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Tetramorium tsushimae, a new invasive ant in North America

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Abstract

Combining molecular and morphological evidence, an invasive ant in Missouri and Illinois, USA, is identified as *Tetramorium tsushimae* Emery, 1925, a polygynous-polycalic species native to East Asia. *T. tsushimae* is recorded as invasive for the first time. RFLP and worker morphometrics provide tools for reliable determination. Mitochondrial DNA data reveal the probable geographic origin of the invasive populations to be Japan.

Introduction

Ants are among the most harmful insect species introduced by man. The majority of alien ants remain confined to human-modified habitats, but a small number, classified as invasive, penetrate natural ecosystems and disturb the communities of native ants and other organisms in a direct or indirect way (review in Holway et al. 2002). A common feature of most of the invasive ants is their polygynous-polycalic social organisation. The resulting absence of intraspecific competition leads to an advantage over other ant species. Besides being an important conservation concern, invasive ants cause enormous costs to human societies. Morphology-based determination of Palaearctic *Tetramorium* species is often ambiguous. There is evidence that some of the described species actually comprise species complexes. *Tetramorium caespitum* (Linnaeus, 1758), an introduced non-invasive ant in North America (McGlynn 1999), constitutes such a species complex in Europe (Steiner et al. 2002).

In 1988, one of us (JCT) discovered a population of an unfamiliar, small, polygynous *Tetramorium* ant in St. Louis, Missouri and first considered it as a polygynous dwarf form of *T. caespitum*. In 2002 the ant was reported as *T. rhenanum* Schulz, 1996 on T. McGlynn's 'Non-native ants' website (McGlynn 2002). The identification remained doubtful, however. *T. rhenanum* was recently shown to be the junior synonym of *T. moravicum* Kratochvil, 1941 (Schlick-Steiner et al. submitted).

The first two authors contributed equally to this work.

In 1988 this *Tetramorium* species occupied only urban habitats in an area of about 30 × 20 km, encompassing the city and inner suburbs of St. Louis (Figure 1). In 1990 this ant arrived in Ballwin and Glencoe, Missouri in west St. Louis County, and by 2004 had come to occupy an area of about 100 × 40 km, encompassing parts of all counties adjacent to St. Louis Co. in both Missouri and Illinois. The polygynous *Tetramorium* is now established also in Columbia, Missouri, 150 km to the west (Figure 1).

No longer confined to human habitats, the species has invaded natural xeric calcareous grassland, albeit only disturbed areas so far. The ant has expelled native ants like terrestrial *Camponotus*, *Formica*, *Myrmica* and *Crematogaster*. Arboreal *Camponotus*, *Tapinoma sessile* (Say 1836) and minute Myrmicinae such as *Temnotho-*

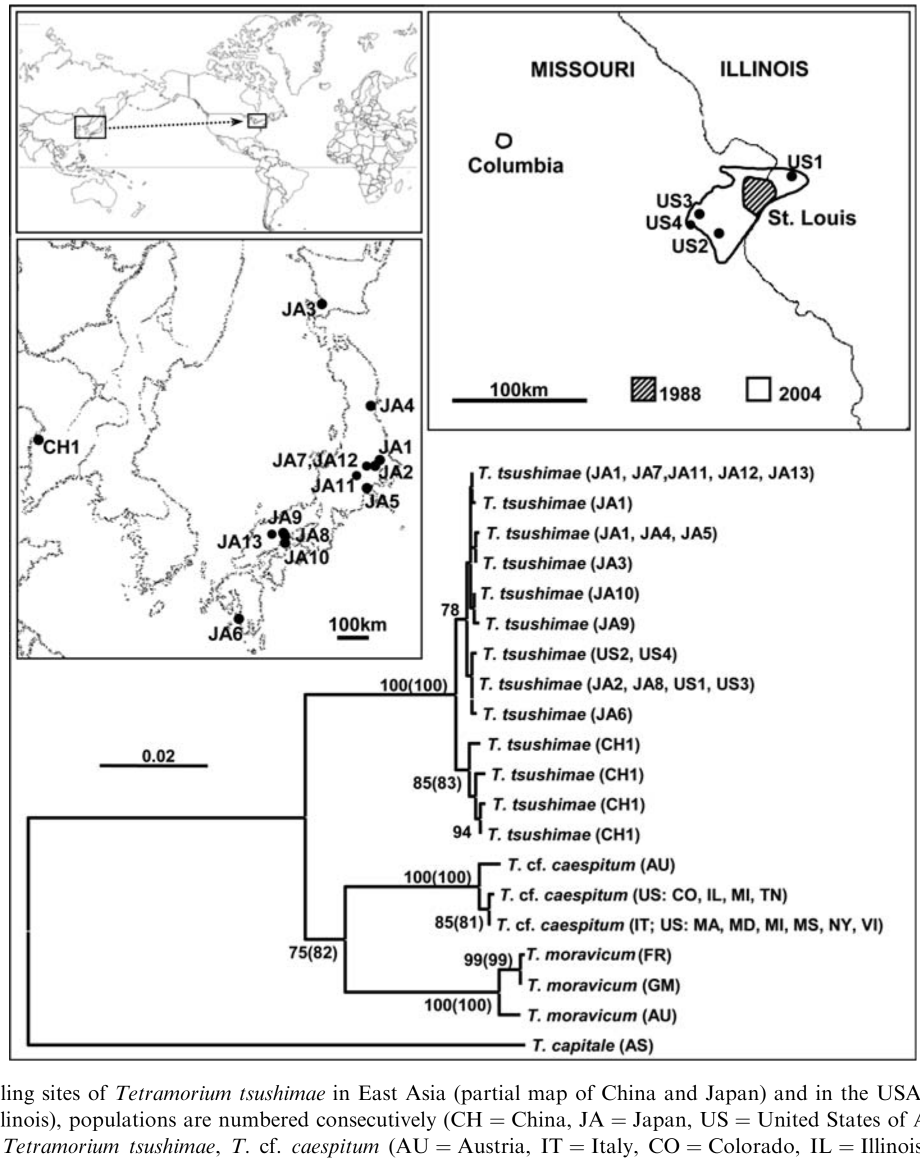

Figure 1. Sampling sites of *Tetramorium tsushimae* in East Asia (partial map of China and Japan) and in the USA (partial map of Missouri and Illinois), populations are numbered consecutively (CH = China, JA = Japan, US = United States of America). Phylogenetic tree of *Tetramorium tsushimae*, *T.* cf. *caespitum* (AU = Austria, IT = Italy, CO = Colorado, IL = Illinois, MA = Massachusetts, MD = Maryland, MI = Michigan, MS = Missouri, NY = New York, VI = Virginia), *T. moravicum* (FR = France, GM = Germany) and *T. capitale* (AS = Australia), based on Neighbour Joining calculated with the Tamura–Nei algorithm of 1113 bp of the COI gene. Bootstrap values >75 are given at nodes, bootstrap values of the MP branches in parentheses.

rax often persist, but in lower abundance than in uninfested areas. *T. caespitum* has been also displaced. By contrast, subterranean *Solenopsis*, *Ponera* and *Brachymyrmex* species appear to be unaffected. This study aims at uncovering the species identity and the geographic origin of the invasive *Tetramorium* populations in North America by combining morphometry and molecular genetics. Furthermore, tools for reliable determination are provided.

Materials and methods

Molecular genetics

Seven samples of the invasive *Tetramorium* species (four sites; Illinois, Missouri; Figure 1), 17 of *T.* cf. *caespitum* (Austria, Italy, USA), three of *T. moravicum* (Austria, France, Germany), 24 of *T. tsushimae* (China, Japan; Figure 1) and one of *Tetramorium capitale* (McAreavey, 1949) (Australia) as outgroup were compared.

Sequences of *T. moravicum* were obtained from another project and deposited in GenBank under the accession numbers AY641705, AY641718 and AY641709 (Schlick-Steiner et al. submitted).

DNA of single individuals of all other specimens was extracted using the Sigma Genelute Extraction kit. PCR was performed in reaction volumes of 50 µl : 4 µl template DNA; 1× reaction buffer; 0.2 mM dNTPs; 0.2 µM forward and reverse primers; 2 U Sigma-Taq DNA polymerase and H_2Odd. PCR was run in a MJ thermocycler using a touchdown program from a T_M of 47–55 °C for 31 cycles. Primers for amplifying the cytochrome oxidase I (COI) gene segment were COI1f 5′-cccccctctattagattattatt-3′ (developed by Schlick-Steiner et al. submitted) and L2-N-3014r (Simon et al. 1994). LCO1490 (Folmer et al. 1994) was used as forward primer for *T. capitale*.

PCR products were purified using the QIAquick PCR purification kit and subsequently sequenced in two directions by the Big Dye termination reaction chemistry. Sequences were aligned with Clustal X (Thompson et al. 1997). For phylogenetic inferences 1113 bp of COI were submitted to distance (neighbour-joining algorithm, NJ) and maximum parsimony (MP) analysis with the software package PAUP (test version 4.0b3a; Swofford 1998). Tamura–Nei distance (Tamura and Nei 1993) was used for NJ trees. MP trees were generated by heuristic search using the tree bisection reconnection (TBR) algorithm and a random taxon addition sequence. Sequences were deposited in GenBank under accession numbers AY641663–AY641664, AY641670–AY641700 and AY641725–AY641728.

Morphometrics

Material

Tetramorium cf. *caespitum* – 69 workers from 23 nests (samples covering the whole known distribution range: Armenia, Austria, Bulgaria, France, Greece, Croatia, Hungary, Italy, Romania, Russia, Slovenia, Spain, Turkey, and 9 states of the USA. Their conspecificity had previously been ascertained by COI sequencing; data not shown).

Tetramorium tsushimae – 36 workers from 12 nests (China and Japan, Figure 1). Additionally, three workers of the type series were assessed (Museo Civico di Storia Naturale, Genova).

Invasive *Tetramorium* sp. – 15 workers from 5 nests (USA).

Twelve morphometric distance characters were measured, two angles calculated (Table 1; Figure 2).

Dry-mounted specimens were fixed on a pin-holding goniometer. A Nikon SMZ 1500 dissecting microscope with a 1.6× planapochromatic lense and a cross-scaled ocular micrometer was used at magnifications 50–320×.

The program package SAS 8.2 was used to classify the morphometric data according to the genetic hypotheses by a discriminant analysis.

Results and discussion

The identity of the invasive Tetramorium *populations and determination tools*

Topologies of the NJ and MP trees were congruent. Disregarding the outgroup (*T. capitale*), mutations were detected at a total of 120 sites (114 being informative), seven of these at the first and 113 at the third codon position (Figure 3). The samples of the invasive US populations

Table 1. Definition of morphometric characters (also see Figure 2) and comparison of the workers of *T.* cf. *caespitum* and *T. tsushimae*.

Morphometric character		*T.* cf. *caespitum* (*n* = 69)	*T. tsushimae* (*n* = 51)
Alpha	Angle between PSSP and PLSP; $\cos \alpha = (\mathrm{PSSP}^2 + \mathrm{PLSP}^2 - \mathrm{PSPL}^2)/2 \cdot \mathrm{PSSP} \cdot \mathrm{PLSP}$	77 ± 5 [68, 89]	68 ± 4 [61, 75]
Beta	Angle between PSMP and PLMP; $\cos \beta = (\mathrm{PSMP}^2 + \mathrm{PLMP}^2 - \mathrm{PSPL}^2)/2 \cdot \mathrm{PSMP} \cdot \mathrm{PLMP}$	53 ± 2 [48, 58]	56 ± 2 [49, 61]
CW	Maximum cephalic width across eyes	816 ± 76 [662, 977]	754 ± 61 [656, 941]
MPSP	Distance from the anteroventral corner of the metapleuron to the tip of the spine	348 ± 41 [274, 458]	354 ± 34 [293, 431]
MW	Maximum mesosoma width, immediately posterior the pronotum corners	517 ± 55 [415, 660]	497 ± 43 [426, 624]
PEW	Maximum width of petiole	263 ± 31 [207, 330]	232 ± 22 [197, 283]
PLMP	Distance from the posterior end of the posterodorsal corner of the propodeal lobe to the anteroventral corner of the caudal margin of the metapleuron	280 ± 27 [224, 357]	259 ± 20 [222, 314]
PLSP	Distance from the posterior end of the posterodorsal corner of the propodeal lobe to the tip of the spine	207 ± 27 [158, 279]	227 ± 25 [179, 280]
PreOcLa	Preocular distance from lateral view; shortest distance from the anterior eye margin to the frontal margin of the gena	204 ± 21 [167, 251]	183 ± 15 [153, 225]
PronHL	Length of the longest hair on the corner of the pronotum	218 ± 25 [162, 272]	178 ± 22 [115, 221]
PSMP	Distance from the centre of the propodeal spiracle to the anteroventral corner of the caudal margin of the metapleuron	216 ± 23 [169, 274]	206 ± 18 [172, 264]
PSPL	Distance from the centre of the propodeal spiracle to the posterior end of the posterodorsal corner of the propodeal lobe	229 ± 25 [181, 288]	224 ± 18 [194, 278]
PSSP	Distance from the centre of the propodeal spiracle to the tip of the spine	157 ± 23 [113, 218]	168 ± 20 [113, 208]

Upper line: arithmetic mean ± standard deviation, lower line, in [#]: minimum and maximum values, *n* = number of measured specimens.

containing two haplotypes fell within the clade of *T. tsushimae* (Figure 1). All species formed well supported monophyletic groups. Maximum intraspecific variation was moderate: *T.* cf. *caespitum* 0.7%, *T. tsushimae* 1.1%, *T. moravicum* 0.9%.

Based on the genetic results the US-invasive *Tetramorium* was pooled with the East Asian *T. tsushimae* for morphometric analysis (Table 1). Workers of *T.* cf. *caespitum* and *T. tsushimae* overlap in all characters, with biggest differences in the angle alpha. On average, *T. tsushimae* is smaller (CW, MW) and its propodeal spines are longer (alpha, PSSP). However, a discriminant analysis with eight characters (alpha, beta, CW, MPSP, MW, PEW, PreOcLa, PronHL) successfully classifies 119 of 120 (99.2%) single workers according to the genetic hypothesis. When nest means of three workers are used, it takes only five characters (alpha, beta, CW, MW, PEW) for a 100% successful discrimination. Subsequently, three workers of the type series of *T. tsushimae* and their means, respectively, were classified by the discriminant functions as '*T. tsushimae*' without exception. Thus, both molecular genetics and morphometry clearly indicate that the invasive species is *T. tsushimae*.

Reliable routine identification and discrimination from the morphologically similar *T.* cf.

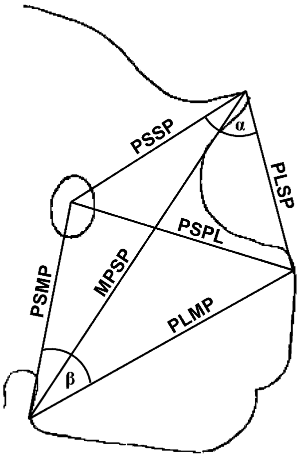

Figure 2. Posterior mesosoma of a *Tetramorium* worker in lateral view with morphometric characters. For verbal definition of characters see Table 1.

caespitum is essential for monitoring the invasive ant species in North America. First signs can be obtained in the field. *T. tsushimae* is polygynous, while *T.* cf. *caespitum* is usually monogynous. For reliable determination two procedures are presented.

(1) Restriction fragment length polymorphism (RFLP): The restriction enzyme *Fok*I cuts the COI fragment in *T. tsushimae* (Figure 3) and results in two fragments 394 and 890 bp but *Fok*I does not cut the COI fragment of *T.* cf. *caespitum*.

(2) Morphometric determination of workers via discriminance function: This facilitates the screening of dried voucher specimens for a reconstruction of the invasion history (cf. Schlick-Steiner et al. 2003). A user-friendly

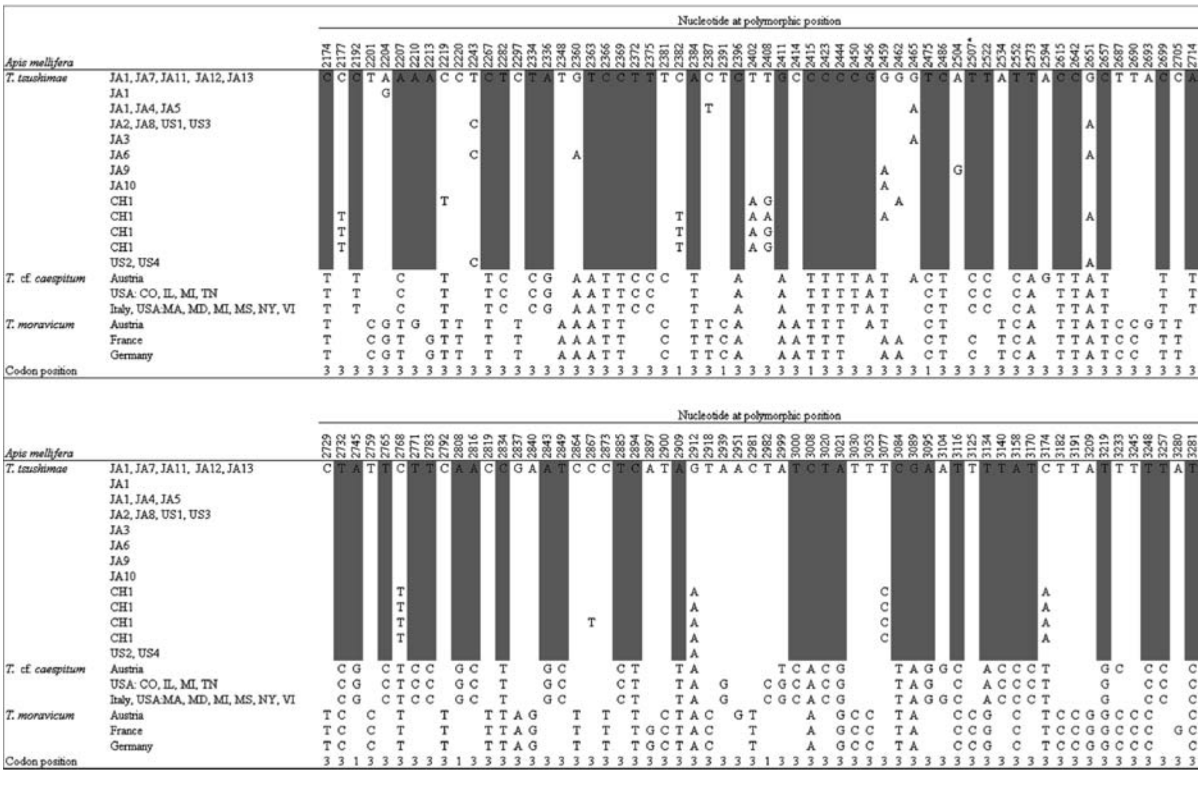

Figure 3. Variable sites of COI sequences of the 19 haplotypes of *T. tsushimae* including the invasive US populations, *Tetramorium* cf. *caespitum*, and *T. moravicum* with equivalent COI sequence positions in *Apis mellifera* (Crozier and Crozier 1993). Codon positions (1–3) are indicated below. Geographic origin: CH = China, JA = Japan, US = USA; CO = Colorado, IL = Illinois, MA = Massachusetts, MD = Maryland, MI = Michigan, MS = Missouri, NY = New York, VI = Virginia. For position of CH1, JA1–JA13 and US1–US4 see Figure 1. Diagnostic sites for *T. tsushimae* against *T.* cf. *caespitum* are shaded grey. *Recognition site of restriction enzyme *Fok*I.

(!) program for morphometric discrimination betweeen *T.* cf. *caespitum* and *T. tsushimae* is accessible at ⟨http://homepage.boku.ac.at/h505t3/DiscANT/⟩. On entering the values into the discriminant mask the species determination and corresponding error-probabilities are given.

Geographic origin and invasion history

The mtDNA data of *Tetramorium tsushimae* suggest that the North American populations originated from Japan (Figure 1): one haplotype found in Illinois and Missouri (sites US1 and US3) is identical with a haplotype found in the Tokyo (JA2) and Okayama (JA8) region. The second North American haplotype, found in Missouri (US2, US4), differs by only 1 bp (Figure 3) and was not found in Japan, either due to a mutation or lack of sampling.

The haplotypes found in the Shenyang region of China (CH1) diverge from the Japanese haplotypes by 0.7–1.1%. This corresponds to a reproductive separation for at least 500,000 years taking the common rate of 2% per million years (DeSalle et al. 1987). However, further studies will be needed to obtain a more detailed picture of the phylogeographic history of *T. tsushimae*.

In Japan *T. tsushimae* is one of the most common ants (Japanese Ant Database Group 2003), with at least some polygynous-polycalic populations that usually occur in urban habitats. This trait is likely to be main reason for the invasiveness of the US-populations. The introduction to the USA may have taken place shortly before 1988, at a time, when the import of soil containing material to the USA still was legal and when plant nurseries still were being shipped from Japan. Another possible invasion history involves the fact that introduced species sometimes exist latently for a certain period after their introduction and then suddenly become invasive and expansive (cf. O'Dowd et al. 2003). This would mean that *T. tsushimae* might have been imported as early as around 1900, when the Asian tree *Pseudolarix amabilis* Nelson was planted in the vicinity of the Japanese Pavilion of the 1904 World's Fair in the Forest Park of St. Louis.

The present spread over North America is probably anthropogenic, and as primary mode the transport of potted plants and rock or soil with queenright colony fragments is suspected. Our data are the first report of *Tetramorium tsushimae* being an invasive species. Like other invasive ants *Tetramorium tsushimae* has the potential to become a pest of major impact. Thus correct identification is extremely important. It allows to trace the local spread and the comparison of the invasive populations with native populations in East Asia may elucidate social and ecological changes due to the introduction of ants in general (Holway et al. 2002). Eventually, the immediate detection of new *T. tsushimae* infestations will improve the prospect of eradication measures (Holway et al. 2002; cf. Krushelnycky et al. 2004).

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