Ann. Zool. Fennici 42: 23–35 ISSN 0003-455X
Helsinki 23 February 2005

Towards DNA-aided biogeography: An example from *Tetramorium* ants (Hymenoptera, Formicidae)

Florian M. Steiner[1,2,*], Birgit C. Schlick-Steiner[1,2], Matthias Sanetra[3], Toshko Ljubomirov[4], Vera Antonova[5], Erhard Christian[1] & Christian Stauffer[2]

1) *Institute of Zoology, Department of Integrative Biology, BOKU, University of Natural Resources & Applied Life Sciences, Vienna, Gregor-Mendel-Str. 33, A-1180 Wien, Austria (*corresponding author's e-mail: h9304696@edv1.boku.ac.at)*
2) *Institute of Forest Entomology, Forest Pathology and Forest Protection, Department of Forest and Soil Sciences, BOKU, University of Natural Resources & Applied Life Sciences, Vienna, Hasenauer Str. 38, A-1190 Wien, Austria (e-mail: christian.stauffer@boku.ac.at)*
3) *Zoology and Evolutionary Biology, University of Konstanz, Universitätsstr. 10, D-78457 Konstanz, Germany (e-mail: matthias.sanetra@uni-konstanz.de)*
4) *Institute of Zoology, Bulgarian Academy of Sciences, 1, Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria (e-mail: tljubomirov@zoology.bas.bg)*
5) *Central Laboratory of General Ecology, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, Bulgaria (e-mail: antonova@ecolab.bas.bg)*

Received 17 June 2004, revised version received 18 Aug. 2004, accepted 25 July 2004

Steiner, F. M., Schlick-Steiner, B. C., Sanetra, M., Ljubomirov, T., Antonova, V., Christian, E. & Stauffer, C. 2005: Towards DNA-aided biogeography: An example from *Tetramorium* ants (Hymenoptera, Formicidae). — *Ann. Zool. Fennici* 42: 23–35.

The increasing level of fine-scale systematics in insects requires sophisticated morphometric tools for species identification. As a consequence, regional species lists and biogeographic data tend to be ambiguous. We explore the use of DNA techniques in an example of palearctic ants of the myrmicine genus *Tetramorium*, in which morphology-based determination is difficult and frequently controversial. Several chorological facts are uncovered by the combined use of morphological characters and mitochondrial DNA sequences: *Tetramorium moravicum* is reported from Bulgaria and Ukraine, *T. hungaricum* from Austria and Bulgaria, while sequence comparisons question published records of *T. semilaeve* and *T. forte* in Eastern Europe. These findings have permitted us to delineate a biogeographic framework for *T. forte*, *T. moravicum*, *T. semilaeve* and *T. hungaricum*.

Introduction

Our knowledge of global biodiversity has increased enormously over the past decades. The number of described species of ants, on the one hand, has risen by 16.4% during the last eight years (Bolton 1995, Agosti 2003). On the other hand, the determination of ant species is becoming extremely difficult (e.g. Seifert 2002, 2003, Csösz & Seifert 2003, Schlick-Steiner *et al.* 2003b). Non-taxonomists often rely upon ambiguous data sets because specialists are not readily available. Recently, DNA-based methods are becoming more commonly used for species distinction. Attempts are being started to catalogue biological diversity by "DNA barcoding" (Hebert *et al*. 2003a, Mason 2003), a method which would help resolve the problem of identifying closely related species (Hebert *et al*. 2003b). Other authors even suggest "DNA taxonomy" (Tautz *et al*. 2003), and, in fact, taxonomic species descriptions by molecular markers alone already exist (Weistheide & Hass-Cordes 2001). Opinions are polarised — some authors are jubilant (Blaxter 2003, Blaxter & Floyd 2003, Pennisi 2003, Stoeckle 2003, Janzen 2004), while others heavily criticise these efforts when used as the sole approach (Dunn 2003, Lipscomb *et al*. 2003, Seberg *et al*. 2003, Sperling 2003, Will & Rubinoff 2004).

The taxonomy of the palearctic species of the ant genus *Tetramorium* is not satisfactory, nor are the available tools for morphological determination (e.g. Sanetra 1996, Seifert 1996, Sanetra *et al*. 1999, Sanetra & Buschinger 2000). Similarity in external morphological characters, particularly in the worker caste, and intraspecific geographic variation are hurdles for reliable species identification. Only a few recent attempts have been made to resolve taxonomic issues in geographically confined regions: Spain (López 1991a, 1991b), Ukraine, Russia (Radchenko 1992), Morocco (Cagniant 1997), and Italy (Sanetra *et al*. 1999). Some species boundaries and phylogenetic hypotheses were established using allozymes (Sanetra *et al*. 1999, Sanetra & Buschinger 2000). Nevertheless, the lack of a comprehensive taxonomic revision renders the use of species names in faunistic and biogeographic studies difficult.

In the eastern parts of Europe, including Hungary, Romania, Bulgaria and Ukraine, the reliability of published records of *Tetramorium* species is rather poor. A total of nine *Tetramorium* species have been published for these countries (Table 1), including *T. caespitum* (Linnaeus, 1758), *T. forte* Forel, 1904, *T. hungaricum* Röszler, 1935, *T. moravicum* Kratochvil, 1941 and *T. semilaeve* André, 1883. *T. caespitum* is supposed to consist of several cryptic species (Steiner *et al*. 2002, 2003). Workers of *T. caespitum* and *T. semilaeve* are sometimes difficult to distinguish (López 1991b, Sanetra *et al*. 1999), yet the sexuals are clearly distinct. *T. semilaeve sensu stricto* appears to have a circum-mediterranean distribution, and thus records from outside this region have been considered doubtful (Sanetra *et al*. 1999). *Tetramorium hungaricum* Röszler, 1935 was originally described from Hungary. It was considered as a variety of *T. semilaeve* by Novák and Sadil (1941) because of the weakly sculptured workers. Although Röszler (1951) raised it to species status, the taxon *hungaricum* was later neglected by most authors (except Collingwood in Franz 1975) until the catalogisation of Röszler's collection by Markó and Csösz (2002). *Tetramorium forte*, described from southern France, belongs to a group of species that can be discerned readily from *T. caespitum* and *T. semilaeve* by the strongly developed

Table 1. Published records of currently valid *Tetramorium* species from Hungary (Röszler 1935, Gallé *et al.* 1998, Sanetra & Buschinger 2000, Csösz *et al.* 2002), Romania (Paraschivescu 1978, Markó & Csösz 2002), Bulgaria (Atanassov & Dlusskij 1992) and Ukraine (Forel 1904, Arnol'di 1968, Apostolov & Likhovidov 1973, Radchenko 1992, Sanetra & Buschinger 2000).

	Hungary	Romania	Bulgaria	Ukraine
T. caespitum (Linnaeus, 1758)	x	x	x	x
T. ferox Ruzsky, 1903			x	
T. forte Forel, 1904[1]		x	x	x
T. hungaricum Röszler, 1935	x			
T. impurum (Förster, 1850)	x	x	x	x
T. meridionale Emery, 1870				x
T. moravicum Kratochvil, 1941	x			x
T. rhenanum Schulz, 1996[2]	x			
T. semilaeve André, 1883	x	x	x	x

[1] Partly published as *Tetramorium taurocaucasicum* Arnol'di, 1968, which was described from Crimea (Ukraine) and which is a junior synonym of *T. forte* according to Radchenko (1992) and Atanassov and Dlusskij (1992).
[2] Species status questioned, synonymy with *T. moravicum* supposed (B. C. Schlick-Steiner *et al.* unpubl. data).

body sculpture especially on the nodes. This feature is also shared by *Tetramorium moravicum*. The latter was described from Moravia (Czech Republic) and characterised in detail by Kratochvil *et al*. (1944). Agosti and Collingwood (1987) later mentioned the occurrence of *T. moravicum* in Bulgaria. Workers are so similar to *T. forte* that Bernard (1968), Dlusskij *et al*. (1990), Atanassov and Dlusskij (1992) and Radchenko (1992) synonymised the two nominal species, before Sanetra *et al*. (1994) reinstated species rank of *T. moravicum*. Schulz (1996) also treated the two taxa as distinct and discussed their distribution. Based on extensive material from different localities, R. Güsten, M. Sanetra & A. Schulz (unpubl. data) found female sexuals of *T. moravicum* and *T. forte* turned to be strikingly different. In summary, this calls for clarifying whether *T. semilaeve* and *T. forte* occur in eastern parts of Europe.

Mitochondrial DNA (mtDNA) is a good molecular marker in insects (Avise *et al*. 1987, Zhang & Hewitt 2003). It has been particularly useful for reconstructing the phylogenetic relationships among closely related taxa and for studying population history. Combining molecular data with morphological analyses reduces the risk of drawing false conclusions. Thus, congruence of genetic and morphological data yields much stronger support for inferred hypotheses about evolutionary patterns than either of these approaches alone (Wiens & Reeder 1997, Wetterer *et al*. 1998, Steiner *et al*. 2004).

In this study we identified *Tetramorium* ants from eastern Europe by combining morphology with mtDNA sequences. The results are discussed with respect to the myrmecofaunae of some eastern European countries and the application of molecular techniques in biogeographic research in general.

Material and methods

Tetramorium ants from Bulgaria, Ukraine, Austria, the Czech Republic, Germany, Hungary and Spain (Table 2) were morphologically determined according to André (1883), Forel (1904), Röszler (1935), Schulz (1996) and Seifert (1996). The *T. forte* sample from Albarracin and the *T. moravicum* samples from Mohelno and Zali's'sa had already been characterised by diagnostic enzyme loci in a previous work (Sanetra & Buschinger 2000: samples T 498, T 207, T 527). The separation of *T. forte* and *T. moravicum* was further corroborated by comparison of the morphologically distinct sexuals from nearby areas. Types of *T. forte* (Muséum d'Histoire Naturelle Genève) and specimens of *T. hungaricum*, the labels of which are identical with the data referred to in Röszler's description (Naturhistorisches Museum Wien), were inspected for comparison. The type material of *T. semilaeve* probably consists of several species (Sanetra *et al*. 1999), and we therefore inspected specimens of *T. semilaeve* from a population in Banyuls-sur-Mer, which was defined as typical by Bondroit (1918) and Emery (1925).

The species investigated in this study belong to the *T. caespitum* group (Bolton 1995). As an outgroup species we selected a member of the *T. striolatum* group, the Australian *T. capitale* (McAreavey, 1949).

All samples were mtDNA-sequenced except 7 samples, which were previously sequenced (GenBank accession numbers AY641656, AY641658, AY641664–AY641666, AY641703, AY641712, AY641714; partly from Steiner *et al*. 2005).

DNA was extracted using the Sigma Genelute Extraction (Saint Louis, MO, USA) kit. PCRs were carried out in reaction volumes of 50 μl containing 4 μl template DNA, 1× reaction buffer, 0.2 mM dNTPs, 0.2 μM forward and reverse primers, 2 U Taq DNA polymerase (Sigma, Saint Louis, MO, USA) and H_2Odd. PCR was run in a MJ thermocycler (MJ Research, MD, USA) using a touchdown program under the following conditions: initial step 1 min at 94 °C, followed by 31 cycles of 1 min at 94 °C, 30 sec at varying annealing temperatures (47–55 °C) and 2 min at 72 °C; completed by a final step of 2 min at 72 °C. Primers used for amplification of the 1280 bp long cytochrome oxidase I (COI) gene segment were "L2-N-3014r" alias Pat 5´-tccaatgcactaatctgccatatta-3´ (Simon *et al*. 1994) and "COI1f" 5´-cccccctctattagattattatt-3´ position 2103 in COI sequence of *Apis mellifera* (Crozier & Crozier 1993).

A total of 1035 bp of COI were used for phylogenetic analysis. PCR products were puri-

Table 2. Examined *Tetramorium* samples. Geographic origin: AU = Austria, BU = Bulgaria, EZ = Czech Republic, GM = Germany, HU = Hungary, SP = Spain, UP = Ukraine. Collectors: AB = Alfred Buschinger, AR = A. Radchenko, BS & FS = B.C. Schlick-Steiner & F.M. Steiner, CC = C.A. Collingwood, EL = E.B. Lopatina, MS = M. Sanetra, RS = R. Schultz, TA = T. Aßmuth, TL = T. Ljubomirov, VA = V. Antonova, VK = V.E. Kipyatkov, XC = X. Cerda, XE = X. Espadaler. Accession numbers of COI sequences in GenBank.

Species	Locality	Coordinates	Altitude	Collector
T. caespitum Linnaeus, 1758	AU: Setzberg vic. Spitz an der Donau	48°21´N, 15°23´E	350 m a.s.l.	BS & FS
	AU: Oberweiden vic. Gänserndorf	48°17´N, 16°49´E	160 m a.s.l.	BS & FS
	GM: Babenhausen vic. Darmstadt	49°57´N, 08°57´E	130 m a.s.l.	AB
T. forte Forel, 1904	SP: Albarracin	40°22´N, 01°34´W	1200 m a.s.l.	TA, MS
	SP: Embalse de la Toba vic. Cuenca	39°38´N, 02°10´W	1400 m a.s.l.	TA, MS
	SP: Doñana National Park	37°07´N, 06°29´W	10 m a.s.l.	XC
T. hungaricum Röszler, 1935	AU: Hackelsberg vic. Winden am See	47°57´N, 16°46´E	180 m a.s.l.	BS & FS
	BU: Petritchka, Kotlovina basin, E Roupite village	41°23´N, 23°13´E	60 m a.s.l.	VA
	BU: Plana mountain, S Kokalyane village	42°34´N, 23°25´E	880 m a.s.l.	TL
	HU: Csiki hegyek vic. Budapest	47°30´N, 19°04´E	200 m a.s.l.	BS & FS
T. moravicum Kratochvil, 1941	AU: Hundsheimer Berg vic. Hainburg an der Donau	48°07´N, 16°56´E	400 m a.s.l.	BS & FS
	BU: Plana mountain, S Kokalyane village	42°34´N, 23°25´E	860 m a.s.l.	TL
	EZ: Mohelno	49°07´N, 16°10´E	300 m a.s.l.	MS
	UP: Zalis‘s‘a vic. Simferopol	44°53´N, 34°06´E	300 m a.s.l.	AB, VK, EL, AR, MS
T. semilaeve André, 1883	SP: Sant Cugat	41°28´N, 02°04´E	150 m a.s.l.	XE
	SP: SW Alcantara	39°36´N, 06°58´W	360 m a.s.l.	RS
	SP: Langas, Lerida	41°37´N, 00°37´E	220 m a.s.l.	CC

fied (Qiaquick PCR purification kit: Qiagen, Hilden, Germany), sequenced in both directions using the Big Dye termination reaction chemistry (Applied Biosystems, Foster City, CA, USA) and analysed with an ABI 377 automated sequencer (Applied Biosystems). Sequence alignment was achieved with Clustal X default settings (Thompson *et al*. 1997). To compare the relationships among sequences, both distance (neighbour-joining algorithm, NJ) and maximum parsimony (MP) analysis were performed using the software package PAUP* (test version 4.0b3a; Swofford 1998). Tamura-Nei distance (Tamura & Nei 1993) was used for the NJ trees. MP trees were generated with a heuristic search using the tree bisection reconnection algorithm and a random taxon addition sequence. Bootstrapping (1000 replicates; Felsenstein 1985) was applied. In order to present the variable sites among the analysed *Tetramorium* samples, a consensus sequence was created using a threshold frequency of 60%.

Results

The Bulgarian and Ukrainian *Tetramorium* samples consisted exclusively of workers. They were morphologically determined as *T. hungaricum* (Bulgaria) and *T. moravicum* (Bulgaria, Ukraine). Our morphological determination of *T. hungaricum* is based mainly on its very small overall body size, the reduced sculpture, the relatively great distance between single carinae on the vertex, the usually low carinae, and the frequent lack of carinae on the dorsolateral part of the head posterior to the eyes. The reduction of sculpture leads to a shiny overall impression of the workers. Our determination of *T. moravicum* is based on a large overall body size, frequently blackish colour, very pronounced sculpture, especially well-developed rugulae on the petiolus and postpetiolus, and a hexagonal microsculpture on the first gastral tergum. The rugulae on the lateral head posterior to the eyes form straight lines vanishing arbitrarily near the posterior margin of the head and do not fuse with each other to form arching lines. The combination of morphological inspections with DNA sequencing facilitates determinations at the species level, providing much greater confidence than if only one method were applied.

Partial regions of the mitochondrial COI gene of the sequenced samples are deposited in GenBank under accession numbers AY641669, AY641720–AY641724 and AY641659–AY641662. Within the COI region of all phylogenetically analysed *Tetramorium* species (1035 bp), mutations occured at a total of 151 sites (140 of them informative), 25 of these are located on the first codon position, one on the second, and 125 on the third codon position (Appendix). Maximum intraspecific variation within all species was low: *T. hungaricum* 0.0%, *T. caespitum* 0.2%, *T. semilaeve* 1.2%, *T. forte* 0.9%, *T. moravicum* 0.1%–0.3%. Interspecific sequence divergence within the genus *Tetramorium* varied from 4.3% to 10.8%. *T. hungaricum* and *T. caespitum* are the closest within the set of data, building a clade as *T. forte* did with *T. moravicum*. In the phylogenetic tree (Fig. 1), these two clades are supported by high bootstrap values.

Discussion

The extents of intraspecific and interspecific sequence divergences of the studied *Tetramorium* species are in the same order of magnitude as found within and between *Lasius* species investigated for the same COI fragment (Steiner *et al*. 2004). We conclude that *T. hungaricum* represents a well-supported taxon which can be characterised by both morphological and molecular features. Additionally, DNA sequencing and identification of diagnostic sites (Appendix) could be the next step towards determinations based on restriction fragment length polymorphism (RFLP). This would provide rapid and cheap routine determinations based on specific sets of enzymes. In the analysed stretch of the COI gene, ten diagnostic sites for *T. hungaricum* against *T. semilaeve* emerged. At one of these (position 2766), the restriction enzyme *Nco*I cuts *T. semilaeve* into two fragments of 666 bp and 614 bp length, but does not cut *T. hungaricum* (Appendix). The literature records from Bulgaria pertaining to *T. semilaeve* probably constitute *T. hungaricum*, although some confusion with *T. caespitum*, to which it appears phylogeneti-

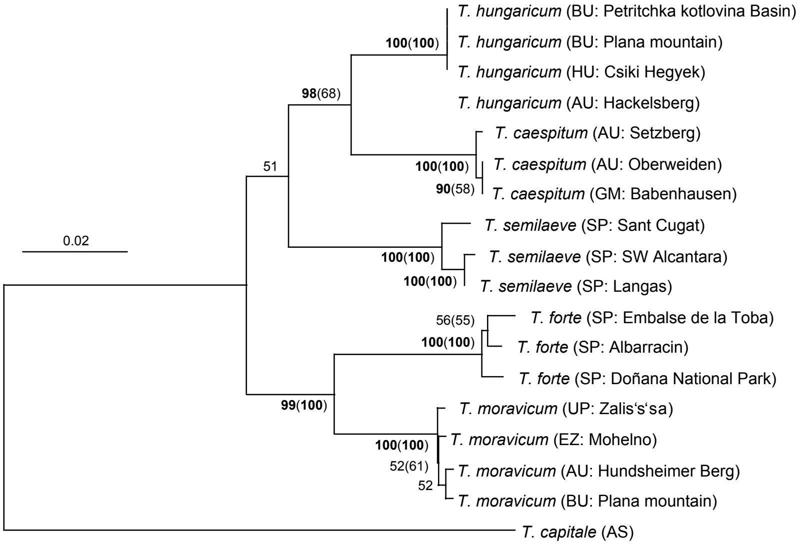

Fig. 1. Phylogenetic tree of *Tetramorium caespitum*, *T. hungaricum*, *T. moravicum*, *T. forte*, *T. semilaeve* and *T. capitale* based on Neighbour Joining calculated with the Tamura-Nei algorithm of 1035 bp of the COI gene. Bootstrap values given at nodes, values > 75 set in boldface. Bootstrap values of the MP branches given in parentheses, except when topology was not congruent and the alternative MP topology was not supported by bootstrap values > 75. Geographic origin: AS = Australia, AU = Austria, BU = Bulgaria, EZ = Czech Republic, GM = Germany, HU = Hungary, SP = Spain, UP = Ukraine.

cally closer (Fig. 1), by the original investigators cannot be ruled out (*see* also López 1991b, Sanetra *et al.* 1999 on the difficulties of reliably distinguishing *T. caespitum* and *T. semilaeve* workers). The phylogenetic proximity of *T. hungaricum* and *T. caespitum* is further corroborated by the large *T. caespitum*-like sexuals of *T. hungaricum*. In practical terms, *T. semilaeve*, listed for Bulgaria by Agosti and Collingwood (1987) and Atanassov and Dlusskij (1992), should be replaced by *T. hungaricum*.

The analysed COI stretch in *T. moravicum* contains thirteen diagnostic sites against *T. forte*, e.g. *Sma*I cuts once in *T. moravicum* but not in *T. forte*. This enables RFLP diagnosis of the two species via two distinguishable DNA fragments (589 bp and 691 bp) in *T. moravicum* (Appendix). Our morphological and genetic evidence re-establishes the occurrence of *T. moravicum* in Bulgaria and confirms its presence in Ukraine (cf. Sanetra & Buschinger 2000). *T. moravicum* was already recorded in Bulgaria by Agosti and Collingwood (1987), but was then excluded from the check-list due to the proposed synonymy with *T. forte* (Atanassov & Dlusskij 1992). Along with other lines of evidence inferred, for instance from allozyme studies (Sanetra *et al.* 1994, Sanetra & Buschinger 2000) and from the morphology of the sexuals (R. Güsten, M. Sanetra & A. Schulz unpubl. data), *T. forte* and *T. moravicum* now clearly turn out to be different species, of which only *T. moravicum* occurs in Eastern Europe (Fig. 2). Literature records of *T. forte* from Bulgaria (Agosti & Collingwood 1987, Atanassov & Dlusskij 1992) and Ukraine (Forel 1904, Arnol’di 1968, Radchenko 1984, the latter two records as *T. taurocaucasicum* Arnol’di, 1968) thus should be corrected to *T. moravicum*.

Based on DNA sequencing it was possible to establish the distribution pattern of several *Tetramorium* species. *T. forte* and *T. moravicum* appear allopatric in origin. While *T. forte* shows an atlanto-mediterranean distribution (confined to southern France, Spain and Morocco), the origin of *T. moravicum* probably lies in the ponto-mediterranean-caucasic refugium (R. Güsten *et al.* unpubl. data). *T. semilaeve*, though morphologically very similar to *T. hungaricum*, has been shown to be confined to areas surrounding the Mediterranean Sea. In stark contrast,

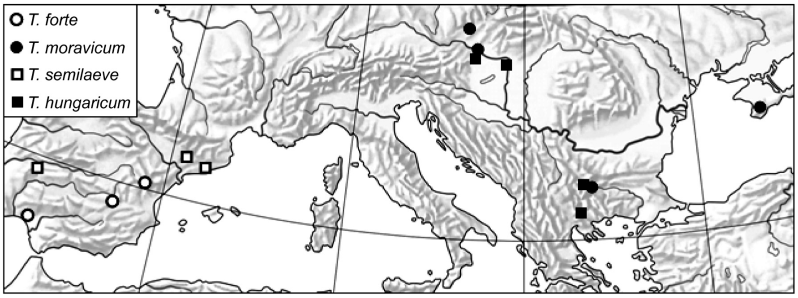

Fig. 2. Partial map of the Western Palearctic Region indicating geographic locations of the sampled populations of *Tetramorium forte* (white circles), *T. moravicum* (black circles), *T. semilaeve* (white squares) and *T. hungaricum* (black squares).

T. hungaricum occurs in the pannonic–balcanic region (ranging from Austria south-eastwards). Hence, biogeographically the two taxa appear well separated (Fig. 2), and records from outside these areas may be considered doubtful. We recommend reinvestigating the records of *T. semilaeve* and *T. forte* from other Eastern European countries such as Hungary (Gallé *et al*. 1998), Romania (Paraschivescu 1978) and Ukraine (Forel 1904, Arnol'di 1968, Apostolov & Likhovidov 1973, Radchenko 1984) with both morphological and molecular methods.

Our DNA-supported biogeographic investigations are a first step towards trustworthy distribution patterns of palearctic *Tetramorium* species. In several other ant genera (*Bothriomyrmex*, *Camponotus*, *Formica*, *Lasius*, *Temnothorax*, *Myrmica*, *Ponera*) the situation remains equally unsatisfactory. The identification of ant species is problematic: Even in the Central European fauna, which is the best studied in the world (Seifert 1999), an estimated 10% to 25% of the total 167 species (Seifert 1996, Seifert 2000, Csösz & Seifert 2003, Schlick-Steiner *et al*. 2003a, 2003b) are occasionally misidentified (Seifert 1996, Schlick-Steiner *et al*. 2003a, B. Seifert pers. comm.). Their biogeographic patterns thus remain blurred.

DNA-aided species identification has become a routine procedure in various disciplines, including diagnostic medicine (e.g. Bettelheim & Beutin 2003, Drobniewski *et al*. 2003), epidemiology (Van Bortel *et al*. 2000, Wells & Sperling 2001), forensic science (e.g. Wilson *et al*. 2003), agriculture (e.g. Bernet *et al*. 2003) and bioengineering (e.g. Kirchner & Tauch 2003). For biogeography the importance of morphological investigations remains unquestioned (Seifert 1999, Van Bortel *et al*. 2000, Seifert 2002, Will & Rubinoff 2004), but a combination with molecular methods would be broadly applicable to all groups of organisms in which morphologically difficult species are abundant, e.g. trematodes (Ryu *et al*. 2000), bivalves (Therriault *et al*. 2004), termites (Clément *et al*. 2001), beetles (Page *et al*. 1997), parasitic wasps (Pinto *et al*. 1997), toads (García-París *et al*. 2003), and also plants (Blomster *et al*. 2000). In our opinion, molecular techniques may prove to be just one more instrument in the toolbox, yet a powerful one.

Acknowledgements

For funding we wish to thank Boku, University of Natural Resources & Applied Life Sciences, Vienna, and the Austrian Federal Ministry for Education, Science & Culture. We are greatly indebted to Dr. Christo Deltchev for help during field work, to Tom Aßmuth, Dr. Alfred Buschinger, Dr. Xim Cerda, Dr. Cedric A. Collingwood, Dr. Xavier Espadaler, Dr. Brian Heterick and Dr. Roland Schultz for providing samples, to Dr. Robert Güsten and Andreas Schulz for sharing unpublished information, to Balint Markó for bibliographic support, to Dr. John Plant for linguistic improvements, and to two anonymous referees.

References

Agosti, D. 2003: The last of its kind? — *Nature* 424: 727.

Agosti, D. & Collingwood, C. A. 1987: A provisional list of the Balkan ants (Hym., Formicidae) and a key to the worker caste. I. Synonymic list. — *Mitt. Schweiz. Entomol. Ges*. 60: 51–62.

André, E. 1883: *Species des Hyménoptères d'Europe et d'Algérie,* vol. 2. — Fr. Bouffaut Impr., Beaune.

Apostolov, L. G. & Likhovidov, V. E. [Апостолов, Л. Г. & Лиховидов, В. Е.] 1973. [Contribution to the fauna and ecology of ants (Hymenoptera, Formicidae) from southeastern Ukraine]. — *Vestn. Zool*. 1973: 60–66. [In Russian].

Arnol'di, K. V. [Арнольди, К. В.] 1968: [Important additions to the myrmecofauna of the USSR, and descriptions of new forms]. — *Zool. Zh.* 47: 1800–1822. [In Russian].

Atanassov, N. & Dlusskij, G. M. 1992: Hymenoptera, Formicidae. — *Fauna Bulgarica* 22: 1–310.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. & Saunders, N. C. 1987: Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. — *Ann. Rev. Ecol. Syst.* 18: 489–522.

Bernard, F. 1968: Les Fourmis (Hymenoptera, Formicidae) d'Europe occidentale et septentrionale. — *Faune de l'Europe et du Bassin Méditerranéen* 3: 1–441.

Bernet, G. P., Bramardi, S., Calvache, D., Carbonell, E. A. & Asins, M. J. 2003: Applicability of molecular markers in the context of protection of new varieties of cucumber. — *Plant Breeding* 122: 146–152.

Bettelheim, K. A. & Beutin, L. 2003: Rapid laboratory identification and characterization of verocytotoxigenic (Shiga toxin producing) *Escherichia coli* (VTEC/STEC). — *J. Appl. Microbiol.* 95: 205–217.

Blaxter, M. 2003: Counting angels with DNA. — *Nature* 421: 122–124.

Blaxter, M. & Floyd, R. 2003: Molecular taxonomics for biodiversity surveys: already a reality. — *TREE* 18: 268–269.

Blomster, J., Hoey, E. M., Maggs, C. A. & Stanhope, M. J. 2000: Species-specific oligonucleotide probes for macroalgae: molecular discrimination of two marine fouling species of *Enteromorpha* (Ulvophyceae). — *Mol. Ecol.* 9: 177–186.

Bolton, B. 1977: The ant tribe Tetramoriini (Hymenoptera: Formicidae). The genus *Tetramorium* Mayr in the Oriental and Indo-Australian Regions, and in Australia. — *Bull. Br. Mus. nat. Hist. (Ent.)* 36: 67–151.

Bolton, B. 1995: *A new general catalogue of the ants of the world*. — University Press, Cambridge, Harvard.

Bondroit, J. 1918: Les Fourmis de France et de Belgique. — *Ann. Soc. Entomol. Fr.* 87: 1–174.

Cagniant, H. 1997: Le genre *Tetramorium* au Maroc (Hymenoptera: Formicidae): cle et catalogue des especes. — *Ann. Soc. Entomol. Fr. (n.s.)* 33: 89–100.

Clément, J.-L., Bagnéres, A.-G., Uva, P., Wilfert, L., Quintana, A., Reinhardt, J. & Dronnet, S. 2001: Biosystematics of *Reticulitermes* termites in Europe: morphological, chemical and molecular data. — *Insectes soc.* 48: 202–215.

Crozier, R. H. & Crozier, Y. C. 1993: The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. — *Genetics* 133: 97–117.

Csösz, S., Markó, B., Kiss, K., Tartally, A. & Gallé, L. 2002: The ant fauna of the Fertö-Hansag national park (Hymenoptera: Formicoidea). — *The fauna of the Fertö-Hansag National Park* 2002: 617–629.

Csösz, S. & Seifert, B. 2003: *Ponera testacea* Emery, 1895 stat.n. — a sister species of *P. coarctata* (Latreille, 1802) (Hymenoptera, Formicidae). — *Acta Zool. Acad. Sci. Hung.* 49: 201–214.

Dlusskij, G. M., Soyunov, O. S. & Zabelin, S. I. 1990: *Ants of Turkmenistan*. — Ylym Press, Ashkhabad.

Drobniewski, F. A., Caws, M., Gibson, A. & Young, D. 2003: Modern laboratory diagnosis of tuberculosis. — *The Lancet Infectious Diseases* 3: 141–147.

Dunn, C. P. 2003: Keeping taxonomy based in morphology. — *TREE* 18: 270–271.

Emery, C. 1925: Notes critiques de Myrmécologie. XI. *Tetramorium caespitum* (L.) — *Ann. Soc. Entomol. Belg.* 64: 177–191.

Felsenstein, J. 1985: Confidence limits on phylogenies: An approach using the bootstrap. — *Evolution* 39: 783–791.

Forel, A. 1904: Note sur les fourmis du Musée Zoologique de l'Academie des Sciences à St. Petersbourg. — *Ezhegodnik Zoologicheskago Muzeya Imperatorskoi Akademii Nauk* 8: 368–389.

Franz, H. 1975: *Die Bodenfauna der Erde in biozönotischer Betrachtung*, vol. I. — Franz Steiner Verlag GmbH, Wiesbaden.

Gallé, L., Csösz, S., Tartally, A. & Kovács, É. 1998: A check-list of Hungarian ants. — *Fol. entomol. hung.* 59: 213–220.

García-París, M., Buchholz, D. R. & Parra-Olea, G. 2003: Phylogenetic relationships of Pelobatoidea re-examined using mtDNA. — *Mol. Phylogen. Evol.* 28: 12–23.

Hebert, P. D. N., Cywinska, A., Ball, S. L. & deWaard, J. R. 2003a: Biological identifications through DNA barcodes. — *Proc. R. Soc. Lond. B* 270: 313–322.

Hebert, P. D. N., Ratnasingham, S. & deWaard, J. R. 2003b: Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. — *Proc. R. Soc. Lond. B* (Suppl.) 270: S96–S99.

Janzen, D. H. 2004: Now is the time. — *Phil. Trans. R. Soc. Lond. B* 359: 731–732.

Kirchner, O. & Tauch, A. 2003: Tools for genetic engineering in the amino acid-producing bacterium *Corynebacterium glutamicum*. — *J. Biotechnol.* 104: 287–299.

Kratochvil, J., Novák, V. & Snoflak, J. 1944: Mohelno. 5. Hymenoptera–Aculeata. Formicidae–Apidae–Vespoidea. — *Archiv des Verbandes für Natur- und Heimatschutz in Mähren* 6: 1–132.

Lipscomb, D., Platnick, N. & Wheeler, Q. 2003: The intellectual content of taxonomy: a comment on DNA taxonomy. — *TREE* 18: 65–66.

López, F. 1991a: Estudio morfológico y taxonómico de los grupos de especies ibéricas del género *Tetramorium* Mayr, 1855 (Hym.: Formicidae). — *Boln. Asoc. esp. Ent.* 15: 29–52.

López, F. 1991b: Variabilidad morfológica y problemas taxonómicos en *Tetramorium caespitum* (Linné, 1758) y *Tetramorium semilaeve* André, 1881 (Hym.: Formicidae). — *Boln. Asoc. esp. Ent.* 15: 65–78.

Markó B. & Csösz, S. 2002: Die europäischen Ameisenarten (Hymenoptera: Formicidae) des Hermannstädter (Sibiu, Rumänien) Naturkundemuseums I.: Unterfamilien Ponerinae, Myrmicinae und Dolichoderinae. — *Ann. Hist. Natur. Mus. Nat.* 94: 109–121.

Mason, B. 2003: Marine survey sees net gain in number of fish species. — *Nature* 425: 889.

Novák, V. & Sadil, J. 1941: Klíc k urcování mravencu strední Evropy se zvlástním zretelem k mravencí zvírene Cech a Moravy. — *Entomologické Listy* 4: 65–115.

Page, M., Nelson, L. J., Blomquist, G. J. & Seybold, S. J. 1997: Cuticular hydrocarbons as chemotaxonomic characters of pine engraver beetles (*Ips* spp.) in the *grandicollis* subgeneric group. — *J. Chem. Ecol.* 23: 1053–1099.

Paraschivescu, D. 1978: Elemente balcanice in mirmecofauna R.S. Romania. — *Nymphaea* 6: 463–474.

Pennisi, E. 2003: Modernizing the tree of life. — *Science* 300: 1692–1697.

Pinto, J. D., Stouthamer, R. & Platner, G. R. 1997: A new cryptic species of *Trichogramma* (Hymenoptera: Trichogrammatidae) from the Mojave desert in California as determined by morphological, reproductive and molecular data. — *Proc. Entomol. Soc. Wash.* 99: 238–247.

Radchenko, A. G. 1984: Ecologo-faunistiskaia karakteristika muraviev (Hymenoptera, Formicidae) Tchernomoskovo zapoviednika i prilegaiuschik territorii. — *Vestn. Zool.* 2: 20–23.

Radchenko, A. G. 1992: Ants of the genus *Tetramorium* (Hymenoptera, Formicidae) in the fauna of the USSR. Part 2. — *Zool. Zh.* 71: 50–58. [In Russian with English summary].

Röszler, P. 1935: Beiträge zur Kenntnis der Ameisenfauna von Siebenbürgen und Ungarn. — *Verhandlungen und Mitteilungen des Siebenbürgischen Vereins für Naturwissenschaften zu Hermannstadt* 83–84: 72–83.

Röszler, P. 1951: Myrmecologisches aus dem Jahre 1938. — *Zool. Anz.* 146: 88–96.

Ryu, J.S., Hwang, U. W., Min, D. Y., Shin, K. S., Nam, S. J. & Lee, O. R. 2000: Molecular identification of *Paragonimus ohirai* and *P. westermani* from Anhui Province, China. — *Parasite* 7: 305–309.

Sanetra, M. 1996: *Systematik, Biologie und Phylogenie westpaläarktischer Ameisen der Tribus Tetramoriini unter besonderer Berücksichtigung molekularer Merkmale (Hymenoptera: Formicidae).* — Ph.D. thesis, Technische Hochschule Darmstadt, Darmstadt.

Sanetra, M. & Buschinger, A. 2000: Phylogenetic relationships among social parasites and their hosts in the ant tribe Tetramoriini (Hymenoptera: Formicidae). — *Eur. J. Entomol.* 97: 95–117.

Sanetra, M., Güsten, R. & Schulz, A. 1999: On the taxonomy and distribution of Italian *Tetramorium* species and their social parasites. — *Mem. Soc. Entomol. Ital.* 77: 317–357.

Sanetra, M., Heinze, J. & Buschinger, A. 1994: Enzyme polymorphism in the ant genus *Tetramorium* Mayr and its social parasites (Hymenoptera: Formicidae). — *Bioch. Syst. Ecol.* 22: 753–759.

Schlick-Steiner, B. C., Steiner, F. M. & Schödl, S. 2003a. A case study to quantify the value of voucher specimens for invertebrate conservation: ant records in Lower Austria. — *Biodiv. Cons.* 12: 2321–2328.

Schlick-Steiner, B. C., Steiner, F. M., Schödl, S. & Seifert, B. 2003b: *Lasius austriacus* sp.n., a Central European ant related to the invasive species *Lasius neglectus*. — *Sociobiology* 41: 725–736.

Schulz, A. 1996: *Tetramorium rhenanum* nov. spec. vom Mittleren Rheintal in Deutschland (Hymenoptera: Formicidae). — *Linz. Biol. Beitr.* 28: 391–412.

Seberg, O., Humphries, C. J., Knapp, S., Stevenson, D. W., Petersen, G., Scharff, N. & Andersen, N. M. 2003: Shortcuts in systematics? A commentary on DNA-based taxonomy. — *TREE* 18: 63–65.

Seifert, B. 1996: *Ameisen: beobachten, bestimmen.* — Naturbuch Verlag, Augsburg.

Seifert, B. 1999: Interspecific hybridisations in natural populations of ants by example of a regional fauna (Hymenoptera, Formicidae). — *Insectes soc.* 46: 45–52.

Seifert, B. 2000: A taxonomic revision of the ant subgenus *Coptoformica* Mueller, 1923 (Hymenoptera, Formicidae). — *Zoosystema* 22: 517–568.

Seifert, B. 2002: How to distinguish most similar insect species — improving the stereomicroscopic and mathematical evaluation of external characters by example of ants. — *J. Appl. Entomol.* 126: 1–9.

Seifert, B. 2003: The ant genus *Cardiocondyla* (Insecta: Hymenoptera: Formicidae) — a taxonomic revision of the *C. elegans*, *C. bulgarica*, *C. batesii*, *C. nuda*, *C. shuckardi*, *C. stambuloffi*, *C. wroughtonii*, *C. emeryi*, and *C. minutior* species groups. — *Ann. Nat. Mus. Wien* 104B: 203–338.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994: Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. — *Ann. Entomol. Soc. Am.* 87: 651–701.

Sperling, F. 2003: *DNA barcoding: Deus ex machina.* — Newsletter of the Biological Survey of Canada (Terrestrial Arthropods) 22(1), available on the web at http://www.biology.ualberta.ca/bsc/news22_2/contents.htm.

Steiner, F. M., Schlick-Steiner, B. C. & Buschinger, A. 2003: First record of unicolonial polygyny in *Tetramorium cf. caespitum* (Hymenoptera, Formicidae). — *Insectes soc.* 50: 98–99.

Steiner, F. M., Schlick-Steiner, B. C., Nikiforov, A., Kalb, R. & Mistrik, R. 2002: Cuticular hydrocarbons of *Tetramorium* ants from Central Europe: Analysis of GC-MS data with Self-Organizing Maps (SOM) and implications for systematics. — *J. Chem. Ecol.* 28: 2569–2584.

Steiner, F. M., Schlick-Steiner, B. C., Schödl, S., Espadaler, X., Seifert, B., Christian, E. & Stauffer, C. 2004: Phylogeny and bionomics of *Lasius austriacus* (Hymenoptera, Formicidae). — *Insectes soc.* 51: 24–29.

Steiner, F. M., Schlick-Steiner, B. C., Trager, J. C., Moder, K., Sanetra, M., Christian, E. & Stauffer, C. 2005: *Tetramorium tsushimae*, a new invasive ant in North America. — *Biol. Inv.* [In press].

Stoeckle, M. 2003: Taxonomy, DNA, and the bar code of life. — *BioScience* 53: 2–3.

Swofford, D. L. 1998: *PAUP*: Phylogenetic analysis using parsimony (*and other methods),* ver. 4.0b3. — Sinauer, Sunderland, Massachusetts.

Tamura, K. & Nei, M. 1993: Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. — *Mol. Biol. Evol.* 10: 512–526.

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H. & Vogler, A. P. 2003: A plea for DNA taxonomy. — *TREE* 18: 70–74.

Therriault, T. W., Docker, M. F., Orlova, M. I., Heath, D. D. & MacIsaac, H. J. 2004: Molecular resolution of the family Dreissenidae (Mollusca: Bivalvia) with emphasis on Ponto-Caspian species, including first report of *Mytilopsis leucophaeata* in the Black Sea basin. — *Mol. Phylogen. Evol.* 30: 479–489.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997: The Clustal-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. — *Nucl. Acids Res.* 25: 4876–4882.

Van Bortel, W., Trung, H. D., Roelants, P., Harbach, R. E., Backeljau, T. & Coosemans, M. 2000: Molecular identification of *Anopheles minimus* s.l. beyond distinguishing the members of the species complex. — *Insect Mol. Biol.* 9: 335–340.

Wells, J. D. & Sperling, F. A. H. 2001: DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). — *For. Sci. Int.* 120: 110–115.

Westheide, W. & Hass-Cordes, E. 2001: Molecular taxonomy: description of a cryptic *Petitia* species (Polychaeta: Syllidae) from the island of Maheacute; (Seychelles, Indian Ocean) using RAPD markers and ITS2 sequences. — *J. Zool. Syst. Evol. Res.* 39: 103–111.

Wetterer, J. K., Schultz, T. R. & Meier, R. 1998: Phylogeny of fungus-growing ants (Tribe Attini) based on mtDNA sequence and morphology. — *Mol. Phyl. Evol.* 9: 42–47.

Wiens, J. J. & Reeder, T. W. 1997: Phylogeny of the spiny lizards (*Sceloporus*) based on molecular and morphological evidence. — *Herpetol. Monogr.* 11: 1–101.

Will, K. W. & Rubinoff, D. 2004: Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. — *Cladistics* 20: 47–55.

Wilson, I. J., Weale, M. E. & Balding, D. J. 2003: Inferences from DNA data: population histories, evolutionary processes and forensic match probabilities. — *J. Roy. Stat. Soc. A* 166: 155–188.

Zhang, D. & Hewitt, G. M. 2003: Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. — *Mol. Ecol.* 12: 563–584.

Appendix. Variable sites of COI sequences of the 16 samples of *Tetramorium hungaricum*, *T. semilaeve*, *T. moravicum*, *T. forte*, and *T. caespitum* with equivalent COI sequence positions in *Apis mellifera* (Crozier & Crozier 1993). Codon positions (1–3) are indicated below. Diagnostic sites for *T. hungaricum* against *T. semilaeve* and *T. moravicum* against *T. forte*, respectively, are set in boldface. • Recognition site of restriction enzyme *Sma*I, * Recognition site of restriction enzyme *Nco*I.

		Nucleotide position																									
Apis mellifera		2175	2178	2181	2182	2193	2194	2196	2202	2205	2208	2209	2211	2221	2244	2247	2250	2262	2268	2283	2298	2301	2304	2314	2319*	2331	2334
Consensus			C		T		A	C	C	A			A		T		A	T	T	T	T	A		T	C	C	C
T. hungaricum	BU: Petritchka kotlovina basin	C		C		T					G	G		C	**C**	C							C		**T**	**T**	
	BU: Plana mountain	C		C		T					G	G		C	**C**	C							C		**T**	**T**	
	HU: Csiki hegyek	C		C		T					G	G		C	**C**	C							C		**T**	**T**	
	AU: Hackelsberg	C		C		T					G	G		C	**C**	C							C		**T**	**T**	
T. caespitum	AU: Setzberg	C		A		T	G				A	G		C		C			A		C		C				
	AU: Oberweiden	C		A		T	G				A	G		C		C			A		C		C				
	GM: Babenhausen	C		A		T	G				A	G		C		C			A		C		C				
T. semilaeve	SP: Sant Cugat	T	G	C	C	C		T			G	G		C		A	T			C			T				A
	SP: Alcantara	T	A	C	C	C		T			A	G		C		A	T		C	C			T				A
	SP: Langas	T	A	C	C	C		T			A	G		C		A	T			C			T				A
T. forte	SP: Embalse de la Toba	T		A		T			T		C	C		T		A		C		C		G	T	C			
	SP: Albarracin	T		A		T			T		C	C		T		A		C		C		G	T	C			
	SP: Doñana National Park	T		A		T			T		C	C		T		A		C		C			T	C			
T. moravicum	EZ: Mohelno	T		A		C				G	**T**	C	**G**	T		A							T				
	AU: Hundsheimer Berg	T		A		C				G	**T**	C	**G**	T		A							T				
	BU: Plana mountain	T		A		C				G	**T**	C	**G**	T		A							T				
	UP: Crimea	T		A		C					**T**	C	**G**	T		A							T				
Codon position		3	3	3	1	3	1	3	3	3	3	1	3	1	3	3	3	3	3	3	3	3	3	1	3	3	3

		Nucleotide position																									
Apis mellifera		2349	2358	2365	2368	2370	2371	2373	2376	2377	2379	2382	2385	2388	2391	2392	2397	2400	2409	2415	2416	2451	2457	2460	2463	2466	2478
Consensus		T	T	A	C				T		A			T				A	T		T		A	T		G	A
T. hungaricum	BU: Petritchka kotlovina basin					A	T	A		T		T	C		T	T	C			T		T			**T**		
	BU: Plana mountain					A	T	A		T		T	C		T	T	C			T		T			**T**		
	HU: Csiki hegyek					A	T	A		T		T	C		T	T	C			T		T			**T**		
	AU: Hackelsberg					A	T	A		T		T	C		T	T	C			T		T			**T**		
T. caespitum	AU: Setzberg		C		T	A	T	A		T		T	T		A	T	A	G	C	T	C	T	G	G	G		G
	AU: Oberweiden		C		T	A	T	A		T		T	T		A	T	A	G	C	T	C	T	G	A	A		G
	GM: Babenhausen		C		T	A	T	A		T		T	T		A	T	A	G	C	T	C	T	G	A	A		G
T. semilaeve	SP: Sant Cugat				T	A	T	A	C	C	T	T	T		T	T	C			T	C	T		A	A	A	
	SP: Alcantara				T	A	T	A	C	C	T	T	T		T	T	C			T	C	T	G	A	A		
	SP: Langas				T	A	T	A	C	C	T	T	T		T	T	C			T	C	T	G	A	A		
T. forte	SP: Embalse de la Toba			G		C	C	T		C		C	C	G	A	C	T			A		C			G	A	
	SP: Albarracin					C	C	T		C		C	C		A	C	T			A		C			G		
	SP: Doñana National Park			G		C	C	T		C		C	C		A	C	T			A		C			G	A	
T. moravicum	EZ: Mohelno	**A**				**T**	C	T		C		C	T		A	C	A			A		C			G		
	AU: Hundsheimer Berg	**A**				**T**	C	T		C		C	T		A	C	A			A		C			G		
	BU: Plana mountain	**A**				**T**	C	T		C		C	T		A	C	A			A		C			G		
	UP: Crimea	**A**				**T**	C	T		C		C	T		A	C	A			A		C			G		
Codon position		3	3	1	1	3	1	3	3	1	3	3	3	3	3	1	3	3	3	3	1	3	3	3	3	3	3

Continued

Appendix. Continued.

		Nucleotide position																									
Apis mellifera		2481	2487	2493	2505	2508	2529	2530	2532	2535	2544	2553	2559	2581	2592	2598	2601	2616	2631	2640	2652	2655	2676	2688	2691•	2694	2706
Consensus		T	T		A		T	T	A	T	T		T	A		A		C		A	A			T	T		C
T. hungaricum	BU: Petritchka kotlovina basin			T	G	C						T			C	**G**	C		C		G	C	T			G	
	BU: Plana mountain			T	G	C						T			C	**G**	C		C		G	C	T			G	
	HU: Csiki hegyek			T	G	C						T			C	**G**	C		C		G	C	T			G	
	AU: Hackelsberg			T	G	C						T			C	**G**	C		C		G	C	T			G	
T. caespitum	AU: Setzberg			T		C						T			C		C		C			C	T			A	
	AU: Oberweiden			T		C						T			C		C		C			C	T			A	
	GM: Babenhausen			T		C						T			C		C		C			C	T			A	
T. semilaeve	SP: Sant Cugat		C	T	G	C	C	C			A	T			T		C		T			T	T			A	
	SP: Alcantara		C	T		C	C	C			A	T		G	T		C		T		G	T	T			A	
	SP: Langas		C	T		C	C	C			A	T			T		C		T		G	T	T			A	
T. forte	SP: Embalse de la Toba	C		C		T			G		C	C	C		T		T	T	A			T	A			A	
	SP: Albarracin	C		C		T				C	C	C	C		T		T	T	A	G		T	A			A	
	SP: Doñana National Park	C		C		T					C	C	C		T		T	T	A			T	A	C		A	
T. moravicum	EZ: Mohelno			C		T						C			T		T		A			T	A	C	**C**	G	**T**
	AU: Hundsheimer Berg			C		T						C			T		T	T	A			T	A	C	**C**	G	**T**
	BU: Plana mountain			C		T						C			T		T	T	A			T	A	C	**C**	G	**T**
	UP: Crimea			C		T						C			T		T	T	A			T	A	C	**C**	G	**T**
Codon position		3	3	3	3	3	3	1	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3

		Nucleotide position																									
Apis mellifera		2715	2733	2754	2766	2769	2784	2796	2800	2808	2813	2817	2820	2823	2826	2827	2835	2841	2847	2860	2865	2868	2874	2880	2883	2892	2895
Consensus		A		A	T	T	T	C	T	A	A	A		T		T			A		T	C		T	T	T	T
T. hungaricum	BU: Petritchka kotlovina basin		T	**G**									C		T		T	**T**		T			C				
	BU: Plana mountain		T	**G**									C		T		T	**T**		T			C				
	HU: Csiki hegyek		T	**G**									C		T		T	**T**		T			C				
	AU: Hackelsberg		T	**G**									C		T		T	**T**		T			C				
T. caespitum	AU: Setzberg	G	T										T		T		C	C		C			C		C		C
	AU: Oberweiden	G	T										T		T		C	C		C			C		C		C
	GM: Babenhausen	G	T										T		T		C	C		C			C		C		C
T. semilaeve	SP: Sant Cugat		T			C	C	A	C			C	C	A	C		C	A	G	T	C	T	C				
	SP: Alcantara		T			C	C	A				C	C	A	T		C	A	G	T	C	T	C				
	SP: Langas		T			C	C	A				C	C	A	T		C	A	G	T	C	T	C				
T. forte	SP: Embalse de la Toba		C		C			T		G		C	C	C	C	C	C	G		C			T	C		C	
	SP: Albarracin		C		C			T		G		C	C	C	C		C	A		C			T	C		C	
	SP: Doñana National Park		C		C			T		G		C	C	C	C		C	A		C			T	C		C	
T. moravicum	EZ: Mohelno		C										T		C		T	G		C			T				
	AU: Hundsheimer Berg		C										T		C		T	G		C			T				
	BU: Plana mountain		C										T		C		T	G		C			T				
	UP: Crimea		C								G		T		C		T	G		C			T				
Codon position		3	3	3	3	3	3	3	1	3	2	3	3	3	3	1	3	3	3	1	3	3	3	3	3	3	3

		Nucleotide position																									
Apis mellifera		2898	2910	2916	2919	2922	2929	2934	2937	2940	2943	2952	2962	2979	2982	2983	2988	3000	3009	3022	3030	3031	3036	3054	3069	3085	3090
Consensus		A	T	C		T			A	A		A	A			T	A	A				T	T	T	C	T	
T. hungaricum	BU: Petritchka kotlovina basin				T		C	A			**G**			C	T			**G**	C	A	C						G
	BU: Plana mountain				T		C	A			**G**			C	T			**G**	C	A	C						G
	HU: Csiki hegyek				T		C	A			**G**			C	T			**G**	C	A	C						G
	AU: Hackelsberg				T		C	A			**G**			C	T			**G**	C	A	C						G
T. caespitum	AU: Setzberg				T		C	A			A			T	C	C			C	A	C				T		A
	AU: Oberweiden				T		C	A			A			T	C	C			C	A	C				T		A
	GM: Babenhausen				T		C	A			A			T	C	C			C	A	C				T		A
T. semilaeve	SP: Sant Cugat			T	C		C	A			A		G	C	C		T		C	A	C		C			C	G
	SP: Alcantara			T	C		C	A			A		G	C	C		T		C	A	C		C			C	G
	SP: Langas			T	C		C	A			A		G	C	C		T		C	A	C		C			C	G
T. forte	SP: Embalse de la Toba	G	C		T	C	T	T			T			T	C				A	G	T				A		A
	SP: Albarracin	G	C		T	C	T	T			T			T	C				A	G	T				A		A
	SP: Doñana National Park	G	C		T	C	T	T	G	G	T			T	C				A	G	T				A		A
T. moravicum	EZ: Mohelno				C		T	T			**C**	**G**		T	T				A	G	T	**C**		**C**			A
	AU: Hundsheimer Berg				C		T	T			**C**	**G**		T	T				A	G	T	**C**		**C**			A
	BU: Plana mountain				C		T	T			**C**	**G**		T	T				A	G	T	**C**		**C**			A
	UP: Crimea				C		T	T			**C**	**G**		T	T				A	G	T	**C**		**C**			A
Codon position		3	3	3	3	3	1	3	3	3	3	3	1	3	3	1	3	3	3	1	3	1	3	3	3	1	3

		Nucleotide position																				
Apis mellifera		3099	3108	3117	3120	3124	3126	3129	3135	3141	3147	3156	3159	3171	3180	3181	3183	3186	3195	3199	3201	3204
Consensus		A	C	T			T	T		T	T	T	C		T	A	T	A	A			T
T. hungaricum	BU: Petritchka kotlovina basin				T	A			T					C					**G**	T	C	
	BU: Plana mountain				T	A			T					C					**G**	T	C	
	HU: Csiki hegyek				T	A			T					C					**G**	T	C	
	AU: Hackelsberg				T	A			T					C					**G**	T	C	
T. caespitum	AU: Setzberg		T		T	A			T					C	A					T	T	
	AU: Oberweiden		T		T	A			T					C	A					T	T	
	GM: Babenhausen		T		T	A			T					C	A					T	T	
T. semilaeve	SP: Sant Cugat	G			T	A			C	C	C		A	T		G		T		T	C	
	SP: Alcantara				T	A			C	C	C	C	A	T		G		T		T	C	
	SP: Langas				T	A			C	C	C	C	A	T		G		T		T	C	
T. forte	SP: Embalse de la Toba		T		C	T		C	A				A	C						G	T	C
	SP: Albarracin		T		C	T		C	A				A	C						G	T	C
	SP: Doñana National Park				C	T		C	A				A	C						G	T	C
T. moravicum	EZ: Mohelno			**C**	**A**	T	**C**		**G**					T			**C**			G	T	
	AU: Hundsheimer Berg			**C**	**A**	T	**C**		**G**					T			**C**			G	T	
	BU: Plana mountain			**C**	**A**	T	**C**		**G**					T			**C**			G	T	
	UP: Crimea			**C**	**A**	T	**C**		**G**					T			**C**			G	T	
Codon position		3	3	3	3	1	3	3	3	3	3	3	3	3	3	1	3	3	3	1	3	3