Tetramorium tsushimae, a new invasive ant in North America

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Abstract
A new invasive species of the ant genus *Tetramorium* is reported from the United States of America, where it has been found in parts of Missouri and Illinois. Both molecular and morphological investigations show that these populations are closely allied to *Tetramorium tsushimae* Emery, 1925, a species native to East Asia. According to mitochondrial DNA sequences the US-invasive populations originated from Japan. We provide RFLP and worker morphometrics as tools for the reliable determination of this species. In its introduced range *T. tsushimae* forms polygynous-polycalic nest associations, thus having the potential to become a major pest species if not controlled properly.

Introduction
Ants are among the most serious pest species introduced by human activities. They are classified as invasive if they have the potential to penetrate natural ecosystems [1]. Several tropical species of the world-wide distributed genus *Tetramorium* are known to maintain introduced populations in the vicinity of human buildings [2], while no invasive ants of this genus have been reported from the palearctic region thus far. An unknown species of *Tetramorium* was found in 1988 in the city area of St. Louis, Missouri, USA. From 1988 to 2004 the introduced population expanded its range considerably to occupy some 4000 km^2 (Fig. 1). At present, the species has also invaded natural habitats where it has expelled numerous native ant species. It displays a polygynous-polycalic social organization, which is a common feature of invasive ants.

Objectives
•Determine the species identity of the US-invasive ants
•Identify the geographic origin of introduction

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, J =Japan, US= United States of America). Phylogenetic tree of *Tetramorium tsushimae*, *T.* cf. *caespitum* (CO=Colorado, IL=Ilnois, MA =Massachusetts, MD=Maryland, MI=Michigan, MS=Missouri, NY=New York, VI=Virginia), and *T. moravicum* (FR=France, GM=Germany) 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.

Figure 2. Posterior mesosoma of a *Tetramorium* worker in lateral view with morphometric characters

Methods
Molecular genetics - Seven samples of the invasive *Tetramorium* species (four sites; Illinois, Misouri), 17 of *T.* cf. *caespitum* (Austria, Italy, USA), three of *T. moravicum* (Austria, France, Germany), 24 of *T. tsushimae* (China, Japan) and one of *T. capitale* (Australia) as outgroup were compared. DNA of single individuals was extracted using the Sigma Genelute Extraction kit. PCR was performed in reaction volumes of 50 : 4 µl template DNA; 1 x reaction buffer; 0.2 mM dNTPs; 0.2 µlM forward and reverse primers; 2 U Sigma-Taq DNA polymerase and H2Odd. PCR was run in a MJ thermocycler using a touchdown programme from a TM of 47–55 °C for 31 cycles. Primers for amplifying the cytochrome oxidase I (COI) gene segment were COI1f 5'-cccccctcattagattattatt-3' and L2-N-3014r PCR products were purified using the QIA-quick PCR purification kit and subsequently sequenced in two directions by the Big Dye terminatio reaction chemistry. Sequences were aligned with Clustal X. 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). Tamura–Nei distance 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. Morphometrics -Twelve morphometric distance characters were measured, two angles calculated (Fig. 2). Dry-mounted specimens were fixed on a pin-holding goniometer. A Nikon SMZ 1500 dissecting microscope with a 1.6x planapochromatic lense and a cross-scaled ocular micrometer was used at magnifications 50–320x. The program package SAS 8.2 was used to classify the morphometric data according to the genetic hypotheses by a discriminant analysis.

Results & Discussion
The identity of the invasive *Tetramorium* populations
The samples of the invasive US populations containing two haplotypes fell within the clade of *T. tsushimae* (Fig. 1). Topologies of the NJ and MP trees were congruent. All species formed well supported monophyletic groups. Mutations were detected at a total of 120 sites (114 being informative), mostly at the third codon position. Maximum intraspecific variation was moderate, ranging from 0.7-1.1% sequence divergence.

Workers of *T.* cf. *caespitum* and *T. tsushimae* overlapped in all morphometric characters, with the biggest differences in the angle alpha. On average, *T. tsushimae* is smaller (CW, MW) and its propodeal spines are longer. However, a discriminant analysis with eight characters (alpha, beta, CW, MPSP, MW, PEW, PreOcLa, PronHl) successfully classified 119 of 120 (99.2%) single workers according to the genetic groupings. Thus, both molecular genetics and morphometry clearly indicate that the invasive species is *T. tsushimae*. Routine identification and discrimination from the morphologically similar *T.* cf. *caespitum* is essential for monitoring the invasive ant species in North America. For reliable determination two procedures are presented:

(1) Restriction fragment length polymorphism (RFLP): The restriction enzyme FokI cuts the COI fragment in *T. tsushimae* and results in two fragments 394 and 890 bp but FokI does not cut the COI fragment of *T.* cf. *caespitum*.
(2) Morphometric determination of workers via discriminance function: Screening of voucher specimens for a reconstruction of the invasion history [3]. A programme for morphometric discrimination of the two species is available at http://homepage.boku.ac.at/h505t3/DiscANT/

Geographic origin and invasion history
The mtDNA data of *T. tsushimae* suggest that the North American populations originated from Japan (Fig. 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.
In Japan *T. tsushimae* is one of the most common ants [4], with at least some polygynous-polycalic populations that usually occur in urban habitats. This trait is very likely to be main reason for the invasiveness of the introduced USA-populations. The introduction to the USA may have taken place shortly before 1988, at a time, when plant nurseries were being shipped from Japan. Our data are the first report of *T. tsushimae* being an invasive species. Like other invasive ants this species has the potential to become a pest of major impact. Thus correct identification is extremely important because it will improve the prospects of eradication measures [1].

References
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