at least tergite I matt, covered by a dense carpet of microscopic pubescence hairs (Fig. 5K, L) Pronotum often without setae 11
- 11 In dorsal view, tergite III shiny, with notably less dense pubescence than tergite I (Fig. 5K) *Formica gagatoides*
- In dorsal view, tergite III matt, with pubescence similarly dense as on tergite I (Fig. 5L) *Formica lemani*

Discussion

Checklist, biogeography and altitudinal patterns

Based on literature review, field work and DNA-barcoding, I conclude that the ant species diversity of Kamchatka is lower than could be assumed based on the published record. Unreliable, earlier species records may be based on incorrect collection information, taxonomic changes or misidentifications of taxonomically difficult taxa. However, Kamchatka is a large peninsula and most of it is poorly explored. Thus, the 12 species recognized here may be a conservative estimate. The low observed number of ant species encountered may be explained by the cold climate of Kamchatka in combination with its geographic isolation. The only land bridge connecting it to the mainland is mountainous and located at around 60°N (Fig. 1A). Most ant species are sensitive to low temperatures and only exceptionally cold resistant species are likely to have colonized the peninsula via this route. The southern part of the landmass is separated from the mainland by several hundreds of kilometers of ocean. Therefore, in contrast to boreal species, species adapted to more temperate climate may not have been able to colonize Kamchatka. This may also explain why only moderate altitudinal variation in ant communities was observed. Of the 9 species encountered in the field, 4 (*F. lemani*, *F. lugubris*, *L. acervorum* and *M. kamtschatica*) were found across the entire altitudinal range observed to be inhabited by ants (0–1000 m). Two species (*F. candida* and *M. displicentia*) were observed only below and 3 (*C. herculeanus*, *F. exsecta* and *F. gagatoides*) only above roughly 500 m a. s. l. (Table 2). The highest number of species was found at an intermediate altitude of around 500 m a. s. l., where all of these species could potentially co-occur. The low species and genetic diversity of ants also support the previous notion, that the fauna of Kamchatka resembles that of an island rather than that of a mainland peninsula (Jones and Solomina 2015).

DNA-barcoding

Similar to the low observed species diversity, genetic diversity and within species divergence in mtDNA was low too (Table 2). This could be a result of an edge effect due to recent colonization of Kamchatka by the investigated species. The exception was *L. acervorum*, which displayed a surprising level of COI diversity, with every sequenced

sample being of a different haplotype and genetic distance ranging up to 2.13% (Table 2). The latter high genetic distance was found due to a divergent *L. acervorum* sample (Figs 2, 3), belonging to a haplogroup previously only known from North America (Smith et al. 2016; Schär et al. 2018) (Fig. 3). The high genetic diversity found within *L. acervorum* in Kamchatka is surprising. Possible explanations include that Kamchatka has been a long term refuge for this species. Alternatively, it could be suggestive of a strong dispersal ability of *L. acervorum*, i. e. wind dispersal of the small sexual castes over long distances. This latter theory is also supported by a previous observation of an *L. acervorum*-haplotype occurring in both Canada and Europe (Ojeda et al. 2021). For the 3 Holarctic species (*C. herculeanus*, *F. gagatoides* and *L. acervorum*), comparison of DNA-barcodes with sequences on BOLD revealed the existence of closely related haplotypes (<1% distance) in North America (*C. herculeanus* and *F. gagatoides*) or in Europe and North America (*L. acervorum*). This suggests more recent gene-flow across Beringia than between West and East Palearctic in these taxa. On the other hand, low or even lack of genetic distances were found for the three *Formica* species with trans-Palearctic distributions (*F. exsecta*, *F. lemani* and *F. lugubris*). This could mean that the latter have expanded their distribution ranges more recently than the former three Holarctic species. It is less likely to be caused by better dispersal ability of *F. exsecta*, *F. lemani* and *F. lugubris*, because they are absent from the Nearctic. The two *Myrmica* species endemic to East Asia (*M. displicentia* and *M. kamtschatica*) have not been represented on BOLD before. Comparison to other BOLD specimens supported the current placement of both taxa within the *M. lobicornis* species group (Radchenko and Elmes 2010) and their separate status from other sequenced members of this group.

Formica lemani or *F. fusca*?

Among the questionable ant species records from Kamchatka, *F. fusca* is the one mentioned by the the most (3) literature sources (Table 1). Two of these sources also mentioned *F. lemani* from Kamchatka. Among the worker specimens examined here, two-thirds showed morphological traits more suggestive of *F. fusca*, while one third was suggestive of *F. lemani* (Fig. 4). Thus, the presence of both species appears plausible at first. However, two observations lead to the conclusion that all of these specimens belong to the same species:

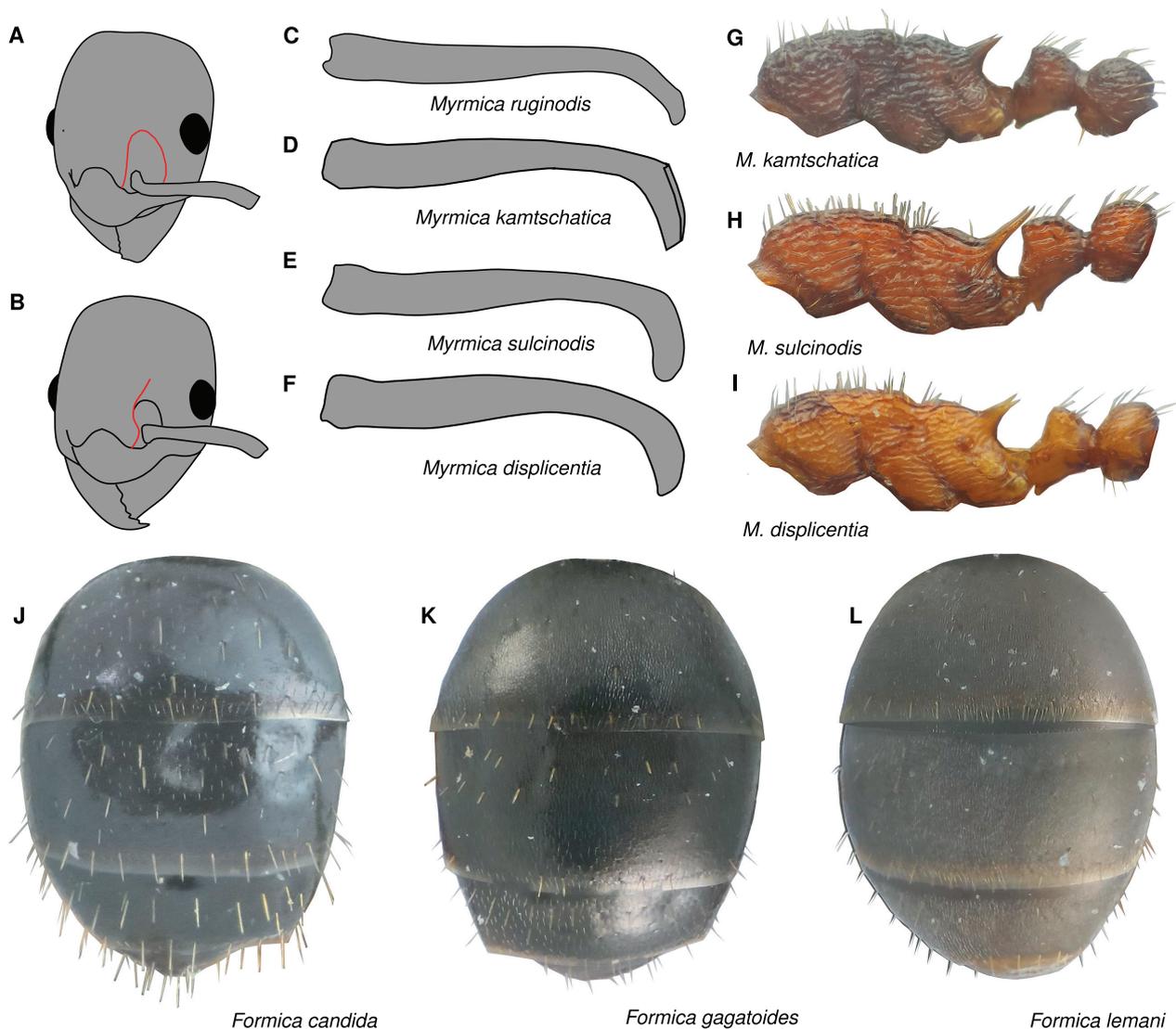


Figure 5. Morphological traits used for the identification of ants found in Kamchatka (see identification key).

1. Specimens with both morphologies are found in the same nests (Fig. 4), and no nest contained exclusively specimens with the traits of *F. lemani*.
2. All but one sequenced colonies had the same COI haplotype (Fig. 2). The remaining sequence differed from the others by just a single nucleotide change (0.15%, Table 2).

Additionally, despite the majority of workers resembling *F. fusca*, it is argued here that the name of this species is *F. lemani* rather than *F. fusca*, unless *F. lemani* would be revealed as a junior synonym of *F. fusca* in the future. This conclusion is entirely based on the morphology of gynes. Gynes of the two taxa are more distinguishable than workers. Workers differ almost only by the number of macro-setae, a trait that can vary within species in Hymenoptera (Sandrock et al. 2011) and therefore should be viewed as insufficient for taxonomic decision making. In gynes, the pubescence density on the first tergite is an additional trait used for discrimination of the two taxa: the first tergite of *F. fusca* gynes is covered by dense pubescence dorsally, leading to a matt appearance, while this body

part is smooth and shiny due to low pubescence density in *F. lemani* gynes (Seifert 2018). The three gynes found from Kamchatka all had a shiny first tergite, along with high numbers of setae typical for *F. lemani* (Fig. 4). Two of these gynes were found in the same nest with workers resembling *F. fusca* (Fig. 4). In previous investigations, *F. fusca* and *F. lemani* could not be separated based on nuclear microsatellite markers (Seppä et al. 2011) and mitochondrial COI sequences (Schär et al. 2018). Similarly, rather low support for heterospecificity of these two taxa was also found for the mitochondrial Cyt-b gene (Antonov and Bukin 2016). It is therefore possible that *F. fusca* and *F. lemani* are morphs of the same species and this should be investigated in the future. If so, the name *F. fusca* Linnaeus, 1758 would have priority over *F. lemani* Bondroit, 1917.

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Supplementary material 1

Collection data, GenBank accession numbers and specimen images

Authors: Sämi Schär

Data type: pdf

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Supplementary material 2

Morphometric data for *Formica lemani* and *F. fusca*

Authors: Sämi Schär

Data type: xlsx

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