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Tetramorium indocile Santschi, 1927 stat. rev. is the proposed scientific name for *Tetramorium* sp. C sensu Schlick-Steiner et al. (2006) based on combined molecular and morphological evidence (Hymenoptera: Formicidae)

Sándor Csősz[a,*], Herbert Christian Wagner[b], Miklós Bozsó[c], Bernhard Seifert[d], Wolfgang Arthofer[b], Birgit C. Schlick-Steiner[b], Florian M. Steiner[b], Zsolt Pénzes[c,e]

[a] MTA-ELTE-MTM, Ecology Research Group, Pázmány Péter sétány 1C, H-1117 Budapest, Hungary
[b] Molecular Ecology, Institute of Ecology, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria
[c] Biological Research Center of Hungarian Academy of Sciences, Institute of Genetics, Temesvári krt 62, Szeged 6726, Hungary
[d] Senckenberg Museum für Naturkunde Görlitz, Am Museum 1, 02826 Görlitz, Germany
[e] University of Szeged, Department of Ecology, Közép fasor 52, Szeged 6726, Hungary

ABSTRACT

The last decade's improvements in taxonomic methods led to a rapid exploration of biodiversity. Among the new species thus discovered there are many well outlined *Operational Taxonomic Units* (OTUs) which, however, have not yet been described in accord with the *International Code of Zoological Nomenclature*. In this paper we revive *Tetramorium indocile* Santschi, 1927 **stat. rev.** from synonymy and propose its scientific name for the Western Palearctic OTU characterized earlier, *Tetramorium* sp. C sensu Schlick-Steiner et al. (2006), based on evidences of molecular phylogeny combined with multivariate analyses of morphometric data. The type series of *T. indocile* was associated with the *Molecular Operational Taxonomic Unit Tetramorium* sp. C with high posterior probability ($p = 1.0$) based on cumulative linear discriminant analysis of morphometric traits. Possible synonyms are excluded as names for it based on biogeographic, and worker-morphometric arguments. In order to avoid further uncertainties, a lectotype for *Tetramorium indocile* Santschi, 1927 is designated hereby. A diagnosis of *T. indocile*, the redescription of the worker caste and the first description of males including the male genital morphology are also provided.

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1. Introduction

Over the past decades, a broad range of conceptual and methodological developments in systematic research contributed to eliminating subjective, unquantified aspects from the characterization of biodiversity at the species level (recent ant examples: Schlick-Steiner et al., 2005; Ferreira et al., 2010; Bagherian Yazdi et al., 2012; Csősz, 2012; Gotzek et al., 2012; Berville et al., 2013; Seifert et al., 2013). The evolutionary entities thus recognized are often designated as *Operational Taxonomic Units* (OTUs) (Blaxter, 2004; Smith et al., 2005; Steiner et al., 2009). Frequently, when morphological characterization of OTUs is attempted, problematic or name-bearing types of candidate taxa are hard to match any of the recognized clusters. As a consequence, OTUs frequently remain being unidentified, i.e., treated under code names instead of scientific names in accord with the *International Code of Zoological Nomenclature* (ICZN) (Ward and Sumnicht, 2012; Ng'endo et al., 2013) and the integration into the accepted system (Schlick-Steiner et al., 2010) is left to forthcoming ventures.

In a phylogenetic analysis of the *Tetramorium caespitum/impurum* complex, Schlick-Steiner et al. (2006) discovered a number of cryptic species, provided new insights in relationships within this group and established a revised classification for the complex. A few taxa, i.e. *T. caespitum* (Linnaeus, 1758), *T. hungaricum* Röszler, 1935 and *T. impurum* (Foerster, 1850) were treated under available scientific names, but the rest of *Operational Taxonomic Units* (OTUs) received code-names, i.e., *Tetramorium* spp. A–E. Subsequently, one of the code-named OTUs, *Tetramorium* sp. A sensu Schlick-Steiner et al. (2006), has been described as *T. alpestre* by Steiner et al. (2010).

However, sooner or later every OTU requires formal taxonomic and nomenclatorial acts that meet the requirements of the ICZN

* Corresponding author. Tel.: +36 70 2536004.
E-mail address: sandorcsosz2@gmail.com (S. Csősz).

in order to make each OTU fully available for fluent scientific discourse. The purpose of the present paper is to continue and update work on mapping phylogenetic lineages established by Schlick-Steiner et al. (2006) to the morphology-based system and to add more details to taxonomy. In doing so, morphology plays a central role, in that only numeric morphology is able to find the correct link between the *Molecular Operational Taxonomical Units* (MOTUs) and name-bearing types, i.e., Zoological Nomenclature (Schlick-Steiner et al., 2007a; Seifert et al., 2013).

The currently accepted approach starts with the exploration of related species via molecular phylogenetics, followed by the practical application of information by morphometric methods. Thus, the nearly 100-year-old type specimens are in general not available for the purpose of molecular investigations, but their position in the morphology-based discriminant space can be defined as can posterior probabilities for the classification as one of the morphologically defined clusters, which before were established based on molecular phylogenies. In more detail, we executed a workflow to find the corresponding OTU for the name-bearing type series of *Tetramorium caespitum* var. *indocile* Santschi, 1927 as follows: *Molecular Operational Taxonomic Units* (a) are recognized from a DNA sequence (cytochrome c oxidase I, COI) based molecular phylogeny. Then (b) the boundaries of OTUs are confirmed by multivariate analyses of continuous morphometric characters, i.e., the results of molecular phylogeny are translated to the language of morphology and the position of type material is inferred within the same discriminant space. Finally, (c) the OTU found to correspond to the type material is described under its scientific name.

We hereby propose the scientific name *Tetramorium indocile* Santschi, 1927 to replace the code name of one of the OTUs sensu Schlick-Steiner et al. (2006), *Tetramorium* sp. C, based on combined evidences of different approaches. The morphology of the male genitalia of related *Tetramorium* species is found to be clearly distinct in most cases. Hence, we also provide the description of the hitherto unknown male genital morphology of *T. indocile* that plays a key role in species and species complex recognition.

2. Materials and methods

2.1. Material investigated

We morphometrically investigated and analyzed 226 workers of 51 *Tetramorium* samples from several European countries (France, Germany, Hungary, Poland, Romania, Russia, Spain, Ukraine), Armenia in Western Asia and Kyrgyzstan in Central Asia using multivariate statistics. The genetically investigated samples formed the core of our morphometric analyses. The genetically as well as the morphologically investigated material (including GenBank accession numbers) is listed in Table 1. All images presented are available online on AntWeb (http://www.antweb.org) and can be uniquely identified with specimen-level code affixed to each pin.

2.2. Type material investigated

Tetramorium caespitum var. *indocile* Santschi, 1927 (U+263F, U+2640, U+2642) [Kyrgyzstan]: type series of workers, gynes and males on two dry pins are labeled as:

TYPE [−] "*T. caespitum* L. v *indocile* Sant" [/] SANTSCHI det. 19 "26" [−] "Semiretschie" [/] "Kisil-Kija Pass." [/] "(Kusnezow)" [−] Sammlung Dr. F. Santschi Kairouan [−] NATURHIST. MUSEUM BASEL [−] "9" [−] (4(U+263F), 1(U+2640), 1(U+2642); thereof we have choosen the lectotype worker, see Section 4.1.); TYPE [−] "*T. caespitum* L. v. *indocile* Sant." [/] SANTSCHI det. 19 "26" [−] "Semiretschie" [/] "Kisil-Kija-Pass" [/] ".Kusnezow." [−] "Ssemiretschie" [/] "Kisil-Kija-Pass W A 14" [/] "15-VIII-1924 N.

Kusnezow" [−] Sammlung Dr. F. Santschi Kairouan (1(U+263F), 1(U+2640)).

Further workers and males mentioned also by Santschi (1927) on two dry pins are labeled as:

"*T. caespitum* L. v. *indocile* Sant-" [/] SANTSCHI det. 19 "26" [−] "Ssemiretschie" [/] "Karkara" [/] "N. Kusnezow." [/] "16.VIII.1924" [−] Sammlung Dr. F. Santschi Kairouan [−] "A9." [−] (3(U+263F), 2(U+2642)); "*Tetramorium caespitum* L. v. *indocile* Sant" [/] SANTSCHI det. 19 "26" [−] "Ssemiretschie" [/] "Issyk-kul." [/] "Dolonolo W. Alo." [/] "(N. Kusnezow)" [−] Sammlung Dr. F. Santschi Kairouan [−] (6(U+263F)).

Type and further material listed above is housed in Naturhistorisches Museum, Basel, Switzerland (NHMB).

2.3. Molecular analyses

Altogether 64 samples were involved in the molecular analyses. Analyses were done in two laboratories (46 samples in Szeged, 18 in Innsbruck) using two slightly different protocols:

(1) Biological Research Center of Hungarian Academy of Sciences, Institute of Genetics lab (Szeged): Samples were stored in 96% ethanol in −20 °C until DNA extraction. Total genomic DNA was extracted from legs by Chelex® 100 (biotechnology grade, BioRad) protocol (Nicholls et al., 2010). Primers used for PCR amplification of a 1280 bp long mitochondrial COI gene segment were COI1f (forward) 5′-CCC CCC TCT ATT AGA TTA TTA TT-3′, Jerry (forward) 5′-CAA CAT TTA TTT TGA TTT TTT GG-3′, and Pat = UEA10 (reverse) 5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′ (see Simon et al., 1994 for protocol and references). Sequences were determined for one or two individuals from each nest. An internal primer was also designed for amplification of problematic samples (TetraR1, reverse) 5′-TCT ACT TAG AGT TGA GAA GTA CC-3′.

The polymerase chain reaction was carried out in 25 μl reaction volumes, containing 30 ng DNA, 1× PCR buffer with KCl, 8 mM $MgCl_2$, 0.2 mM of each dNTP, 120 nM of each primer and 1 U recombinant Taq DNA polymerase (all PCR reagents provided by Thermo Scientific) using an MJ Research PTC-200 Thermal Cycler. The touchup PCR reactions were run for 36 cycles with the following parameters: an initial 2 min. denaturation at 94 °C; then cycled at 94 °C for 30 s. (denaturation), 45–51 °C for 90 s. (annealing), and 72 °C for 90 s. (extension). PCR was completed with a 10 min. final extension at 72 °C.

PCR products were electrophoresed on 1% agarose gels, stained with ethidium bromide, and visualized by UV transillumination. Successfully amplified products were purified using Millipore Ultrafree®-DA PCR purification columns according to the manufacturer's protocol and sequenced from both directions with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 310 machine using the PCR primers applied for the target segment amplification.

(2) Molecular Ecology lab (Innsbruck): Samples were stored in 96% ethanol in −20 °C until DNA extraction. Total genomic DNA was extracted from gasters with the Sigma GenElute Mammalian DNA Extraction Kit. Primers used for PCR amplification of a 1280 bp long COI gene segment were COI1f and Pat = UEA10 (see above). Sequences were determined for one or two individuals from each nest.

The polymerase chain reaction was carried out in 15 μl reaction volumes, containing 50 ng template DNA, 1× MyTaq buffer, 200 nM of each primer and 0.4 U MyTaq DNA polymerase (Bioline) using a VWR Unocycler equipped with a 96 well standard gradient block. The PCR reactions were run with an initial denaturation at 95 °C for 3 min., followed by 35 cycles of 95 °C for 30 s, 48 °C for 45 s and 72 °C for 2 min, followed by a 10 min final extension at 72 °C.

PCR products were electrophoresed on 2% agarose gels, stained with GelRed, and visualized by UV transillumination. Successfully

Table 1

List of samples investigated by morphometric character analysis. Taxon name, sample code, locality, sampling date, and collectors' names are given. Geographic coordinates LAT and LON and elevation are provided in decimal format. Elevation: altitude in meters above sea level. GenBank accession numbers of haplotypes are provided. New haplotypes are shown in bold. The results of confirmatory LDA with geometric mean of posterior probabilities for nest samples are also shown.

Taxon	Code	Locality	Latitude (North)	Longitude (East)	Elevation	Date of collecting	Collector	GenBank	Haplotype name	Posterior P				
										T. caespitum	TB	TC	TU4	*T. hungarcum*
Tetramorium caldarium	Egy 003	Egypt: Hurghada	27.239	33.836	14	16.04.2008	Csősz S.	KC921199	H01					
Tetramorium caespitum	Pl 014	Poland: Janowiec, distr. Putawy	51.417	21.967	137	11.06.2003	Czechowska W.	AY909106	HT1	0.934	0.000	0.066	0.000	0.000
Tetramorium caespitum	Pl 013	Poland: Janowiec, distr. Putawy	51.417	21.967	137	11.06.2003	Czechowska W.	AY909106	HT1	1.000	0.000	0.000	0.000	0.000
Tetramorium caespitum	AA 360	Hungary: Bátaapáti	46.217	18.6	171	02.09.2006	Csősz S.	AY909106	HT1	0.993	0.000	0.007	0.000	0.000
Tetramorium caespitum	Ger 026	Germany: Augsfeld	50.16	10.55	342	22.07.2001	Csősz S.	AY909106	HT1	0.996	0.000	0.004	0.000	0.000
Tetramorium chefketi	Rus 129	Russia: Krasnodarskiy Kray, Gelendzhik 2 km SSE	44.573	38.075	33	05.06.2006	Csősz S.	KC905182	H02					
Tetramorium feroxide	Rus 009	Russia: Stavropolskiy Kray, Neftekumsk, Achikulak	44.552	44.838	68	08.06.2006	Csősz S.	KC905183	H03					
Tetramorium ferox	Sr 001b	Slovakia: Sudovce	48.83	18.53	724	01.06.2008	Csősz S.	KC905184	H04					
Tetramorium hungaricum	AA 309	Hungary: Budapest, Sas mountain	47.5	19.033	192	08.05.2005	Csősz S.	AY909289	HT108	0.000	0.140	0.000	0.002	0.858
Tetramorium hungaricum	AA 310	Hungary: Budapest, Sas mountain	47.5	19.033	192	08.05.2005	Csősz S.	AY909289	HT108	0.000	0.211	0.000	0.000	0.789
Tetramorium hungaricum	Rus 089	Russia: Stavropolskiy Kray, Novoselitskoye 5 km E	44.75	43.44	172	11.06.2006	Csősz S.	KC905185	H05	0.000	0.021	0.000	0.001	0.978
Tetramorium hungaricum	Rus 118	Russia: Krasnodarskiy Kray, Gelendzhik	44.573	38.075	33	04.06.2006	Csősz S.	KC905185	H05	0.000	0.001	0.000	0.011	0.988
Tetramorium hungaricum	Rus 119	Russia: Krasnodarskiy Kray, Gelendzhik	44.573	38.075	33	04.06.2006	Csősz S.	KC905186	H06	0.000	0.053	0.001	0.011	0.936
Tetramorium hungaricum	Rus 120	Russia: Stavropolskiy Kray, Stavropol, Ruskij les	45.04	41.973	577	11.06.2006	Csősz S.	KC905187	H07	0.000	0.057	0.000	0.002	0.941
Tetramorium hungaricum	AA 320	Hungary: Budapest, Sas mountain	47.5	19.033	192	20.05.2005	Csősz S.	KC905188	H08	0.000	0.004	0.000	0.000	0.996
Tetramorium hungaricum	Ro 038	Romania: Dobrogea, Măcin, Pricopanu mountain	45.25	28.15	212	01.06.2005	Csősz S.	KC905189	H09	0.000	0.001	0.000	0.000	0.999
Tetramorium hungaricum	Ro 069	Romania: Dobrogea, Horia	44.533	26.8	63	01.06.2005	Csősz S.	KC905190	H10	0.000	0.001	0.000	0.000	0.999

Table 1 (*Continued*)

Taxon	Code	Locality	Latitude (North)	Longitude (East)	Elevation	Date of collecting	Collector	GenBank	Haplotype name	Posterior P				
										T. caespitum	TB	TC	TU4	*T. hungarcum*
Tetramorium hungaricum	Rus 020	Russia: Stavropolskiy Kray, Neftekumsk, Achikulak	44.552	44.838	68	08.06.2006	Csősz S.	KC905185	H05	0.000	0.005	0.000	0.001	0.994
Tetramorium hungaricum	Rus 037	Russia: Stavropolskiy Kray, Neftekumsk, Achikulak	44.552	44.838	68	09.06.2006	Csősz S.	KC905185	H05	0.000	0.002	0.000	0.000	0.998
Tetramorium hungaricum	Rus 036	Russia: Stavropolskiy Kray, Neftekumsk, Achikulak	44.552	44.838	68	09.06.2006	Csősz S.	KC905185	H05	0.000	0.003	0.000	0.000	0.997
Tetramorium hungaricum	AA 341	Hungary: Paks	46.633	18.867	88	02.09.2006	Csősz S.	KC905191	H11	0.000	0.029	0.000	0.000	0.971
Tetramorium indocile	Rus 105	Russia: Stavropolskiy Kray, Aleksandrovskoye 2 km W	44.65	43	443	11.06.2006	Csősz S.	AY909384	HT82	0.026	0.000	0.974	0.000	0.000
Tetramorium indocile	AA 319	Hungary: Budapest, Sas mountain	47.5	19.033	192	20.07.2005	Csősz S.	AY909295	HT87	0.001	0.000	0.999	0.001	0.000
Tetramorium indocile	AA 361	Hungary: Vértesboglár	47.433	18.533	161	12.10.2007	Csősz S.	AY909261	HT83	0.001	0.000	0.999	0.000	0.000
Tetramorium indocile	AA 362	Hungary: Vértesboglár	47.433	18.533	161	12.10.2007	Csősz S.	AY909261	HT83	0.001	0.000	0.998	0.001	0.000
Tetramorium indocile	AA 364	Hungary: Vértesboglár	47.433	18.533	161	12.10.2007	Csősz S.	AY909261	HT83	0.006	0.000	0.992	0.001	0.000
Tetramorium indocile	Kyr 001	Kyrgyzstan: Issyk-Kul	42.461	76.18	1648	08.06.2006	Borowiec M.	KC905192	H12	0.001	0.000	0.999	0.000	0.000
Tetramorium indocile	i135	Kyrgyzstan: Tien Shan	41.1963	75.7352	1960	17.07.2000	Schultz R.	AY909218	HT80	0.000	0.000	1.000	0.000	0.000
Tetramorium indocile	i136	Kyrgyzstan: Tien Shan	41.4964	76.4247	2300	21.07.2000	Schultz R.	AY909377	HT83	0.001	0.000	0.998	0.000	0.000
Tetramorium indocile	i139	Kyrgyzstan: Issyk-Kul	42.1488	76.7398	1980	23.07.2000	Schultz R.	AY909221	HT81	0.001	0.000	0.998	0.001	0.000
Tetramorium indocile	16855	France: Pamproux	46.3836667	−0.04913333	110	31.05.2009	Lenoir A.	KF377601		0.000	0.000	0.994	0.006	0.000
Tetramorium indocile	17428	Ukraine: Krym, Čatyr-Dag	44.7916667	34.3055556	1120	21.05.2009	Tarnawski D.	–		0.000	0.000	1.000	0.000	0.000
Tetramorium indocile	17853	Spain: Reocin de los Molinos	42.8613889	−4.06388889	850	20.06.2010	Garcia-Silvares J. C.	KF377600		0.007	0.000	0.992	0.001	0.000
Tetramorium indocile	17854	Spain: Reocin de los Molinos	42.8613889	−4.06388889	850	20.06.2010	Garcia-Silvares J. C.	KF377599		0.000	0.002	0.670	0.316	0.011
Tetramorium indocile	i537	Ukraine: Krym, Simferopol	44.8833333	34.1	400	13.08.1995	Buschinger A., Kipyatkov V. E., Lopatina E. B., Radchenko A., Sanetra M.	AY909384	HT82	0.000	0.000	1.000	0.000	0.000

Species	Code	Locality	Lat	Long	Alt	Date	Collector	GenBank	Haplotype					
Tetramorium indocile	i541	Ukraine: Krim, Aj-Petri	44.5	34.1666667	1200	16.08.1995	Buschinger A., Kipyatkov V. E., Lopatina E. B., Radchenko A., Sanetra M.	AY909261	HT83	0.000	0.000	1.000	0.000	0.000
Tetramorium indocile	i543	Ukraine: Krym, Aj-Petri	44.5	34.1666667	1200	16.08.1995	Buschinger A., Kipyatkov V. E., Lopatina E. B., Radchenko A., Sanetra M.	AY909263	HT84	0.000	0.000	1.000	0.000	0.000
Tetramorium indocile	i563	Ukraine: Krim, Simferopol	44.8833333	34.1	400	13.08.1995	Buschinger A., Kipyatkov V. E., Lopatina E. B., Radchenko A., Sanetra M.	AY909268	HT82	0.000	0.002	0.653	0.037	0.308
Tetramorium indocile	i599	Spain: Reocin de los Molinos	39.6333333	−2.16666667	1400	27.05.1995	Sanetra M.	KF377598		0.000	0.019	0.710	0.135	0.137
Tetramorium indocile	i644	Armenia: Kechut	39.8	45.6666667	2100	04.07.2002	Wuermli M.	AY909281	HT85	0.000	0.000	1.000	0.000	0.000
Tetramorium indocile	i647	Armenia: Landjik/Maralik	40.5769444	43.8602778	2000	12.07.2002	Wuermli M.	AY909283	HT85	0.005	0.000	0.992	0.003	0.000
Tetramorium indocile	i655	Armenia: Drakhtik	40.5688889	45.2319444	1950	17.08.2002	Wuermli M.	AY909389	HT85	0.000	0.000	0.992	0.004	0.004
Tetramorium indocile	i726	Russia: Troitsk	54.0666667	61.25	200	25.07.2002	Esyunin S. L.	AY909295	HT87	0.000	0.000	1.000	0.000	0.000
Tetramorium indocile	i728	Russia: Troitsk	54.0666667	61.25	200	25.07.2002	Esyunin S. L.	AY909296	HT89	0.000	0.000	0.978	0.022	0.000
Tetramorium indocile	TM34	Hungary: Budapest	47.5	19.07	300	24.06.2000	Schlick-Steiner B. C., Steiner F. M.	AY909327	HT90	0.000	0.000	1.000	0.000	0.000
Tetramorium indocile	**type series**	**Kyrgyzstan: Ssemiretschie, Kisil-Kija'pass**	**40.27**	**72.13**	**2100**	**1926**	**Kusnezow N.**			**0.000**	**0.000**	**1.000**	**0.000**	**0.000**
Tetramorium moravicum	Rus 005	Russia: Stavropolskiy Kray, Stavropol, Ruskij les	45.04	41.973	577	11.06.2006	Csősz S.	KC905193	H13					
Tetramorium moravicum	Rus 156	Russia: Krasnodarskiy Kray, Goryachiy Klyuch 15 km SE	44.605	39.024	85	05.06.2006	Csősz S.	KC905194	H14					
Tetramorium moravicum	Sr 001a	Slovakia: Sudovce	48.83	18.53	722	01.06.2008	Csősz S.	AY641705	MORA1					
Tetramorium sp. B	Ro 150	Romania: Greci	44.55	23.117	221	29.05.2005	Csősz S.	AY909137	HT58	0.000	0.953	0.000	0.001	0.046
Tetramorium sp. B	Ro 151	Romania: Greci	44.55	23.117	221	29.05.2005	Csősz S.	AY909137	HT58	0.000	0.857	0.000	0.000	0.143
Tetramorium sp. B	Ro 152	Romania: Greci	44.55	23.117	221	29.05.2005	Csősz S.	–		0.000	0.901	0.000	0.000	0.099
Tetramorium sp. B	Ro 153	Romania: Greci	44.55	23.117	221	29.05.2005	Csősz S.	–		0.000	0.949	0.000	0.000	0.050

Table 1 (*Continued*)

Taxon	Code	Locality	Latitude (North)	Longitude (East)	Elevation	Date of collecting	Collector	GenBank	Haplotype name	Posterior P				
										T. caespitum	TB	TC	TU4	*T. hungarcum*
Tetramorium sp. D	Rus 067	Russia: Stavropolskiy Kray, Stavropol, Ruskij les	45.04	41.973	577	11.06.2006	Csősz S.	KC905195	H15					
Tetramorium sp. D	Rus 153	Russia: Krasnodarskiy Kray, Goryachiy Klyuch 15 km SE	44.605	39.024	85	05.06.2006	Csősz S.	KC905196	H16					
Tetramorium sp. D	Rus 184	Russia: Stavropolskiy Kray, Yessentuki, Potkumot 1 km E	44.76	44.98	45	11.06.2006	Csősz S.	–						
Tetramorium sp. D	Rus 048	Russia: Krasnodarskiy Kray, Goryachiy Klyuch, 15 km SE	44.605	39.024	85	05.06.2006	Csősz S.	KC905197	H17					
Tetramorium sp. D	Rus 053	Russia: Stavropolskiy Kray, Stavropol, Ruskij les	45.04	41.973	577	11.06.2006	Csősz S.	KC905198	H18					
Tetramorium sp. D	Rus 101	Russia: Stavropolskiy Kray, Alexandrovskoe 2 rkm W	44.65	43	443	11.06.2006	Csősz S.	KC905199	H19					
Tetramorium sp. D	AA 363	Hungary: Vértesboglár	47.433	18.533	161	12.10.2007	Csősz S.	KC905200	H20					
Tetramorium sp. D	AA 373	Hungary: Vértesboglár	47.433	18.533	161	18.05.2008	Csősz S.	KC905201	H21					
Tetramorium sp. E	Ctc1	Romania: Dobrogea, Celig dere	45.183	28.667	26	29.05.2005	Csősz S.	KC905202	H22					
Tetramorium sp. E	Ro 030	Romania: Dobrogea, Măcin, Pricopanu mountain	45.25	28.15	212	01.06.2005	Csősz S.	AY909259	HT75					
Tetramorium sp. E	Ro 037	Romania: Dobrogea, Măcin, Pricopanu mountain	45.25	28.15	212	01.06.2005	Csősz S.	KC905203	H23					
Tetramorium sp. E	Ro 045	Romania: Dobrogea, Greci	44.55	23.117	221	29.05.2005	Csősz S.	AY909059	HT79					
Tetramorium sp. E	AA 308	Hungary: Budapest, Sas mountain	47.5	19.033	192	08.05.2005	Csősz S.	AY909059	HT79					
Tetramorium sp. E	Rus 136	Russia: Krasnodarskiy Kray, Gelendzhik, 2 km SSE	44.573	38.075	33	05.06.2006	Csősz S.	KC905204	H24					
Tetramorium sp. U4	Rus 013	Russia: Stavropolskiy Kray, Neftekumsk, Achikulak	44.552	44.838	68	08.06.2006	Csősz S.	AY909297	U4	0.000	0.000	0.009	0.991	0.000

Species	Sample	Locality				Collector	GenBank	Haplotype					
Tetramorium sp. U4	Rus 090	Russia: Stavropolskiy Kray, Novoselitzkoye 5 km E	44.75	43.44	172	11.06.2006	Csősz S.	AY909297	U4	0.000	0.003	0.004	0.944	0.049
Tetramorium sp. U4	Rus 099	Russia: Stavropolskiy Kray, Alexandrovskoye 2 km W	44.65	43	443	11.06.2006	Csősz S.	KC905205	H25	0.000	0.000	0.000	1.000	0.000
Tetramorium sp. U4	Ro 047	Romania: Greci	44.55	23.117	221	29.05.2005	Csősz S.	AY909297	U4	0.000	0.005	0.051	0.868	0.076
Tetramorium sp. U4	Rus 022	Russia: Stavropolskiy Kray, Neftekumsk, Achikulak	44.552	44.838	68	08.06.2006	Csősz S.	AY909297	U4	0.000	0.000	0.001	0.998	0.001
Tetramorium goniommoide	Rus 015	Russia: Stavropolskiy Kray, Neftekumsk, Achikulak	44.552	44.838	68	08.06.2006	Csősz S.	KC905206	H26					
Tetramorium goniommoide	Rus 021	Russia: Stavropolskiy Kray, Neftekumsk, Achikulak	44.552	44.838	68	08.06.2006	Csősz S.	–						

amplified products were purified by a 15 min digestion with 2 U Exonuclease I (Thermo Scientific) and 0.1 U FastAP (Thermo Scientific) at 37 °C, followed by a 15 min incubation at 80 °C in order to inactivate remaining enzymes. The purified PCR products were Sanger sequenced on an ABI 3730 capillary sequencer at the Comprehensive Cancer Center DNA Sequencing Facility, University of Chicago, USA, using the PCR primers.

2.4. Phylogenetic reconstruction

Sequences were aligned manually without any difficulties as they have the same length. Of the amplified region, 1115 bp with acceptable sequence quality for all samples were further used. The sequences of the new haplotypes were deposited in GenBank (for accession numbers, see Table 1). Fully overlapping sequences were obtained from the relevant studies of Steiner et al. (2005, 2010) and Schlick-Steiner et al. (2006, 2007b). Appropriate models of sequence evolution were determined using MrModeltest 2.3 (Nylander, 2004) for each codon position separately using the Akaike Information Criterion. The second codon position was excluded from the following analyses as at this position no informative variation was found within the ingroup. A general time-reversible model with gamma distributed rates across sites (GTR + G) was suggested for the first and third position.

Phylogeny was estimated in a Bayesian framework using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) with the selected model types and default priors. The first and third codon positions were defined as unlinked partitions. During the analysis, search was performed in two independent runs with four simultaneous chains. Chains were run for five million generations, sampling every 200th generation. The first 35% (8750 samples) were discarded as burn-in. Tetramorium caldarium (Roger, 1857) was defined as outgroup (GenBank accession number KC921199, this study). A 50% majority rule consensus tree is presented with posteriori probabilities (Fig. 2). In the interpretation of the tree we followed earlier authors (e.g., Huelsenbeck and Rannala, 2004) and regarded just nodes with a posterior probability of $p > 0.95$ as significantly supported.

2.5. Morphometric character recording and terminology (Fig. 1A–D)

Measurements were made with an Olympus SZX9 stereomicroscope at a 150× magnification by SC and with a LEICA M165 C stereomicroscope at a 360× magnification by HCW Nest means of 1–13 (median = 5) workers were built for the analysis. Morphometric data recording was taken on the same pool of nest samples that were analyzed genetically. Type material was investigated by morphometric approaches only, since molecular investigation was not available. Cases with missing values were omitted from the analyses.

The morphometric investigation was restricted to workers. The definitions and abbreviations for the measured characters are as follows:

CL length of head in full-face view, measured in straight line from anteriormost point of median clypeal margin to midpoint of posterior margin of head. Concavity of posterior margin and an emargination on the frontal clypeus reduce CL.

CS cephalic size; calculated from arithmetic mean of CL and CW. It is used as a less variable indicator of body size.

CW maximum width of head in full-face view, including compound eyes.

EH minimum diameter of compound eye (Fig. 1C).

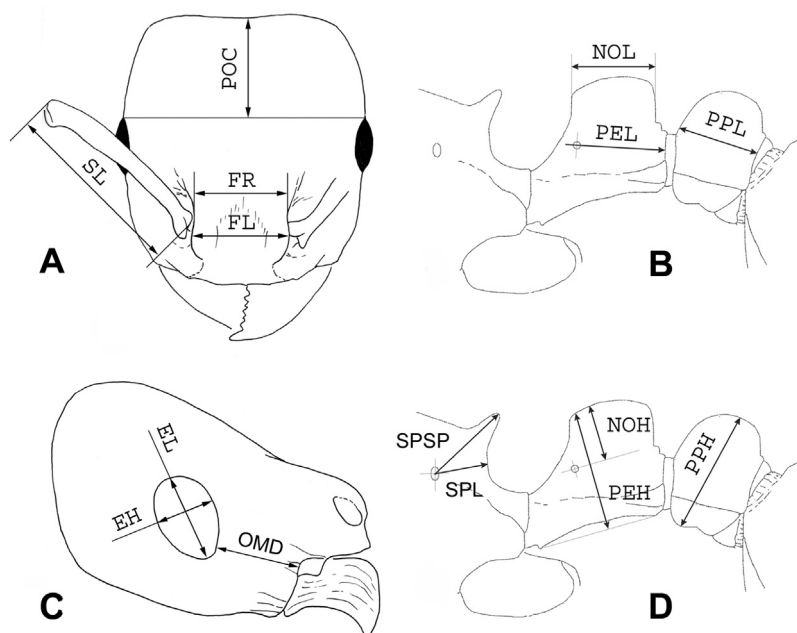EL maximum diameter of compound eye (Fig. 1C).

Fig. 1. Measurement lines for metric characters. (A) Head in dorsal view, measurement lines for POC, FL, FR and SL. (B) Petiole and postpetiole in lateral view, measurement lines for NOL, PEL and PPL. (C) Head in lateral view, measurement lines for EL, EH, and OMD. (D) Propodeum, petiole and postpetiole in lateral view, measurement lines for, NOH, PEH, PPH, SPSP, and SPL (after Csősz and Schulz, 2010).

EYE   eye size index, calculated from arithmetic mean of EL and EH, divided by CS.

FL   maximum distance between external borders of frontal lobes (Fig. 1A). If FL is not defined, because frontal carinae converge anterior to the FR level, FL is measured as distance between carinae inflection points; these inflection points are between concave (caudal) and convex (frontal) carinae margin.

FR   minimum width of frons between frontal carinae (Fig. 1A). If FR is not defined, because frontal carinae converge, FR = FL.

ML   diagonal length of mesosoma measured in lateral view from the anteriormost point of pronotal slope to posterior (or postero-ventral) margin of propodeal lobes; equivalent measuring also possible in dorsal view.

MW   maximum width of pronotum from above.

NOH   maximum height of petiolar node (Fig. 1D).

NOL   length of petiolar node (Fig. 1B).

OMD   oculo-malar space. The minimal distance between anterior (lower) margin of the compound eye and the mandibular junction in profile (Fig. 1C).

PEH   maximum height of petiole (Fig. 1D).

PEL   distance between posteriormost point of petiole and petiolar spiracle (Fig. 1B).

PEW   maximum width of petiole in dorsal view.

POC   postocular distance. Measured from reference line fitted on posterior margin of compound eyes to the mid-point of the posterior margin of head (Fig. 1A). Concavity of posterior margin reduces POC.

POTCos   number of post-oculo temporal costulae is counted along a reference line perpendicular from the posteriormost border of eye to the ventralmost side of head in lateral view. Costulae just touching the measuring line are counted as 0.5. This meristic character is not involved in discriminant analyses, but handled as a numeric descriptive character in the differential diagnosis.

PPH   maximum height of postpetiole in lateral view (Fig. 1D).

PPL   maximum length of postpetiole in lateral view (Fig. 1B).

PPW   maximum width of postpetiole in dorsal view.

SL   maximum length of scape, measured from proximal point of scape lobe to distal end of scape (Fig. 1A).

SPL   minimal distance between center of propodeal spiracle and propodeal declivity measured in lateral view (Fig. 1D).

SPST   maximum length of propodeal teeth from center of propodeal spiracle to tip of spine, measured in the same focal level (Fig. 1D).

The above morphometric traits were tested for repeatability (see Csősz et al., 2007; Csősz and Schulz, 2010); each character has passed tests of repeatability.

Nomenclature of surface sculpturing in this paper follows categories of Harris (1979).

2.6. Multivariate analyses

We were particularly interested to know the position of the *T. indocile* type series (and single members of the type series) in the multidimensional discriminant space of the species of the *T. caespitum/impurum* complex sensu Schlick-Steiner et al. (2006) using multivariate statistics of 21 continuous morphometric traits. Cumulative Linear Discriminant Analysis (LDA) was applied to confirm morphological separation of Molecular Operational Taxonomical Units suggested by the mtDNA phylogeny. LDA was performed using R statistics on workers.

2.7. Male genitalia

The genitalia of some males were investigated by SC and HCW and drawn of one representative example (sample code 17853, Table 1, Fig. 4). Terminology of male genitalia follows Collingwood (1979).

3. Results

3.1. Molecular phylogenetics

Sequences were obtained from 64 nests, i.e., we did not obtain sequences from four nests (Rus21, Rus184, RO152 and RO153). The GenBank accession numbers of haplotypes are given in Table 1. The final alignment included 66 sequences and 743 characters. Most of the MOTUs defined by Schlick-Steiner et al. (2006) were recovered with high support (Fig. 2), including *Tetramorium indocile = Tetramorium* sp. C (posterior probability 1.00).

3.2. Morphometric confirmation of MOTUs and position of type material of Tetramorium caespitum var. indocile Santschi, 1927

We considered the relevant available names of taxa that could potentially be conspecific with the OTU *Tetramorium* sp. C. Bolton's Catalog with a list of *Tetramorium* taxon names formed the backbone of our endeavor to find the link of *Tetramorium* sp. C to its correct species name. Reading the original descriptions of all available names listed, we excluded many names based on biogeographical and morphological arguments. We analyzed type specimens of the remaining taxa.

Seven species included in the molecular analyses (*T. chefketi* Forel, 1911; *T. ferox* Ruzsky, 1903; *T. feroxoide* Dlussky and Zabelin, 1985; *T. forte* Forel, 1904; *T. moravicum* Kratochvil, 1941; *T. semilaeve* André, 1883 and *T. goniommoide* Poldi, 1979) could be excluded from in-depth morphological analysis based on some striking descriptive characteristics: They either had continuous imbricate microsculpture on the 1st gaster tergite or lacked long sinuous postbuccal hairs on the ventral head or showed a combination of both characteristics. These species are clearly not members of the *Tetramorium caespitum/impurum* complex. Neither of the two characteristics applied to the workers of the *T. indocile* type series.

Further sculpture characteristics, in particular dense longitudinal costulae on the postoculo-temporal area (POTCos) excluded two further MOTUs, *Tetramorium impurum* (POTCos: 9.91 ± 1.7 [8.0, 13.0]) and *Tetramorium* sp. E sensu Schlick-Steiner et al. (2006) (POTCos: 10.25 ± 1.6 [7.0, 13.0]) as being conspecific with *T. indocile* type series (POTCos: 4.36 ± 2.25 [0.0, 7.0]). *Tetramorium* sp. D sensu Schlick-Steiner et al. (2006) shows a clinal pattern in postoculo-temporal surface sculpturing (POTCos). In Asia, Eastern Europe and the Pannonian region, surface sculpturing is comparatively strong (POTCos: 11.24 ± 2.27 [6.5, 15.0]) whereas it is less pronounced in the Mediterranean (POTCos: 6.48 ± 3.19 [3.5, 14.5]). Thus, because at the type locality of *T. indocile* (Kyrgysia) surface sculpturing of *Tetramorium* sp. D is considerably stronger than that of *T. indocile* populations, the exclusion of conspecificity of *Tetramorium* sp. D and the type material is feasible. Distribution of *T. indocile* does not overlap with the high-altitude *T. alpestre* Steiner et al., 2010, therefore the latter was also eliminated from this step.

Eventually, samples of five MOTUs were involved in the cumulative discriminant analysis based on the lack of strictly deviating descriptive characteristics between them and the type series of *T. indocile*. These were as follows: *T. caespitum*, *T. hungaricum*, *Tetramorium* sp. B sensu Schlick-Steiner et al. (2006), *Tetramorium* sp. C and *Tetramorium* sp. U4 sensu Schlick-Steiner et al. (2006). Cumulative LDA (Table 2) using 20 morphometric traits confirmed the morphological separation of the five selected MOTUs with high support: 93.4% of the individuals (96.4% of *T. caespitum*, 91.7% of *T.* sp. B, 91.2% of *Tetramorium* sp. C, 90.0% of *Tetramorium* sp. U4 and 96.1% of *T. hungaricum* workers) and 100% of the nest samples were correctly classified. The geometric means of posterior probabilities for nest samples are given in Table 1.

The workers of *Tetramorium caespitum* var. *indocile* Santschi, 1927 type material (*n* = 13) were treated as wildcards in the

Table 2
Unstandardized row coefficients of Roots 1–4 achieved by discriminant D(20) analysis run on *T. caespitum*, *T.* sp. B, *T.* sp. C (= *T. indocile*), *T.* sp. U4 and *T. hungaricum*. The explanation how to use the values for a determination is given in the text (Section 4.5.).

	Root 1	Root 2	Root 3	Root 4
CL	−0.0388	0.0411	0.0043	−0.0351
CW	−0.0039	−0.0094	−0.0198	−0.0210
FR	0.0148	0.0690	−0.0095	−0.0491
FL	0.0082	−0.0597	−0.0486	0.0074
SL	0.0295	−0.0334	−0.0035	0.0097
ML	0.0319	0.0230	0.0098	0.0214
MW	−0.0430	−0.0623	0.0174	−0.0026
PEW	−0.0005	0.0504	−0.1054	0.0271
PEH	0.0161	0.0033	0.0425	0.0340
NOH	−0.0362	−0.0171	−0.0482	−0.0382
NOL	−0.0265	−0.0244	−0.0551	−0.0405
PEL	0.0543	−0.0565	0.0401	0.0294
PPW	0.0341	0.0145	0.0935	0.0103
PPL	0.0395	−0.0150	0.0077	0.0024
PPH	−0.0123	−0.0191	−0.0478	0.0400
SPL	0.0305	−0.0392	0.0854	−0.1201
SPST	0.0156	0.0315	−0.0191	0.0235
EL	−0.0841	−0.0652	−0.0369	0.0411
EH	−0.0324	0.0267	0.0485	−0.0135
OMD	−0.0025	0.0712	0.0509	−0.0079
Constant	−4.1145	2.1472	2.8964	11.4002

analysis (i.e., added in the analysis without group labels) and cumulative LDA used to predict their position within one of the analyzed OTUs. The type series was classified as *Tetramorium* sp. C with posterior probability *p* = 1.0. Conspecificity of *T. indocile* type series with *T.* sp. C is also corroborated by the geographic distribution of *Tetramorium* sp. C provided by Schlick-Steiner et al. (2006).

4. Redescription of *Tetramorium indocile* Santschi, 1927 stat. rev. (Figs 3A–C and 4A–C)

Tetramorium caespitum var. *indocile* Santschi, 1927: 53 (U+263F, U+2640, U+2642) [Kyrgyzstan]: Lectotype (U+263F)

and paralectotype (U+263F,U+263F), (U+2640,U+2640) and (U+2642,U+2642) (for details see Section 4.1.); subspecies of *T. caespitum* sub "*T. caespitum indocilae*": Pisarski, 1967: 402; Raised to species rank: Pisarski, 1969: 304; Junior synonym of *T. caespitum*: Radchenko, 1992: 50.

Morphometric characters are given in μm, numeric characters or ratios are followed by the mean and minimum, maximum values are given in brackets.

4.1. Lectotype designation of Tetramorium indocile Santschi, 1927

In order to avoid any further nomenclatural problems lectotype designation for the type series is essential. We investigated 5 workers, one gyne and one male mounted on two pins. The lectotype worker by the present designation is labeled as: TYPE [−] "*T. caespitum* L. v *indocile* Sant [/] SANTSCHI det. 19 "26" [−] "Semiretschie" [/] "Kisil-Kija Pass" [/] "(Kusnezow)" [−] Sammlung Dr. F. Santschi Kairouan [−] NATURHIST. MUSEUM BASEL [−] "9" [−]. The lectotype is positioned on the proximal end of the third card from the top. The lectotype worker is considered the best preserved one, a leg of a paralectotype worker is caught by its mandibles (Fig. 3A–C), but it does not hinder examination.

Morphometric data of the lectotype worker in μm:
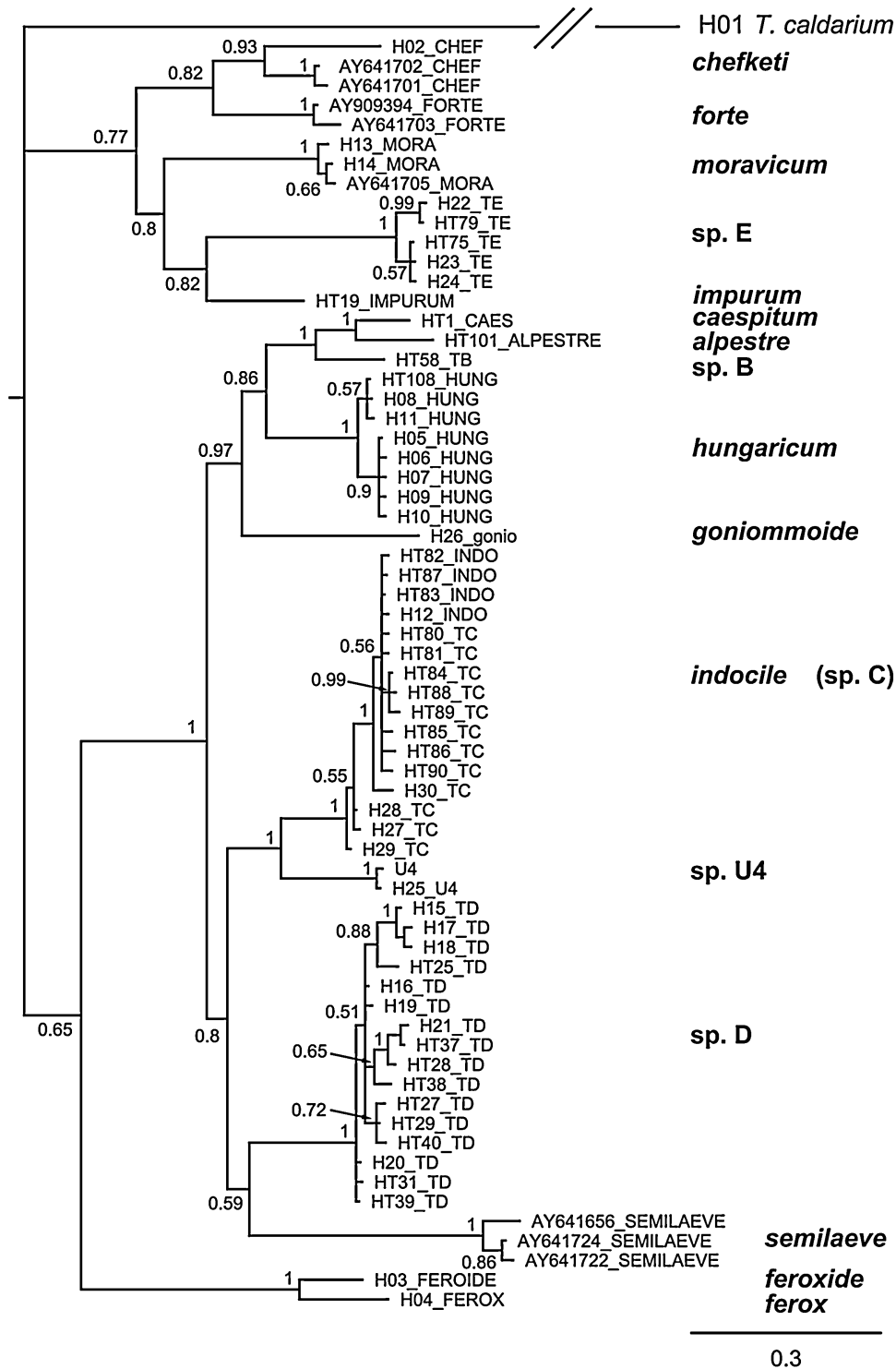CL: 685; CW: 675; FR: 270; FL: 270; SL: 525; ML: 790; MW: 435; PEW: 220; PEH: 255; NOH: 150; NOL: 130; PEL: 160; PPW:

Fig. 2. Phylogenetic reconstruction. 50% majority-rule consensus tree is presented based on Bayesian analysis. Posterior probabilities are shown left above the nodes. If this position is taken, posterior probabilities are shown left under the nodes. If both positions are taken, an arrow shows the connection. Branch length of the outgroup (*T. caldarium*) is shortened.

280; PPL: 180; PPH: 255; SPL: 100; SPST: 140; EL: 130; EH: 100; OMD: 175; POC: 275; POTCos: 3.5.

4.2. Redescription of worker (Fig. 3a–c)

Small to medium size, CS 696 [610,850]. Whole body and appendages dark brown to black. Head slightly longer than broad, CL/CW 1.03 [0.98, 1.07], with very weakly convex or straight sides, feebly concave or straight occipital margin and rounded occipital corners. Eyes moderately large, EYE 0.171 [0.148, 0.186]. Frons moderately narrow, FR/CS 0.38 [0.36, 0.40], frontal lobes as wide as frons, or slightly wider, FL/FR 1.01 [1.0, 1.04]. Scape moderately long, SL/CS 0.77 [0.70, 0.80]. Promesonotal dorsum convex, metanotal groove shallow. Propodeal teeth short SPST/CS 0.19 [0.14, 0.24]. Dorsal surface of petiole rounded backward.

General appearance finely rugulose, interstices usually feebly microreticulate, or rarely smooth. Head dorsum and occiput longitudinally costate-costulate, interstices microreticulate, dull.

Postoculo-temporal area of head with a few longitudinal costulae: 5 [3,9], ground sculpture generally smooth and shiny, or feebly microreticulate. Number of costae between sides of frontal lobes: 14 [10,16], interstices feebly microreticulate, dull.

Mesosoma dorsum longitudinally rugulose, number of longitudinal rugulae at the widest part of mesosoma: 19 [17,21], ground surface smooth and shiny. Mesopleuron longitudinally rugulose and microreticulate. Dorsum of petiolar node smooth. Width of smooth median area on dorsum of petiole: 125 [100,170]. Dorsum of postpetiole longitudinally rugulose and finely microreticulate. Width of smooth median area on dorsum of postpetiole: 185 [140,260].

Polygonal microsculpture absent or sporadic on 1st gastral tergite. Ventral surface of head with a row of short setae mixed with very long sinuous or C-shaped hairs arising just posteriorly to buccal cavity (see Figs. 3 and 4).

4.3. Morphology of male (Fig. 4a–c)

Whole body and appendages black. Head with convex sides, rounded occipital margin and widely rounded occipital corners. Scutum 1.5× wider than head. Propodeal teeth reduced to a pair of longitudinal ridges running parallel on the dorso-caudal slope of propodeum. Dorsal crest of petiolar node with a medially not depressed blunt ridge. Head, mesosoma and waist coarsely sculptured, ground surface microreticulate, dull. Head longitudinally rugulose, ground surface microreticulate. 10 antenna segments. Mesonotum and scutellum longitudinally costulate. Sides of mesosoma with longitudinal rugae, anepisternum longitudinally costulate, katepisternum, particularly on the median part, smooth and shiny. Dorsum of petiolar node microreticulate that is superimposed by feeble irregular rugulae, postpetiole costulate ground sculpture microreticulate. First gastral tergite shiny.

In lateral view, paramere shows peakish apex and apodeme positioned more posterior. Apex and apodeme positioned at almost same transversal level, both with roughly rectangular profile of apical paramere and pronounced apodeme (Fig. 4B). In posterior view, two small sharp corners at the caudalmost position of the paramere are visible (Fig. 4C). In ventral view, gripping jaw of paramere appears very sharp (Fig. 4D).

4.4. Geographic distribution

According to the present data T. indocile is widely distributed in steppe-like habitats from Kyrgyzstan (E 76.74°) to Spain (W 04.06°), but seems to be generally rare in Southern and Western Europe (Table 1).

4.5. Differential diagnosis

Workers of T. indocile differ from workers of Tetramorium chefketi, T. ferox, T. feroxoide, T. forte, T. moravicum, T. semilaeve and T. goniommoide by the lack of continuous imbricate microsculpture of 1st gaster tergite or having a number of long sinuous postbuccal hairs on the ventral head or both. Low density of longitudinal costulae on the postoculo-temporal area helps to distinguish T. indocile workers (POTCos: 4 ± 1.83 [0,8]) of Tetramorium sp. D from Pannonian, Eastern European and Asian populations, Tetramorium sp. E and T. impurum (POTCos: 12 ± 1.35 [10,16]). The distribution of T. indocile does not overlap with the high-mountain T. alpestre, but often stronger petiolar sculpture and denser sculpture of postoculo-temporal head of the latter would help separate these two species.

Due to the fact that T. indocile workers are very similar to that of T. caespitum, T. hungaricum, Tetramorium sp. B and Tetramorium sp. U4 sensu Schlick-Steiner et al. (2006) in their overall appearance, i.e., diagnostic characters and sculpture, their determination
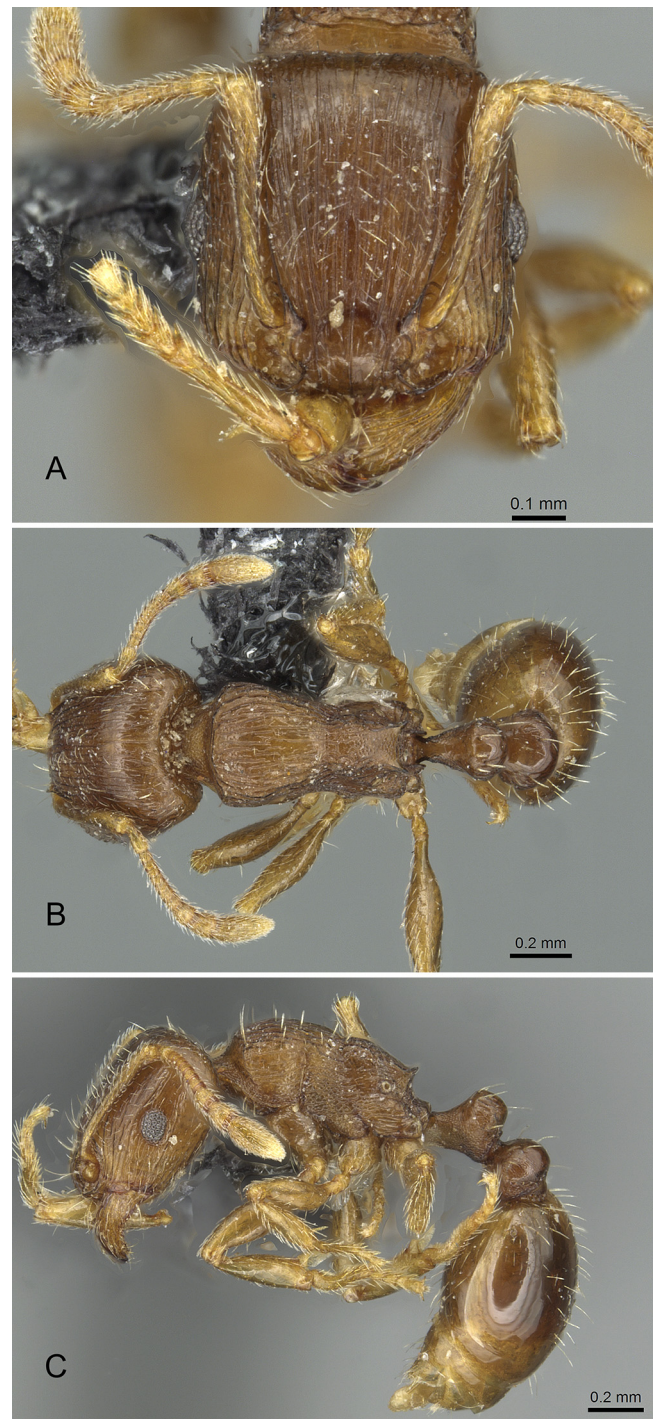

Fig. 3. The lectotype worker of Tetramorium indocile in (A) frontal, (B) dorsal and (C) lateral view. Images are also available online on AntWeb with specimen-level code CASENT0913998.

based on macro-morphological characterization is extremely difficult. Therefore, application of discriminant formulas of particular roots (Table 2) is recommended to separate T. indocile from the most similar OTUs as follows: each morphometric value [μm] from the characters in the first column has to be multiplied by the value in the respective root column which is in the same row. Then, the sum of all 20 products and the constant of the root column is calculated. The result can be compared with the values in Table 3 to determine the species identity of the individual or nest mean sample. Root 1 yields high separation of T. indocile from Tetramorium sp. B

Table 3
Discriminant D(20) scores for individuals and nest means of *T. caespitum*, *T.* sp. B, *T.* sp. C (= *T. indocile*), *T.* sp. U4 and *T. hungarcum* achieved by Roots 1–4.

		Root 1	Root 2	Root 3	Root 4
T. caespitum	Ind (*n* = 28)	3.75 ± 1.1 [1.07, 5.75]	−2.10 ± 1.1 [−4.98, −0.20]	0.17 ± 1.0 [−2.24, 2.31]	−0.57 ± 0.9 [−2.33, 1.50]
	nsm (*n* = 4)	3.68 ± 0.75 [2.62, 4.39]	−2.04 ± 1.0 [−3.02, −0.91]	0.26 ± 0.4 [−0.27, 0.54]	−0.51 ± 0.3 [−0.80, −0.12]
T. sp. B	Ind (*n* = 24)	−2.19 ± 0.7 [−3.58, −0.82]	−1.26 ± 0.7 [−2.97, 0.08]	0.22 ± 0.9 [−1.38, 2.40]	1.76 ± 0.7 [0.60, 3.46]
	nsm (*n* = 4)	−2.19 ± 0.07 [−2.27, −2.12]	−1.26 ± 0.4 [−1.62, −0.89]	0.22 ± 0.5 [−0.46, 0.70]	1.76 ± 0.1 [1.66, 1.84]
T. sp. C (= *indocile*)	Ind (*n* = 68)	2.07 ± 1.2 [−1.18, 4.81]	1.14 ± 1.1 [−0.89, 4.83]	0.48 ± 1.0 [−1.89, 2.57]	0.27 ± 1.1 [−2.76, 3.34]
	nsm (*n* = 24)	1.95 ± 1.01 [−0.34, 4.02]	1.59 ± 1.2 [−0.09, 4.83]	0.42 ± 0.6 [−0.62, 2.37]	0.53 ± 1.0 [−1.45, 3.34]
T. sp. U4	Ind (*n* = 30)	0.02 ± 0.9 [−2.34, 1.40]	0.30 ± 1.0 [−1.45, 2.49]	−2.35 ± 1.3 [−4.82, 0.59]	−0.01 ± 1.1 [−2.11, 2.18]
	nsm (*n* = 5)	−0.09 ± 0.53 [−0.77, 0.54]	0.37 ± 0.4 [−0.10, 0.90]	−2.26 ± 0.8 [−3.12, −1.23]	0.00 ± 0.4 [−0.55, 0.39]
T. hungarcum	Ind (*n* = 76)	−2.55 ± 0.8 [−4.58, −0.47]	0.03 ± 0.9 [−2.68, 1.98]	0.37 ± 0.9 [−2.29, 1.89]	−0.58 ± 1.0 [−2.74, 2.26]
	nsm (*n* = 13)	−2.55 ± 0.44 [−3.44, −1.63]	0.00 ± 0.7 [−1.53, 1.21]	0.27 ± 0.7 [−1.13, 1.20]	−0.54 ± 0.8 [−1.71, 0.80]

Ind: individuals, nsm: nest sample means. Number of cases (*n*), mean ± SD, [Min, Max] is given.
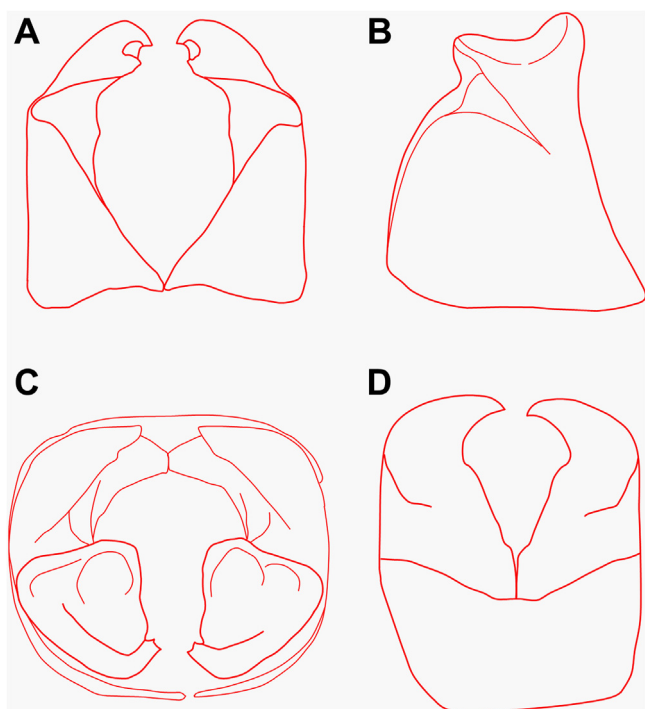

Fig. 4. Male genitalia of *Tetramorium indocile* in (A) dorsal, (B) lateral, (C) posterior and (D) ventral view.

and *T. hungaricum* at both individual and nest sample mean level. Root 2 provides nearly perfect discrimination of *T. indocile* and *T. caespitum*, and Root 3 separates perfectly *Tetramorium* sp. U4 and *T. indocile*.

The male genitalia are clearly different from those of other species of the complex (see Schlick-Steiner et al., 2006: 263).

5. Discussion

Based on our results of molecular phylogeny and the quantitative morphological analyses, we reveal that *Tetramorium* sp. C sensu Schlick-Steiner et al. (2006) is conspecific with *Tetramorium indocile* Santschi, 1927. We thus propose *Tetramorium indocile* Santschi, 1927 as the scientific name for the latter OTU.

Due to the very similar morphologies of *Tetramorium* species, particularly of *T. caespitum*, *T. hungaricum*, *T. indocile* and *Tetramorium* sp. B, separation of these species is extremely difficult and at the moment no easier methods can be provided for certain identification than the given D(11) function. Perhaps, future research will bring us closer to a more accurate mode of determination.

Some further OTUs, i.e., *Tetramorium* sp. B, *Tetramorium* sp. D, *Tetramorium* sp. E and *Tetramorium* sp. U4, remain unidentified until now. Before these OTUs can be linked to scientific names, species delimitation based on denser sampling of the entire Western Palearctic and an in-depth multidisciplinary approach is needed.

The morphometric character set used in this study proved taxonomically informative like in previous studies (Csősz et al., 2007). The newly defined character POTCos turned out very relevant in the qualitative assessment done here and might be generally useful for species delimitation of the *T. caespitum/impurum* complex in future studies.

Finally, we showed for the first time, by the molecular phylogeny (node support for the clade of the entity *Tetramorium* sp. U4 as termed by Schlick-Steiner et al., 2006 is 1.0, Fig. 2), that *Tetramorium* sp. U4 probably is a distinct species indeed, different from *T. indocile*. In the earlier paper by Schlick-Steiner et al. (2006), this issue remained unresolved due to the overly small sample size in that study.

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