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Phylogenetic relationships among social parasites and their hosts in the ant tribe Tetramoriini (Hymenoptera: Formicidae)

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Abstract. The phylogenetic relationships among Palaearctic species of the ant genus *Tetramorium* and its social parasites of the genera *Strongylognathus*, *Anergates* and *Teleutomyrmex*, were investigated electrophoretically at 21 presumptive enzyme loci. The data set comprising 33 species was analysed with distance (UPGMA, Neighbor-joining and least squares statistics) and parsimony methods (independent allele, minimum turnover and mutation coding) in order to rule out analysis-dependent effects. Several groupings were consistently resolved by all procedures. Observed branching patterns support the placement of the three parasite genera and their hosts into the Palaearctic species group of *Tetramorium* (tribe Tetramoriini). The genus *Strongylognathus* forms a monophyletic group in which the slave-makers of the *S. huberi* group constitute the sister group of the inquilines *S. testaceus* and *S. karawajewi* (*S. testaceus* group). Most species of the *S. huberi* group show very low genetic differentiation. However, little consensus has been found with regard to which *Tetramorium* species are the closest relatives of *Strongylognathus*.

According to the electrophoretic data, social parasitism in Palaearctic tetramoriine ants has evolved independently at least twice. Though inquilinism once arose from slave-making ancestors in *Strongylognathus*, the extreme inquilines *Anergates atratulus* and *Teleutomyrmex schneideri* are clearly set apart from the *Strongylognathus* clade in phylogenetic analyses. Thus, extreme inquilinism cannot be regarded as the endpoint of a single parasitic lineage in the Tetramoriini. In these highly advanced inquilines, evolutionary rates at allozyme loci appear to be higher than those of their *Tetramorium* hosts. The results do not unambiguously reveal whether *Anergates* and *Teleutomyrmex* arose jointly or independently from *Tetramorium* ancestors. However, a combined analysis using all available evidence supports the former hypothesis. The finding that the *Tetramorium* parasites are not the closest relatives of their respective host species is discussed in relation to current theories for the evolution of social parasitism.

INTRODUCTION

Social parasites of the widespread ant genus *Tetramorium* are predominantly Palaearctic in distribution (Bolton, 1976), the only exception being two inquilines known from the Afrotropical zoogeographical region (Bolton, 1980). In the Palaearctis, species of the three genera of social parasites, *Strongylognathus*, *Anergates* and *Teleutomyrmex*, display a wide array of differing parasitic traits. Dulotic or slave-making ants kill or drive off the resident queen(s) and later rely on raiding neighbouring host colonies for their supply of workers. Inquilines are usually workerless parasites (but some have retained a reduced worker caste) whose queens either coexist with the host queen or become the only reproductives within the nest. The latter type of parasitism is here referred to as queen-intolerant inquilinism, but was termed pseudoinquilinism by Douwes (1990) and excluded from inquilinism by Bourke & Franks (1991).

All of the approximately 25 currently recognized species of the genus *Strongylognathus* bear remarkable saber-shaped mandibles (Bolton, 1976). While slave-making behaviour is characteristic for the members of the *S. huberi* group (sensu Bolton, 1976; Forel, 1905; Kutter, 1920; Sanetra & Buschinger, 1996), in the *S. testaceus* group (comprising only two species) inquilinism has most likely evolved via degeneration of slave raiding (e.g. Wasmann, 1905; Kutter, 1969; Acosta & Martínez, 1982). *Teleutomyrmex schneideri* also lives as an inquiline alongside the *Tetramorium* host queen, but without maintaining its own worker caste (Kutter, 1950; Stumper, 1951). *Teleutomyrmex*, often called the "ultimate parasite" (e.g. Hölldobler & Wilson, 1990), displays the most outstanding adaptations ever recorded for ant social parasites. With their highly specialized tarsal claws the flattened *Teleutomyrmex* queens attach themselves to the much larger host queen, where they are efficiently tended and fed by the host workers (Kutter, 1950; Stumper, 1951; Collingwood, 1956). A clearly different strategy occurs in the workerless parasite *Anergates atratulus* since the infested nests are devoid of host queens (e.g. Wasmann, 1908; Wheeler, 1910). The occurrence of these varying degrees in socially parasitic behaviour raises the question of whether parasitism arose only once in the evolution of tetramoriine ants or whether it has had multiple origins. Possible transitions of different parasitic relations from one type to another would also be interesting to discover.

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Recently, some progress has been made toward understanding the evolutionary history of the Tetramoriini (Sanetra et al., 1994; Baur et al., 1996), but a thorough phylogenetic analysis has not been presented so far. A severe problem in the use of the genera *Tetramorium* and *Strongylognathus* for phylogenetic studies is the lack of comprehensive taxonomical revisions. Though a number of revisions dealt with *Strongylognathus* in different geographical regions (Pisarski, 1966; Baroni Urbani, 1969; Radchenko, 1991), a satisfactory taxonomy for the entire genus has yet to be produced. The species level taxonomy in the host genus *Tetramorium* is also not well understood, due to the high degree of morphological similarity and considerable intraspecific variability of the species within the group. In the revisionary monographs of Bolton (1977, 1979, 1980) the taxa of the Old World Tropics and the New World have been studied extensively, but the Palaearctic species of the genus (*T. caespitum* group sensu Bolton, 1977) remain largely unresolved. Only a few recent studies have dealt with the taxonomy and identification of Palaearctic *Tetramorium* in detail (López, 1991a, b; Radchenko, 1992a, b; Sanetra et al., 1999).

In the last decade, the subject of social parasite evolution in bees, wasps and ants has received great attention (e.g. Buschinger, 1990; Bourke & Franks, 1991; Carpenter et al., 1993; Choudhary et al., 1994; Heinze, 1991, 1998; Ward, 1996; Lowe & Crozier, 1997). The main topic of debate has been how close social parasites are related to their respective host species with regard to a hypothesis proposed by Emery (1909), now known as Emery's rule. Ant parasites have generally been found to be close relatives of their hosts (loose form of Emery's rule according to some authors, e.g. Ward, 1989; Bourke & Franks, 1991). Since the enormous spread of molecular methods, a number of studies have further revealed that social parasites often fall into monophyletic groups which are closely attached to their host species' groups (Choudhary et al., 1994; Baur et al., 1995, 1996; Pedersen, 1996; Lowe & Crozier, 1997). Thus, the application of Emery's rule in the strict sense, that is each parasite is the sister species of its respective host, appears confined to a number of inquilines scattered through various groups of ants (Hölldobler & Wilson, 1990; Buschinger, 1990). For instance, according to Wilson (1984), there are nine inquiline species known in the ant genus *Pheidole* which seem to have evolved independently and may well be immediate derivatives of their host species. As subsequent speciation events might have occurred in both the host and parasite lineage, Emery's rule has recently been interpreted as implying that the sister group of the parasite clade includes all host species (Ward, 1996; Heinze, 1998).

Another controversial issue, which is related but not identical to the phylogenetic relationships of hosts and parasites, concerns the origin of social parasitism. Social parasites may have arisen through either allopatric or sympatric speciation (e.g. Hölldobler & Wilson, 1990; Buschinger, 1990; Bourke & Franks, 1991; Choudhary et al., 1994; Ward, 1996). The interspecific hypothesis suggests that two species evolved allopatrically, and then the host-parasite relationship developed upon secondary contact (Wilson, 1971; termed social deception hypothesis by Carpenter et al., 1993). A quite different evolutionary pathway leading to parasitism, the intraspecific hypothesis, has been formulated by Buschinger (1990) and Bourke & Franks (1991). Under this model, social parasites evolved sympatrically from within populations of their hosts by assortative mating of intraspecific variants. According to some authors (West-Eberhardt, 1990; Bourke & Franks, 1991; Heinze, 1998) the two competing hypotheses may even be combined, and thus need not be regarded as mutually exclusive. Evolutionary studies of social parasitism still promise to shed new light upon this long-standing discussion, especially when dealing with the large number of ants' parasites having yet unresolved phylogenies.

In the current study we focus on the evolution of social parasites in the myrmicine ant tribe Tetramoriini. The genetic relationships of the three parasite genera, *Strongylognathus*, *Anergates* and *Teleutomyrmex*, and a large number of host and non-host *Tetramorium* species were analysed using allozymes. We will provide the first phylogenetic trees for this group in order to find out whether parasitism has arisen multiple times or only once. We will also address if parasites and their respective hosts are sister species.

MATERIAL AND METHODS

Taxa and sampling

The species included in this study are listed in Table 1. Owing to the problematic nature of species names in the genus *Tetramorium*, only species which could be reliably attributed to described taxa were considered. Species rank of *T. diomedeum* and *T. punctatum* is given in Sanetra et al. (1999). The investigated *Tetramorium* species endemic to the Palaearctic belong to the morphologically well defined *T. caespitum* group (Bolton, 1977) and can therefore be assumed to be monophyletic. The Oriental species *T. insolens*, *T.* cf. *centum*, *T. lanuginosum*, *T. kheperra* (the latter two formerly placed in the genus *Triglyphothrix* Forel, 1890 now being a synonym of *Tetramorium*), the Afrotropical *T. acutisetum*, *T. simillimum*, *T. caldarium* and the New World species *T. hispidum* were designated as outgroups.

Even though similar taxonomic problems have been encountered in species of socially parasitic *Strongylognathus*, all available samples were included because of the rareness of this genus. In most cases, assignment to the described taxa has been established by comparisons with the type material. Our *Strongylognathus* samples from Crimea clearly belong to *S. christophi* and not to *S. arnoldii* Radchenko, 1985, because of the shape of the volsella given as a discriminating feature in the only available key (Radchenko, 1991). The separation of *S. testaceus* and *S. karawajewi* was achieved by examination of the sculpturing on the head surface.

As described in Sanetra et al. (1994), *T.* "Czech Republic" and *T.* cf. *semilaeve*, from which only a single colony each had been available, turned out to constitute unusually small and weakly sculptured *T. caespitum* (see also López, 1991b). Though standing in contrast to the findings of López (1991a), *T.* "S. Nevada" is very likely a geographic variant of *T. impurum*.

TABLE 1. Species included in this study.

Outgroups
- *Tetramorium lanuginosum* Mayr, 1870
- *Tetramorium* cf. *centum* Bolton, 1977
- *Tetramorium kheperra* (Bolton, 1976)
- *Tetramorium acutisetum* Santschi, 1921
- *Tetramorium caldarium* (Roger, 1857)
- *Tetramorium simillimum* (Smith, 1851)
- *Tetramorium insolens* (Smith, 1861)
- *Tetramorium hispidum* (Wheeler, 1915)

Palaearctic *Tetramorium* species
- *Tetramorium caespitum* (L., 1758)
- *Tetramorium impurum* (Förster, 1850)
- *Tetramorium moravicum* Kratochvíl, 1941
- *Tetramorium rhenanum* Schulz, 1996
- *Tetramorium forte* Forel, 1904
- *Tetramorium marocanum* de Haro & Collingwood, 1994
- *Tetramorium chefketi* Forel, 1911
- *Tetramorium biskrense* Forel, 1904
- *Tetramorium brevicorne* Bondroit, 1918
- *Tetramorium semilaeve* André, 1883
- *Tetramorium punctatum* Santschi, 1927
- *Tetramorium meridionale* Emery, 1870
- *Tetramorium ferox* Ruzsky, 1903
- *Tetramorium diomedeum* Emery, 1908

Social parasites
- *Strongylognathus huberi* group
  - *Strongylognathus huberi* Forel, 1874
  - *Strongylognathus christophi* Emery, 1889
  - *Strongylognathus kratochvili* Šilhavý, 1937
  - *Strongylognathus alpinus* Wheeler, 1909
  - *Strongylognathus italicus* Finzi, 1924
  - *Strongylognathus destefanii* Emery, 1915
  - *Strongylognathus silvestrii* Menozzi, 1936
- *Strongylognathus testaceus* group
  - *Strongylognathus testaceus* (Schenck, 1852)
  - *Strongylognathus karawajewi* Pisarski, 1966
- Extreme inquilines
  - *Anergates atratulus* (Schenck, 1852)
  - *Teleutomyrmex schneideri* Kutter, 1950

T. "Crete 1" resembles *T. hippocrate* Agosti & Collingwood, 1987 (Poldi, in litt.), but is not included in this analysis. *T.* "Crete 2" is most likely *T. diomedeum*, of which the typical form occurs in southern Italy (see also Sanetra et al., 1999). Differences between typical *T. semilaeve* and *T.* "Tenerife" are slight at best and the latter form probably does not deserve taxonomic recognition. It still remains an open question, however, as to whether *T. semilaeve* from the western (nominotypical form) and the eastern Mediterranean (*T.* "Crete/Rhodes" in Sanetra et al., 1994) are different species (see Sanetra et al., 1999).

Field collections were carried out mainly in Central Europe, the Mediterranean region and in the western part of Asia (see Appendix 1). Nest samples were aspirated and transported back to the laboratory alive, where most of the material was preserved at –80°C. Additionally, some nest fragments were maintained alive. Three to five individuals from each colony were analysed for each enzyme. However, some of the enzymes could not be scored in colonies where too few individuals had been collected, as was the case in some of the socially parasitic and outgroup species.

Allozyme electrophoresis

Enzymes were either separated by vertical slab gel electrophoresis (Mighty-Small, Hoefer Scientific Instruments) in polyacrylamide [aldehydoxidase (*Ao*, EC 1.2.1.5.), glucose-6- phosphate dehydrogenase (*G6pdh*, EC 1.1.1.49), malate dehydrogenase (NADP$^+$) (*Mdhp*, EC 1.1.1.40), phosphoglucomutase (*Pgm*, EC 5.4.2.2), xanthine dehydrogenase (*Xdh*, EC 1.1.1.204)] as described in Sanetra et al. (1994) or on thin layer cellulose acetate plates ["Titan III" (76 by 76 mm), Helena Laboratories, Beaumont, Texas]. The latter method is highly suitable for small arthropods and is accurately described in Richardson et al. (1986). Single workers or females were crushed in distilled water and the extract was immediately applied to the gels. The tray buffers used were 0.01 M Tris-Maleate-EDTA pH 7.4 [for glucose-6-phosphate isomerase (*Gpi*, EC 5.3.1.9), glycerol-3-phosphate dehydrogenase (*G3pdh*, EC 1.1.1.8), isocitrate dehydrogenase (*Idh*, EC 1.1.1.14), malate dehydrogenase (*Mdh-1*, EC 1.1.1.37)], 0.01 M Tris-Maleate-EDTA pH 8.3 [for aconitase (*Acoh*, EC 4.2.1.3), arginine kinase (Ark, EC 2.7.3.3), hexokinase (*Hk*, EC 2.7.1.1)], 0.1 M Tris-Maleate-EDTA pH 7.0 [for adenylate kinase (*Ak*, EC 2.7.4.3), pyruvate kinase (*Pk*, 2.7.3.3)], 0.015 M Tris-Borate pH 7.5 [for phosphogluconate dehydrogenase (*Pgdh*, EC 1.1.1.44)], 0.05 M Tris-Maleate pH 7.8 (for *Mdh-2*). All plates were run at 8°C and at 200–250 V; running times varied from 15 to 50 min. Enzymes were subsequently visualized using standard histochemical stains (e.g. Richardson et al., 1986; Murphy et al., 1996). Staining solutions for *Ak*, *Hk* and *Pk* were prepared as agar overlays.

Enzyme and locus nomenclature mainly follow Murphy et al. (1996). Differing enzyme variants were designated alphabetically according to increasing electrophoretic mobility towards the anode. When the isozyme product of more than one locus was present on a gel, numerical designations were given to each locus. Due to the many new electromorphs discovered in this study the terminology used in Sanetra et al. (1994, 1999) has been altered; here we provide a synonymic list of corresponding enzyme variants:

Ao: *s*→*c*, *m*→*d*, *f*→*e*; *Gpi*: *e*→*e*, *v*→*f*, *s*→*g*, *m*→*h* (but *m* in *T. moravicum*, *T. brevicorne*, *T. meridionale* and *T.* "S. Nevada" is now *g*), *f*→*i*, *x*→*j*; *G3pdh-1*: *s*+*m*→*b*, *f*→*c* (but *f* in *Anergates* is now *a*), *x*→*d*; *Idh*: *v*→*d*, *s*→*f*, *m*→*g*, *f*→*h* (but *f* in *T. insolens* is now *d*), *x*→*j*; *Mdh-1*: *v*→*b*, *s*→*d*, *f*→*e*, *u*→*g*; *Mdhp*: *s*→*b*, *m*→*d* (but *m* in *Teleutomyrmex* is now *c*), *f*→*e*, *x*→*f*, *u*→*h*; *Pgm-1*: *v*→*d*, *s*→*e*, *m*→*f*, *f*→*g*, *x*→*h*; *Pgm-2*: *e*→*b*, *v*→*c*, *s*→*d*, *m*→*e*, *f*→*g*, *x*→*h*, *u*→*i*; *Sod*: *s*→*a*, *f*→*b*; *Xdh*: *v*→*a*, *s*→*b*/*c*, *f*→*d*.

Data analysis

The genetic variability within species was evaluated using the parameters expected mean heterozygosity per locus (H_{exp}), mean number of alleles per locus (A) and percentage of polymorphic loci (P_{99}) following the 99% criterion. Thus, a locus was considered polymorphic if the frequency of the most common allele did not exceed 0.99. Standard errors were obtained by jackknifing over loci (Shoemaker et al., 1992). Allele frequencies were obtained from the sampled genotypes by weighting the frequencies within colonies equally.

The enzyme character data (Table 2) were subjected to a set of phenetic and cladistic analyses (but *Xdh* electromorphs *b*, *c* are not distinguished in coding) using the computer programs Hennig86 (Version 1.5; Farris, 1988), NTSYS-pc (Version 1.60; Rohlf, 1990) and Phylip (Version 3.572c for Windows; Felsenstein, 1995). Allele frequencies were used to estimate pairwise genetic distances, following the methods of Cavalli-Sforza & Edwards (1967), Nei (1972), Rogers (1972) and Prevosti et al. (1975). Genetic distances were clustered using UPGMA to yield a distance phenogram (Sneath & Sokal, 1973), and additionally analysed with Neighbor-joining (Saitou & Nei, 1987) and least squares statistics (Fitch & Margoliash, 1967) as implemented in the programs Neighbor and Fitch in Phylip (Felsenstein, 1995,

TABLE 2. Allele frequencies at the polymorphic loci investigated (values <0.005 are reported exactly, while all others are rounded).

Locus	*Acoh-1*					*Ak*					*Ao*				*Ark*				*Gpi*							
	b	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*d*	*e*	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*d*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
T. caespitum	1.00					0.08	0.63	0.29				0.97	0.02	0.01		1.00				0.01			0.002		0.96	0.03
T. impurum	1.00					0.04	0.65	0.31				0.89	0.11			1.00					0.002		0.01		0.98	0.01
T. moravicum	1.00					0.06	0.94					1.00				1.00							0.18		0.82	
T. rhenanum	1.00					0.19	0.81					1.00				1.00									1.00	
T. forte			0.70		0.30		0.23	0.52	0.25			1.00				1.00								0.97	0.03	
T. marocanum			0.04		0.96		0.25	0.75			0.03	0.97				0.97	0.03								1.00	
T. chefketi					1.00		1.00					1.00				1.00									1.00	
T. biskrense			1.00					0.02		0.98		1.00				1.00									0.92	0.08
T. brevicorne	1.00						0.75	0.25				1.00				1.00							1.00			
T. semilaeve	0.30		0.70				0.36	0.64			0.26	0.74				1.00					0.27	0.43		0.01	0.29	
T. punctatum	1.00						0.50	0.50				1.00				1.00									1.00	
T. meridionale	0.67		0.33			0.13	0.87				0.20	0.80				1.00							0.20		0.80	
T. ferox	1.00						1.00					1.00				1.00								0.50	0.50	
T. diomedeum	0.50		0.50				0.50	0.50			0.49	0.51				1.00						0.01			0.97	0.02
S. huberi	1.00					0.50	0.50					1.00				1.00									1.00	
S. christophi	1.00					0.75	0.25					1.00				1.00									1.00	
S. kratochvili	1.00					0.50	0.50					1.00				1.00									1.00	
S. alpinus	1.00					0.25	0.75					1.00				1.00									1.00	
S. italicus	1.00					0.50	0.50					1.00				1.00									1.00	
S. destefanii	1.00						1.00				0.67	0.33				1.00								0.33	0.67	
S. silvestrii	1.00						1.00					1.00				1.00								0.64	0.36	
S. testaceus	1.00					0.44	0.56					1.00				1.00									1.00	
S. karawajewi	1.00					0.42	0.58					1.00				1.00									1.00	
A. atratulus	1.00					0.20	0.78	0.02				1.00						1.00					0.92		0.08	
T. schneideri		1.00					0.55	0.45						1.00		1.00					1.00					
T. lanuginosum	1.00						0.50	0.50					1.00		1.00				1.00							
T. cf. *centum*				1.00					1.00				1.00		1.00				1.00							
T. kheperra			1.00					1.00					1.00		1.00				1.00							
T. acutisetum			1.00				0.50	0.30		0.20				1.00		1.00							1.00			
T. caldarium			1.00										1.00		1.00										1.00	
T. simillimum			1.00									1.00			1.00								1.00			
T. insolens												1.00			1.00								0.25		0.75	
T. hispidum												1.00											1.00			

TABLE 2 (continued).

Locus	*G3pdh-1*				*G3pdh-2*		*Hk-1*				*Hk-2*					*Idh*								
	a	*b*	*c*	*d*	*a*	*b*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*e*	*a*	*b*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
T. caespitum			1.00		1.00		0.06	0.55	0.39				1.00											1.00
T. impurum			1.00		1.00			0.26	0.74				0.69		0.31									1.00
T. moravicum			1.00		1.00		0.15	0.55	0.30				1.00									1.00		
T. rhenanum			1.00				0.25	0.35	0.40				0.90		0.10							1.00		
T. forte			1.00				0.20	0.28	0.52				1.00									1.00		
T. marocanum			1.00						1.00				1.00									1.00		
T. chefketi			1.00					0.25	0.75				1.00									1.00		
T. biskrense		0.31	0.69					1.00				1.00						0.01			0.94			0.05
T. brevicorne			1.00				0.49	0.51				1.00						0.02			0.98			
T. semilaeve		0.01	0.98	0.01			1.00				1.00							0.002		0.79	0.19	0.01	0.004	
T. punctatum				1.00			0.25	0.75				1.00								0.67	0.33			
T. meridionale			1.00				0.42	0.58				1.00								0.30	0.70			
T. ferox			1.00						1.00				1.00									1.00		
T. diomedeum		0.50	0.50				0.50		0.50		0.50		0.50							0.50		0.50		
S. huberi			1.00					0.86	0.14						1.00							1.00		
S. christophi			1.00			1.00		0.56	0.44						1.00							1.00		
S. kratochvili			1.00			1.00		0.64	0.36						1.00							1.00		
S. alpinus			1.00			1.00		0.42	0.58				0.19		0.81							1.00		
S. italicus			1.00					0.50	0.50						1.00							1.00		
S. destefanii			1.00					0.38	0.62				0.17		0.83							1.00		
S. silvestrii			1.00					0.41	0.59				0.13		0.87							1.00		
S. testaceus			0.99	0.01	1.00			0.24	0.76				0.31		0.69							1.00		
S. karawajewi		0.50	0.50					0.67	0.33				0.22		0.78							1.00		
A. atratulus	0.86	0.14			1.00		0.37	0.63					1.00							1.00				
T. schneideri		1.00			1.00			0.44	0.56						1.00						1.00			
T. lanuginosum				1.00				0.50	0.50				1.00							1.00				
T. cf. *centum*			1.00													1.00								
T. kheperra			1.00														1.00							
T. acutisetum			1.00						1.00				0.50		0.50				1.00					
T. caldarium				1.00						1.00				1.00			1.00							
T. simillimum				1.00				0.50	0.50			1.00				1.00								
T. insolens			1.00															1.00						
T. hispidum			1.00															1.00						

TABLE 2 (continued).

Locus	*Mdh-1*							*Mdhp*									*Pgdh*					*Pgm-1*						
	b	*c*	*d*	*e*	*f*	*g*	*h*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*a*	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*d*	*e*	*f*	*g*
T. caespitum	0.003		0.99					0.98			0.02							1.00								0.06	0.92	0.02	
T. impurum			1.00								1.00							1.00					0.01			0.05	0.91	0.03	
T. moravicum			1.00								1.00							1.00				0.01				0.22	0.77		
T. rhenanum			1.00								1.00							1.00								0.80	0.20		
T. forte			1.00								1.00							1.00									0.96	0.04	
T. marocanum			1.00								1.00							1.00									1.00		
T. chefketi			1.00								1.00							1.00								0.33	0.67		
T. biskrense			1.00								0.88	0.12						1.00								0.80	0.20		
T. brevicorne			1.00										1.00					1.00									0.91	0.09	
T. semilaeve			0.99	0.003							1.00							1.00								0.05	0.77	0.18	
T. punctatum			1.00								1.00							1.00									1.00		
T. meridionale			1.00								0.83		0.17					1.00									0.80	0.10	
T. ferox			0.50	0.50							1.00						0.50	0.50									1.00		
T. diomedeum			0.50	0.50							0.72		0.28				0.50	0.50									0.36	0.64	
S. huberi			1.00							1.00							1.00										1.00		
S. christophi			1.00							1.00							1.00								0.16	0.84			
S. kratochvili			1.00							1.00							1.00										1.00		
S. alpinus			1.00							1.00							1.00										1.00		
S. italicus			1.00							1.00							1.00										1.00		
S. destefanii			1.00							1.00							1.00										1.00		
S. silvestrii			1.00							1.00							1.00										1.00		
S. testaceus			1.00								1.00							1.00								0.95	0.05		
S. karawajewi			1.00								1.00							1.00									1.00		
A. atratulus				1.00								1.00						0.25	0.63		0.12							1.00	
T. schneideri				1.00					1.00									1.00											1.00
T. lanuginosum			1.00										1.00							1.00					1.00				
T. cf. *centum*			1.00												1.00			1.00							1.00				
T. kheperra			1.00												1.00					1.00				1.00					
T. acutisetum					1.00									1.00				1.00							1.00				
T. caldarium							1.00							1.00						1.00				1.00					
T. simillimum					1.00											1.00					1.00				0.25	0.75			
T. insolens						1.00								0.50	0.50			1.00											1.00
T. hispidum		1.00											1.00														1.00		

TABLE 2 (continued).

Locus	*Pgm-2*									*Pk*									*Sod*		*Xdh*						
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*a*	*b*	*a*	*b*	*c*	*d*	*e*	*f*	*g*
T. caespitum				0.44	0.52		0.04						1.00							1.00		0.52	0.48				
T. impurum		0.01		0.02	0.90		0.07						0.63			0.37				1.00		0.05	0.95				
T. moravicum					1.00								1.00							1.00			1.00				
T. rhenanum					1.00								1.00							1.00			1.00				
T. forte					0.48		0.52								1.00					1.00			1.00				
T. marocanum					1.00										1.00					1.00			1.00				
T. chefketi					1.00										1.00					1.00			1.00				
T. biskrense					1.00													1.00		1.00			1.00				
T. brevicorne					0.87		0.13				1.00									1.00			1.00				
T. semilaeve	0.003				0.94		0.06						1.00							1.00		0.31	0.69				
T. punctatum					1.00								1.00							1.00		1.00					
T. meridionale					1.00						1.00									1.00			1.00				
T. ferox					1.00								1.00							1.00			1.00				
T. diomedeum				0.01	0.99								1.00							1.00		0.40	0.60				
S. huberi							1.00			1.00										1.00			1.00				
S. christophi							1.00			1.00										1.00			1.00				
S. kratochvili							1.00			1.00									1.00				1.00				
S. alpinus							1.00			1.00										1.00			1.00				
S. italicus							1.00			1.00										1.00			1.00				
S. destefanii							1.00			1.00										1.00			1.00				
S. silvestrii							1.00			1.00										1.00			1.00				
S. testaceus							0.45	0.55		1.00										1.00			1.00				
S. karawajewi							0.58	0.42		1.00										1.00			1.00				
A. atratulus				1.00								1.00								1.00				1.00			
T. schneideri			1.00											1.00						1.00			1.00				
T. lanuginosum					1.00							1.00								1.00						1.00	
T. cf. *centum*					1.00									1.00						1.00				1.00			
T. kheperra					1.00								1.00							1.00				1.00			
T. acutisetum			1.00														1.00			1.00				1.00			
T. caldarium						1.00														1.00					1.00		
T. simillimum				1.00																1.00				1.00			
T. insolens									1.00											1.00	1.00						
T. hispidum							1.00													1.00							1.00

1997). The User Tree option in Fitch was used to compute %SD (as a measure for goodness of fit) in order to evaluate tree topologies produced by other methods. A subset of two representative outgroup species was selected for distance analyses. The robustness of the topologies of distance trees was assessed using 100 bootstrap replicates (Felsenstein, 1985), but the resulting values were not interpreted in a strict statistical sense (for discussion see Sanderson, 1989; Bremer, 1994; Siddall, 1995).

Coding of allozyme data for cladistic parsimony analyses was accomplished using three different strategies, because there has been some debate as to the best way to analyse these types of data (e.g. Wiens, 1995; Swofford et al., 1996). In the first procedure, electromorphs were coded as input for Hennig86 in a presence/absence fashion (Mickevich & Johnson, 1976), with alleles treated as characters under a "corrective weighting scheme" that applied the same weight to each locus (Moran et al., 1990). Following the recommendations of Maddison et al. (1984) and Nixon & Carpenter (1993), outgroup (*T. insolens* and *T. hispidum* omitted because of a large number of missing values, see Table 2) and ingroup were simultaneously resolved to seek a globally most parsimonious solution.

For the procedures described in the following a hypothetical ancestral taxon was synthesized by outgroup analysis (Kluge & Farris, 1969; Watrous & Wheeler, 1981; Maddison et al., 1984) using the information from all available outgroup taxa. Ancestral state assessments were obtained by the generalized principle that electromorphs found in both the ingroup and the outgroup are plesiomorphic (e.g. Richardson et al., 1986; Baverstock et al., 1979; Patton & Avise, 1983).

In the second approach, character states were ordered into transformation series (or character state trees) under the assumptions of the minimum allele turnover model (Mickevich & Mitter, 1981, 1983); a few unconnectable states were left unordered (see also Carpenter et al., 1993). New states were allowed as ancestral condition in branching character state trees (Mardulyn & Pasteels, 1994). Polarization of these character states followed the taxonomic outgroup and functional outgroup criteria of Watrous & Wheeler (1981), as expanded by Farris (1982). Branching character state trees (see Appendix 2) were recoded and prepared as input matrices for Hennig86 using ordinal coding (Mickevich & Weller, 1990).

The mutation model for coding allozyme data in conjunction with the "quadraphenic evaluation procedure" (Murphy, 1993) was used in the last approach. A more effective application resulted from combined analysis of the electrophoretic data and some non-molecular characters, which are 1. mandibles elongate and saber-shaped, 2. mandibles reduced and edentate, 3. sting strongly reduced, non functional, 4. adelphogamy, males pupoidal and reduced in number, 5. parasitism.

Trees were constructed from these data matrices with the Hennig86 commands *mhennig*;bb**; which find exact solutions on most occasions (Farris, 1988; Platnick, 1989). In some instances successive weighting (Farris, 1969; Carpenter, 1988) was applied to optimize the results under the criterion of minimum homoplasy (Retention-Index from Farris, 1989). The Jackknife monophyly index (JMI) developed by Siddall (1995) was performed with Lanyon.exe in Random Cladistics (Siddall, 1994). JMI values were utilised to infer which clades are more (or less) stable than others within and among the most parsimonious trees and in the consensus tree. Character states were optimized and mapped onto the trees with accelerated optimization as implemented in Clados (Version 1.6.1; Nixon, 1998). This option prefers reversals over parallelism if the two are "equally parsimonious".

RESULTS

Allozyme variation

Allozyme phenotypes of putative heterozygotes were in accordance with those expected on the basis of the quarternary structure of the enzymes given by Murphy et al. (1996) except for *Xdh* (see Sanetra et al., 1994). The products of 21 presumptive gene loci were obtained for the taxa summarized in Table 1. Of these loci, three (*Acoh-2*, *G6pdh*, *Mdh-2*) were either monomorphic for all ingroup samples or the electromorphs could not be reliably scored. Allele frequency data for the remaining 18 polymorphic loci are presented in Table 2. Among these loci, *Acoh-1*, *Gpi*, *Hk-1/2* and *Pgm-1/2* displayed the highest levels of polymorphism, whereas others, like *Mdh-1* and *G3pdh-1*, showed only occasional variation. Electromorphs extremely different in their electrophoretic mobility were mostly restricted to *Anergates*, *Teleutomyrmex* and/or the outgroup taxa, sometimes even occurring at otherwise monomorphic loci.

TABLE 3. Diagnostic enzyme electromorphs in Palaearctic *Tetramorium* species.

	Acoh-1	*G3pdh-1*	*Hk-2*	*Idh*	*Mdhp*	*Pk*
T. caespitum	*b*	*c*	*c*	*g*	*b, e**	*d*
T. impurum	*b*	*c*	*c, e*	*g*	*e*	*d, g*
T. moravicum	*b*	*c*	*c*	*h*	*e*	*d*
T. rhenanum	*b*	*c*	*c, e*	*h*	*e*	*d*
T. forte	*d, f*	*c*	*c*	*h*	*e*	*f*
T. marocanum	*d, f*	*c*	*c*	*h*	*e*	*f*
T. chefketi	*f*	*c*	*c*	*h*	*e*	*f*
T. biskrense	*d*	*b, c*	*b*	*d, g, j*	*e, f*	*i*
T. brevicorne	*b*	*c*	*b*	*d, g*	*g*	*b*
T. semilaeve	*b, d*	*b, c, d*	*a*	*d, f, g, h, i*	*e*	*d*
T. punctatum	*b*	*d*	*b*	*f, g*	*e*	*d*
T. meridionale	*b, d*	*c*	*b*	*f, g*	*e, g*	*b*

* Very rare, could be due to hybridization with *T. impurum*.

Several species of *Tetramorium* can be distinguished by diagnostic electromorphs which are most likely due to fixed allelic differences (see Table 3). At certain loci (especially *Gpi*, *G3pdh-1*, *Mdh-1*), all *T. diomedeum* and *T. ferox* individuals had the typical banding patterns of heterozygotes, a phenomenon which deserves closer examination (see also Sanetra et al., 1999). These diagnostic enzyme variants proved to be a powerful tool for the reliable identification of *Tetramorium* worker samples that were often very difficult to classify as species morphologically (see also López, 1991a; Sanetra et al., 1994, 1999). Immature stages of the parasitic genera *Strongylognathus*, *Anergates* and *Teleutomyrmex* may also be detected among their host brood by using some of the loci reported in this study. In the late summer the larvae of *A. atratulus* can be tentatively identified in the field because of their coloration and shape. However, in many cases these larvae cannot be easily reared until pupation, thus making electrophoretic tests effective for species confirmation.

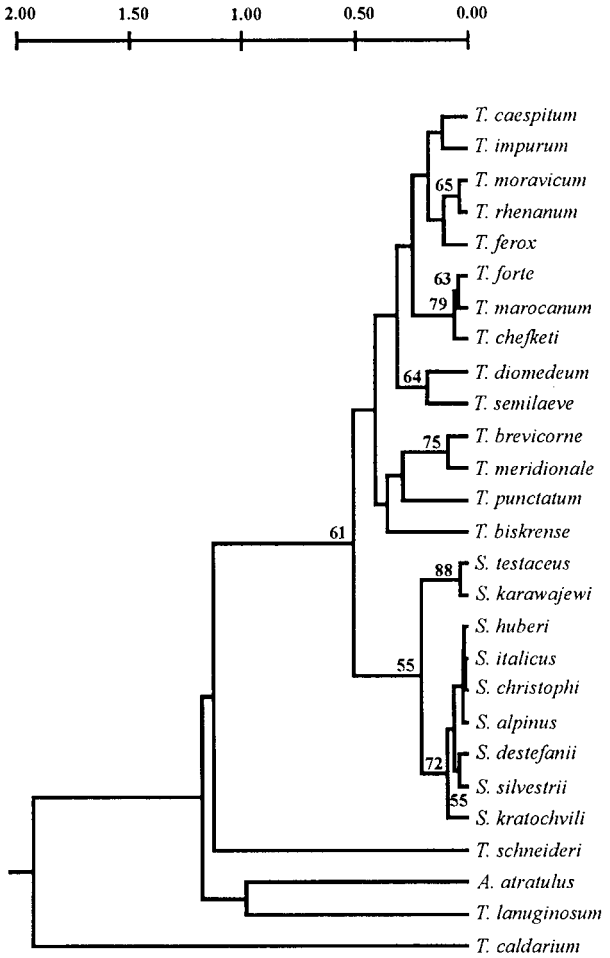

Fig. 1. UPGMA phenogram using Nei's (1972) genetic distance. % SD = 33.07. Bootstrap values are indicated only for groupings supported at least at the 50% level.

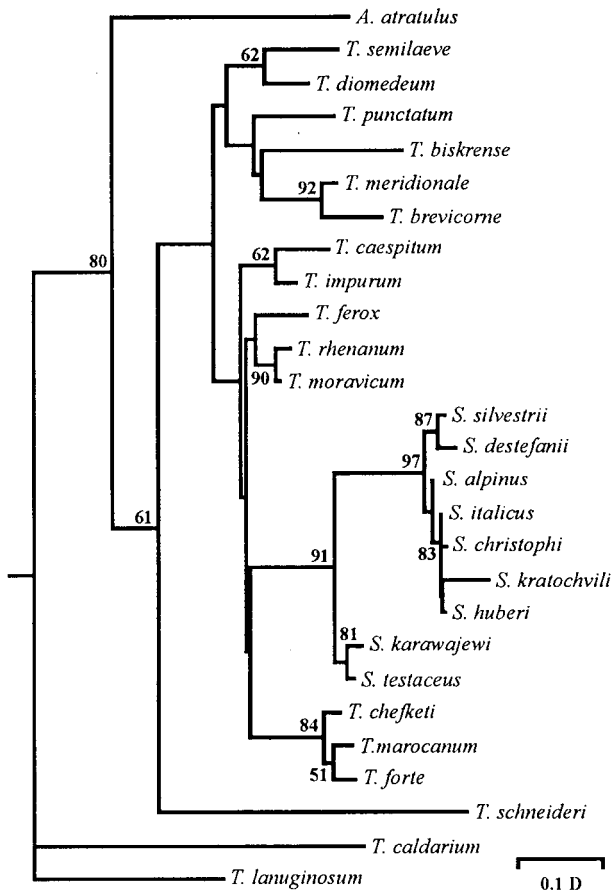

Fig. 2. Fitch-Margoliash tree with shortest tree length using Cavalli-Sforza's (1967) chord distance. % SD = 8.85. Branch lengths drawn proportional to genetic distance. Bootstrap values are indicated only for groupings supported at least at the 50% level.

Intraspecific variability

The average percentage of polymorphic loci ($P_{99} \pm$ SE) was 27.9 ± 2.1 for the Palaearctic *Tetramorium* species, 14.7 ± 1.7 for the genus *Strongylognathus* and 16.7 ± 1.8 for the extreme inquilines *Anergates* and *Teleutomyrmex*. The highest mean number of alleles per locus (with a maximum of four alleles) occurred in *Tetramorium impurum* (1.76 ± 0.056) and *T. caespitum* (1.71 ± 0.050), whereas fewer alleles were detectable in *Strongylognathus* (1.16 ± 0.023), *Anergates* (1.29 ± 0.033) and *Teleutomyrmex* (1.05 ± 0.015). The estimates of heterozygosity ($H_{exp} \pm$ SE) ranged from 0.064 ± 0.008 for the parasitic species to 0.093 ± 0.008 for the *Tetramorium* species. The P_{99} values of the social parasites were significantly lower than those in the free-living *Tetramorium* species, as judged by the fact that the 95% confidence intervals did not overlap. Confidence intervals of H_{exp} showed only a marginal overlap of 0.003.

Phenetic and phylogenetic inferences

UPGMA cluster analyses using four different genetic distances yielded very similar results. In fact, the use of Nei's distance and Roger's distance led to exactly the same topology. In a phenogram constructed with Nei's D (Fig. 1), the extreme inquilines *Anergates* and *Teleutomyrmex* attain D values >1 and are thus distinctly set apart from the remaining Palaearctic species. On the other hand, among *Strongylognathus* and *Tetramorium* species, pairwise genetic distance ranges from 0.19–0.92 (on average 0.56) indicating closer relationships of these genera. Bootstrap analysis shows that all *Strongylognathus* species are grouped with relatively low support, whereas the *S. huberi* and the *S. testaceus* group within *Strongylognathus* are given higher support values. The nearest cluster attached to *Strongylognathus* comprises all *Tetramorium* host and non-host species sampled from the western Palaearctic.

Trees obtained from pairwise chord distances employing the algorithms of Fitch (Fig. 2) and Neighbor had topologies virtually identical to one another. The lowest percent standard deviation (% SD) of branch lengths used as criterion for goodness of fit is 8.85 for the Fitch-Margoliash method and 10.61 for Neighbor-joining. The results of these rate-independent analyses share the general pattern with the UPGMA phenogram, specifically that *Anergates* and *Teleutomyrmex* branch off separately near the basal node of the tree. The genus *Strongylognathus* forms a distinct species assemblage in which

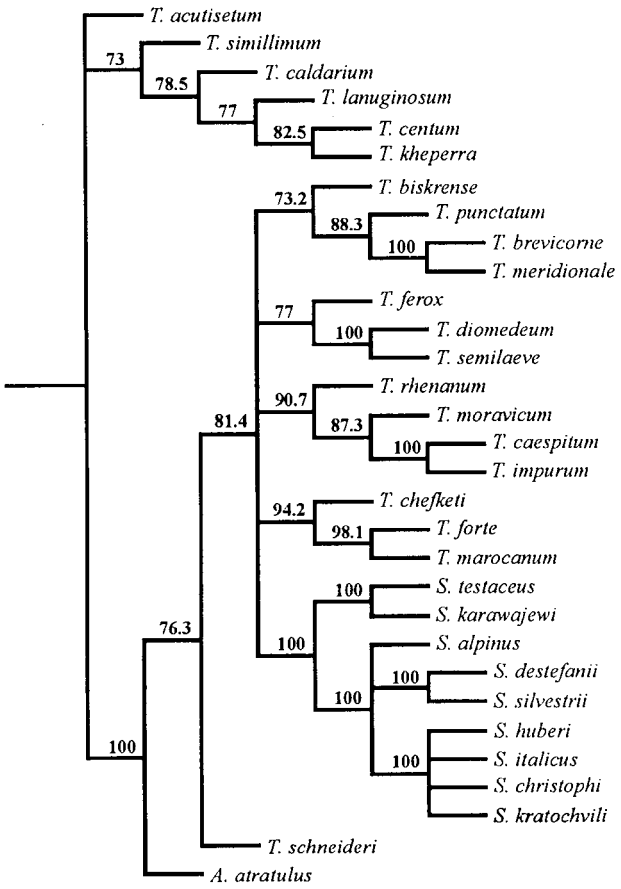

Fig. 3. Strict consensus tree (unweighted length l 230, ci 40, ri 58) of six cladograms (l 226, ci 41, ri 59) resulting from parsimony analyses using independent allele coding. Outgroups and ingroup were resolved simultaneously. Jackknife monophyly indices (Siddall, 1995) are shown at each node to indicate relative stability of clades.

the *S. huberi* and the *S. testaceus* group are sister groups. Most of the species in the *S. huberi* group are genetically very similar (see branch lengths in Figs 1–2). In contrast to the phenogram obtained by UPGMA, *Strongylognathus* appears within the main cluster of Palaearctic *Tetramorium*, suggesting the group (*chefketi* + (*forte* + *marocanum*)) as its closest free-living relatives (Fig. 2). The branch including the two most commonly used host species, (*caespitum* + *impurum*), is placed near to *Strongylognathus*. However, the relative positions of the *Tetramorium* clusters varied considerably (they occurred in less than 50% of bootstrap replicates), as indicated by missing values at the respective nodes.

Maximum parsimony analysis of discrete characters using independent allele coding resulted in six equally parsimonious cladograms. One of these was arbitrarily chosen to demonstrate optimization of cladogram characters (Fig. 4). The strict consensus tree (Fig. 3) shows several features that have already occurred in some of the previously discussed trees, such as the monophyly of *Strongylognathus* and the relatively distant, but separate, positions of *Anergates* and *Teleutomyrmex*. Interrelationships among Palaearctic *Tetramorium* species are only partially resolved into four major groups, which are equally closely attached to the *Strongylognathus* clade. Thus, according to this evolutionary model the sister group of *Strongylognathus* may either include all *Tetramorium* ingroup species or may be composed of different subsets of those four groups (for a possible example see Fig. 4). The relative clade stability was estimated with the Jackknife Monophyly Index (JMI), which assigns a value to each clade according to its frequency of occurrence in pseudoreplicates. A comparison of single taxon removal (Siddall, 1995) further revealed that *T. biskrense* is the most problematic taxon, as its exclusion reduces the number of equally parsimonious solutions to only two trees. Then, a (*chefketi* + (*forte* + *marocanum*)) clade forms the sister group to *Strongylognathus*, as previously seen in the Fitch-Margoliash tree.

Parsimony analysis with minimum turnover coding resulted in three cladograms, which were also stable to successive weighting. The consensus tree (Fig. 5) is far better resolved than that with independent allele coding (Fig. 3). On the former cladogram, *Anergates* and *Teleutomyrmex* are placed as sister genera, an interesting result which is supported by a relatively high JMI value. The nearby position of this clade to the ingroup species (*T. semilaeve* + *T. diomedeum*) is also remarkable. In accordance with all other trees, *Strongylognathus* is monophyletic with a clear distinction between the *S. testaceus* and the *S. huberi* group. The species pair (*T. moravicum* + *T. rhenanum*) appears most closely attached to the *Strongylognathus* clade. However, JMI values indicate that the clades forming sister taxa to the parasites are less stable than those including the parasitic species.

The final solution of the "quadraphenic evaluation procedure" (Murphy, 1993) using all available evidence (biochemical, morphological and life-habits) is shown in Fig. 6. We could obtain several primary and secondary branchings which resulted from taxonomic and functional outgroup analysis, respectively. These phylogenetic inferences are exclusively based on shared mutations and are regarded as the most reliable. Several of these groupings, especially the two independent originations of socially parasitic clades, *Strongylognathus* and (*Anergates* + *Teleutomyrmex*), are congruent with those seen in Fig. 5, but contrast with the remaining trees that suggest *Anergates* and *Teleutomyrmex* have evolved independently. Sister group relationships of the parasites also show only minor deviations from the results with the minimum turnover coding (Fig. 5). Third order branchings were obtained by relaxing the strict definition of the apomorphic state and allowing additional groupings by shared losses of alleles, which are considered of lesser importance for phylogenetic inference. The latter include the establishment of the species pair (*T. moravicum* + *T. rhenanum*). Moreover, some of the previously generated clades are supported by characters of this order, for instance *Anergates* and *Teleutomyrmex* have both lost the otherwise very common alleles *Mdh-1 (d)* and *G3pdh-1 (c)*. Branchings of the fourth order are the least preferable and were produced by mapping those characters onto the tree that could not be

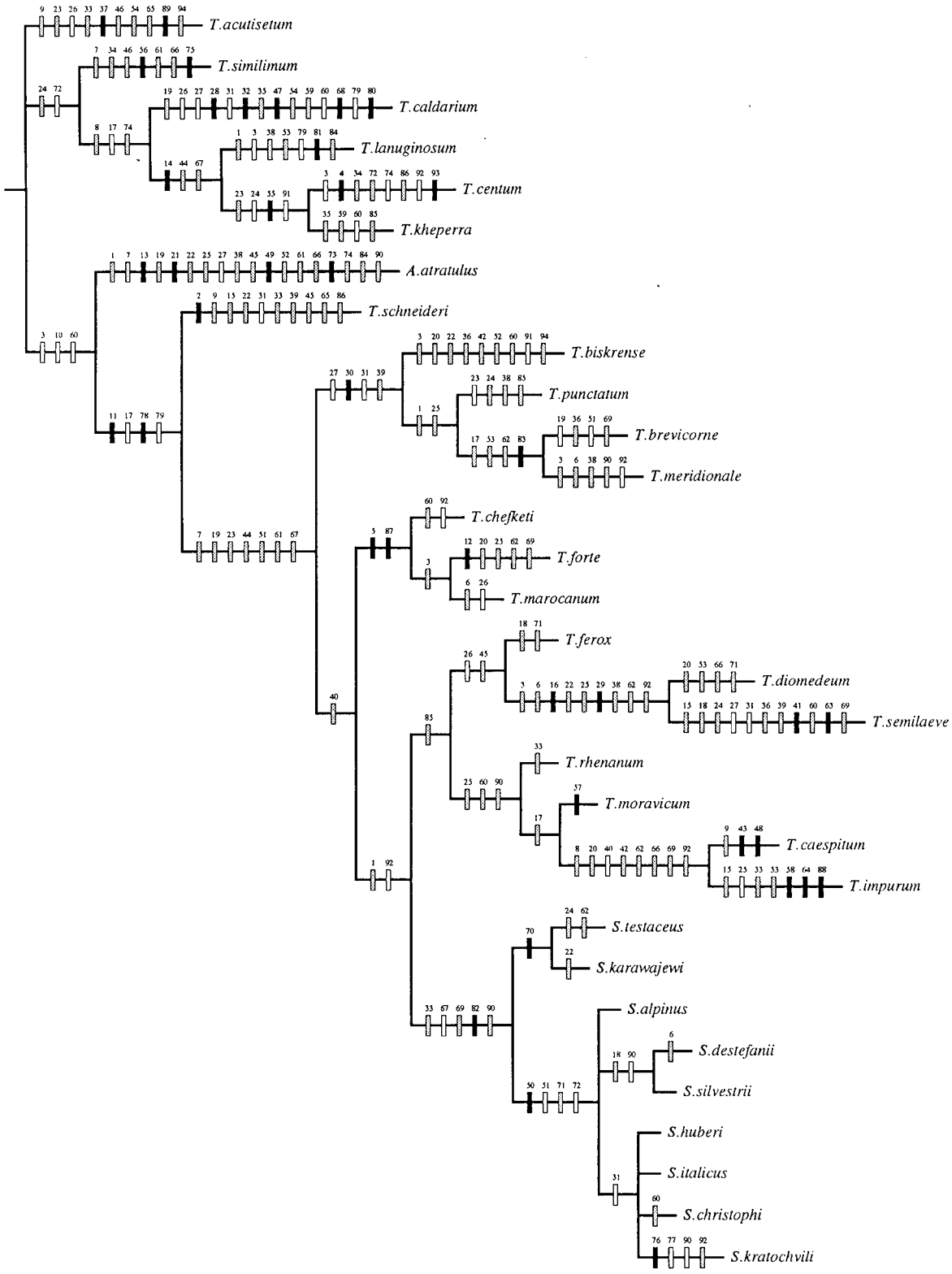

Fig. 4. Character optimization on one of six most parsimonious cladograms (l 226, ci 41, ri 59) resulting from parsimony analyses using independent allele coding. Optimization is accelerated transformation as implemented in Clados software (Nixon, 1998). Character state changes are marked with hashmarks as follows: Filled – unique forward changes, grey-scaled – convergences, open – reversals. Numbers above the hashmarks refer to the following character numbers: 1–5: *Acoh-1 (b-c-d-e-f)*; 6–9: *Ao (b-c-d-e)*; 10–13: *Ark (a-b-c-d)*; 14–20: *Gpi (c-e-f-g-h-i-j)*; 21–24: *G3pdh-1 (a-b-c-d)*; 25–28: *Hk-1 (a-b-c-d)*; 29–33: *Hk-2 (a-b-c-d-e)*; 34–42: *Idh (a-b-d-e-f-g-h-i-j)*; 43–47: *Mdh-1 (b-d-e-f-h)*; 48–56: *Mdhp (b-c-d-e-f-g-h-i-j)*; 57–62: *Pgm-1 (a-b-c-d-e-f)*; 63–70: *Pgm-2 (a-b-c-d-e-f-g-h)*; 71–75: *Pgdh (a-b-c-d-e)*; 76–77: *Sod (a-b)*; 78–81: *Xdh (b/c-d-e-f)*; 82–89: *Pk (a-b-c-d-e-f-g-h)*; 90–94: *Ak (a-b-c-d-e)*.

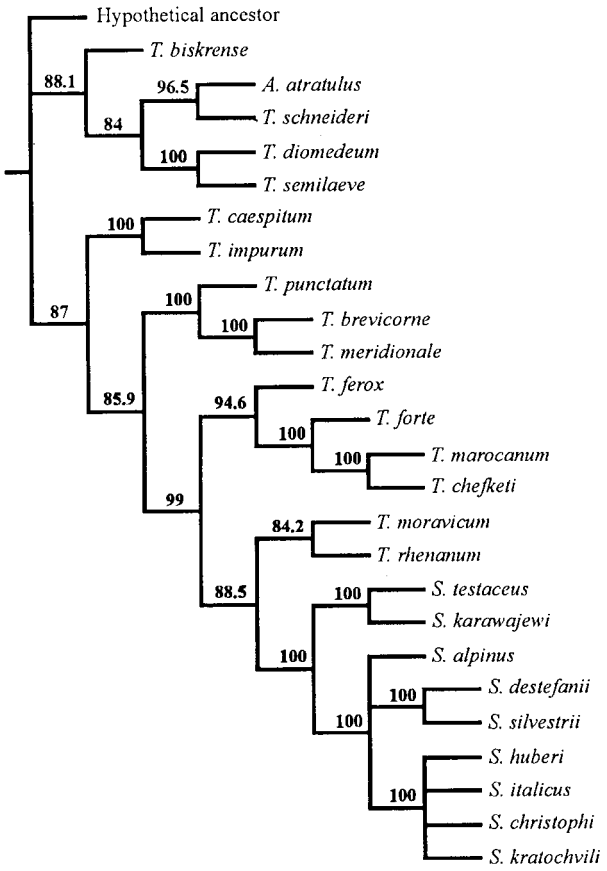

Fig. 5. Strict consensus tree of three cladograms (l 210, ci 54, ri 66) resulting from parsimony analyses using minimum turnover coding and a hypothetical ancestor. Jackknife monophyly indices (Siddall, 1995) are shown at each node to indicate relative stability of clades.

ordered or polarized. Only electromorphs at the *Pk* locus fall into this class leading to a single new branch including (*T. brevicorne* + *T. meridionale*).

DISCUSSION

Relationships within the Tetramoriini

Phylogenetic relationships among tetramoriine ants derived from different tree building methods consistently revealed that the species of the parasite genus *Strongylognathus* fall into one clade. This is not a surprise because the characteristic saber-shaped mandibles, by which the genus is easily distinguished amongst the Tetramoriini, appear as an autapomorphy relative to *Tetramorium*. Thus, the electrophoretic evidence confirms that these peculiar mandibles are not merely an adaptive convergence to parasitism but constitute a reliable guide to phylogeny. Nonetheless there have been a few accounts indirectly suggesting the polyphyly of *Strongylognathus*. In a previous allozyme study with fewer characters, a single origin of *Strongylognathus* was not recovered (Sanetra et al., 1994). Baroni Urbani (1969) asserted that the Asian *S. koreanus* arose independently from the species of the western Palaearctic. However, from a morphological viewpoint there seems to be no justification to treat *S. koreanus* differently from the other species of the *S. huberi* group. Thus, the monophyly of the genus *Strongylognathus* seems hardly disputable, as it has been corroborated by both biochemical and morphological characters.

The sister group relationship between the *S. huberi* and the *S. testaceus* group is of particular interest because two different types of parasitic relations occur within these groups. Obligate dulosis is predominant among species of the *S. huberi* group. The only known exception is *S. kratochvili*, which is supposed to live as an inquiline (Kratochvíl, 1940), while the two species of the *S. testaceus* group evidently are true inquilines which tolerate the host queen (Sanetra et al., 1999; Sanetra & Buschinger, unpublished; Kipyatkov, pers. comm.). From the available information it seems clear that the inquilines in *Strongylognathus* are derived from slave-making ancestors (e.g. Wasmann, 1905; Kutter, 1969; Acosta & Martínez, 1982), although this evolutionary pathway usually leads to queen-intolerant inquilinism (Buschinger, 1990). The transition from slave-making to queen-tolerant inquilines is unique among social parasites, and has previously been questioned for various reasons (Buschinger, 1986). Given the internal phylogeny of *Strongylognathus* presented in this study, the presumably dulotic ancestor (see above) must have split very early into one lineage maintaining dulotic behaviour and another giving rise to inquilinism. Accordingly, if the supposition that *S. kratochvili* is an inquiline were true, inquilinism must have arisen two times independently in the evolutionary history of *Strongylognathus*. The comparably high phylogenetic age of the inquilinous *S. testaceus* group is supported by morphological peculiarities and the wide range of the species *S. testaceus*. In comparison, the morphologically and genetically homogeneous species of the *S. huberi* group show the patchy geographic occurrence typical for social parasites.

The parasitic *Strongylognathus* could have speciated together with their *Tetramorium* hosts, as coevolution is particularly likely to take place between parasites and their hosts (Ridley, 1996). Adaptive radiation of *Tetramorium* host species may have simultaneously driven the parasites to cospeciate, although the transferring of parasites to new hosts (host shift) is another reasonable possibility. Several studies have demonstrated a significant tendency towards cospeciation in host-parasite evolution with only a few examples of host shifts (e.g. Brooks, 1979; Fain, 1994; Hafner et al., 1994). Phylogenies of the relevant tetramoriine taxa could not be resolved reliably enough to draw any firm conclusions about whether cospeciation or host shifts have played a primary role in shaping the evolutionary patterns in *Strongylognathus*. The unusual taxonomic diversity in the *S. huberi* group (monophyletic groups of social parasites seldom comprise more than five taxa), might be the result of cospeciation, which is likely to increase species numbers in particular clades (Thompson, 1989, 1994). Other widely distributed slave-makers, such as *Epimyrma*, have also specialized on different host species (Buschinger, 1989). Nevertheless, the limited degree of host specificity in *Strongylognathus*

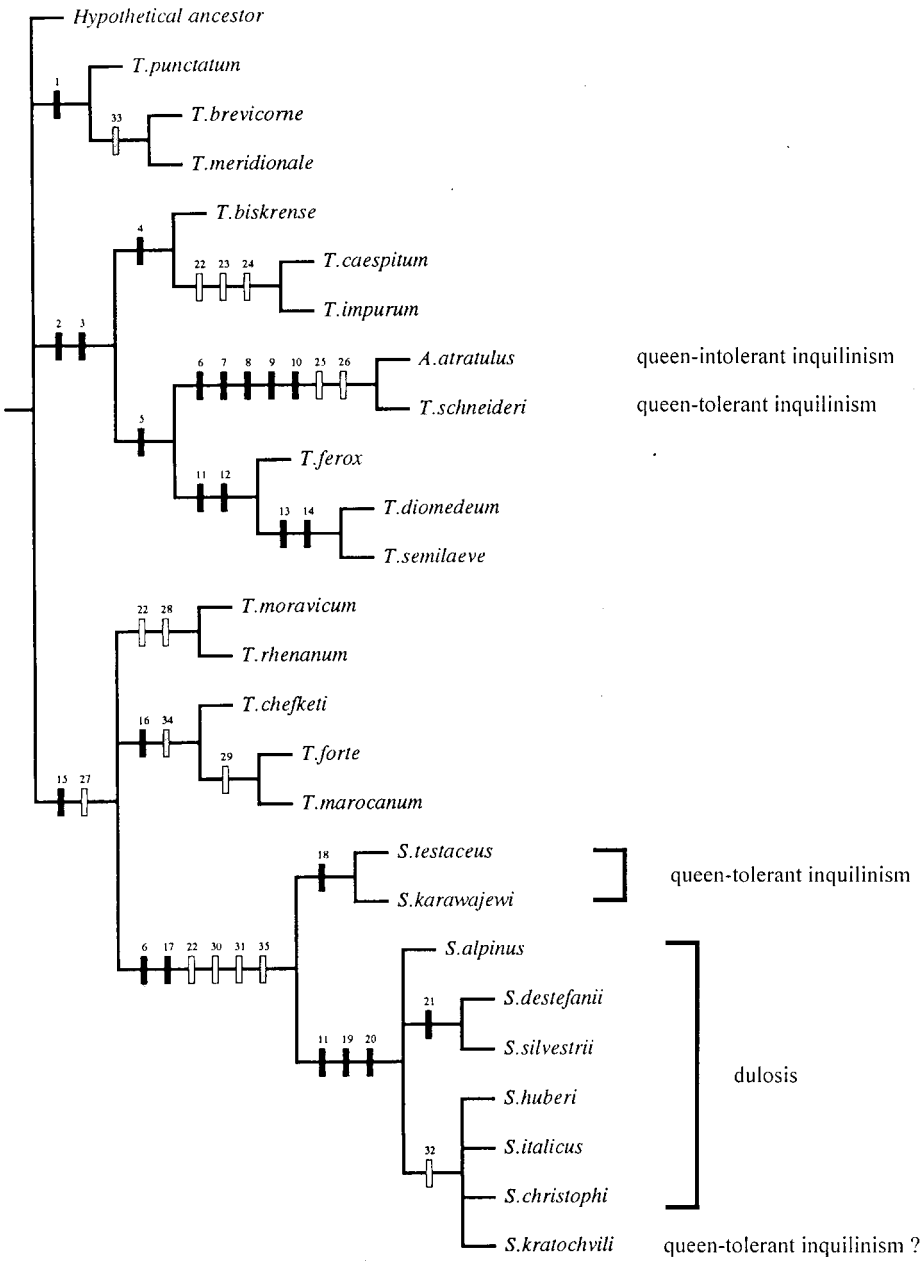

Fig. 6. Final result of the "quadraphenic evaluation procedure" by Murphy (1993) with the inclusion of characters other than allozymes. Numbers above the hashmarks refer to the following character numbers of the four different orders: Filled – first and second order, shared gains (also including some homoplasious changes): 1: *Hk-2 (b)*, 2: *G3pdh-1 (b)*, 3: *Gpi (j)*, 4: *Idh (j)*, 5: *Mdh-1 (e)*, 6: parasitism, 7: mandibles reduced and edentate, 8: sting reduced, non functional, 9: adelphogamy, males pupoidal and reduced in number, 10: two apomorphic ITS-1 base substitutions, 11: *Pgdh (a)*, 12: *Gpi (h)*, 13: *Hk-2 (a)*, 14: *Gpi (f)*, 15: *Idh (h)*, 16: *Acoh-1 (f)*, 17: mandibles elongate and saber-shaped, 18: *Pgm-2 (h)*, 19: *G3pdh-2 (b)*, 20: *Mdhp (d)*, 21: *Gpi (h)*. Open – third order, shared losses: 22: *Acoh-1 (d)*, 23: *G3pdh-1 (b)*, 24: *Idh (d,g)*, 25: *Mdh-1 (d)*, 26: *G3pdh-1 (c)*, 27: *Pgm-2 (d)*, 28: *Pgm-2 (g)*, 29: *Pgm-1 (d)*, 30: *Gpi (g)*, 31: *Pgm-2 (d,e)*, 32: *Hk-2 (c)*. Grey-scaled – fourth order, unordered characters: 33: *Pk (b)*, 34: *Pk (f)*, 35: *Pk (a)*.

(which means a wider spectrum of host species, see Table 4), especially in the *S. testaceus* group, suggests that at least some host shifts have occurred.

Since *Strongylognathus* has been established as a clade nested within the genus *Tetramorium*, the latter becomes paraphyletic. Hence, the question arises if the hosts and social parasites should be taxonomically separated. Many authors (e.g. Wilson, 1984; Carpenter et al., 1993; Ward, 1996) prefer to include hosts and parasites into a single genus when phylogenetic relationships appear as they do in this case. However, if all species included in this study were treated as a single genus, the reader not familiar with the Tetramoriini would have difficulties in keeping track of which species are the parasites and which are free-living. An aggravating problem in this case is that *Strongylognathus* Mayr, 1853 would have priority over *Tetramorium* Mayr, 1855, and the approximately 400 *Tetramorium* species then would have to be transferred to *Strongylognathus*. With respect to nomenclatural stability, we suggest that *Strongylognathus* be retained as

TABLE 4. Parasitic life habits and host species in the studied Palaearctic Tetramoriini.

Parasite	Type of parasitism	Host species	References for host species
S. huberi	dulosis	*T. caespitum*, *T.impurum*	Baroni Urbani, 1962; Sanetra et al., 1999; present study
S. christophi	dulosis	*T. caespitum*, *T.* cf. *impurum*	Radchenko, 1991; present study
S. kratochvili	degenerate dulosis (?), tolerant of host queen (?)	*T. moravicum*, *T. caespitum*	Novák & Sadil, 1941; Sanetra et al., 1994; Werner, in litt.
S. alpinus	dulosis	*T. caespitum*, *T. impurum*	Kutter, 1920; Sanetra et al., 1994, 1999
S. italicus	dulosis (probable)	*T. impurum*	Sanetra et al., 1999; present study
S. destefanii	dulosis	*T. semilaeve*, *T. impurum*	Menozzi, 1921; Sanetra et al., 1999
S. silvestrii	dulosis	*T. semilaeve*, *T. lucidulum*	Buschinger & Douwes, 1993; Sanetra et al., 1994
S. testaceus	queen-tolerant inquilinism	*T. caespitum*, *T. impurum*, *T. moravicum*, *T. ferox*, *T. brevicorne*	Novák & Sadil, 1941; Seifert, 1996; Sanetra et al., 1994, 1999
S. karawajewi	queen-tolerant inquilinism	*T. caespitum*, *T.* cf. *impurum*, *T. moravicum*, *T. karakalense*, *T. inerme*, *T. perspicax*	Radchenko, 1991; present study
A. atratulus	queen-intolerant inquilinism	*T. caespitum*, *T. impurum*, *T. moravicum*, *T. diomedeum*	Sanetra et al., 1994, 1999; Buschinger, 1995; Werner, in litt.
T. schneideri	queen-tolerant inquilinism	*T. impurum*	Buschinger, 1985, 1987, 1995; Sanetra et al., 1994

a separate genus at least until the phylogeny of the large and diverse host genus *Tetramorium* is better understood. *Anergates* and *Teleutomyrmex*, though radically different from *Tetramorium* in anatomical characters, provide similar cases in that their phylogenetic positions render the genus of their hosts paraphyletic.

The placement of *Anergates* and *Teleutomyrmex* differed according to which evolutionary model was used. While distance methods show that *Anergates* and *Teleutomyrmex* split off separately near the base of the tree, parsimony analyses using a hypothetical ancestor suggest a common origin within the remaining ingroup species. There is other evidence advocating the occurrence of common ancestry (see also character mapping in Fig. 6). First, two shared-derived base substitutions in the ITS-1 and 5.8S rRNA sequences have been reported by Baur et al. (1996). Secondly, both *Anergates* and *Teleutomyrmex* have developed a very similar form of workerless parasitism including manifold adaptations, such as physogastry, reduced mouthparts and specialized males (Kutter, 1969; Bolton, 1976). Although the latter apply well to the "inquiline syndrome" (Wilson, 1971), which refers to a specific set of characteristics convergently acquired by many inquilines, this observation cannot be taken as a simple generalization about parasite evolution. Admittedly, each of the above-mentioned characters alone is of limited value, but in its entirety all available evidence supports a common evolutionary origin of *Anergates* and *Teleutomyrmex*.

Regardless of the method, the phylogenetic hypothesis in which *Anergates* and *Teleutomyrmex* are descendants of dulotic or inquilinous *Strongylognathus* ancestors is never supported. Therefore, in contrast to the suggestions of some earlier authors (e.g. Wasmann, 1905, 1909; Kutter, 1969), extremely degenerate inquilinism does not represent the final step in the evolution of dulotic tetramoriine parasites, but rather evolved directly from free-living *Tetramorium* species. This finding also reflects the general scarcity of transitions found among different types of parasitic relations in ants (Buschinger, 1990). For instance, secondary inquilinism in *Strongylognathus* refers to such a progression. If both *Anergates* and *Teleutomyrmex* indeed had a common ancestor, an evolutionary relationship between queen-tolerant and queen-intolerant inquilinism would be evident. Buschinger (1990) suggested that *Anergates* might represent an inquiline having secondarily specialized on the exploitation of already orphaned *Tetramorium* colonies. It seems unlikely, but not impossible, that queen-tolerance in *Teleutomyrmex* is derived from that latter strategy. In the formicixenine genus *Doronomyrmex* it is also unknown which parasite type is ancestral (Bourke & Franks, 1991), queen-intolerant inquilinism in *D. goesswaldi* or queen-tolerant inquilinism in *D. pacis* and *D. kutteri*.

The extreme inquilines *Anergates* and *Teleutomyrmex* are somewhat enigmatic parasites because current theories in evolution do not seem consistent with at least some of the molecular data. Evolutionary rates of the enzyme loci appear very high compared to their *Tetramorium* hosts. This is particularly surprising in light of the low divergence of ITS-1 sequences in this group (Baur et al., 1996). A number of studies have shown that rates of molecular evolution vary considerably among hosts and parasites belonging to different taxonomic groups (e.g. Baverstock et al., 1985; Hafner et al., 1994), but are supposed to be stable among closely related species with similar life histories (see Hillis et al., 1996). Therefore, extreme specialization of parasites and high dependence on their hosts may affect patterns of molecular evolution. One would expect that certain selection pressures on parasites might be reduced, because they spend nearly their whole life cycle in the stable conditions of a hosts' nest. Under these circumstances increased accumulation of mutations could occur in some parts of the genome. This idea is indeed supported by the large genetic distances relative to *Tetramorium*, according to which the origin of *Anergates* and *Teleutomyrmex* would date back to a very early stage in tetramoriine evolution (see Figs 1–2). On the other hand, geographic allozyme variation and heterozygosities are considerably lower than in the

free-living *Tetramorium* species which suggests that positive selection has occurred. The adaptive value of allozymes has been demonstrated in several groups of insects (e.g. Barnes & Laurie-Ahlberg, 1986; Watt et al., 1986) and at least correlated phenotypic effects are known to occur within ant colonies (Keller & Ross, 1993). The phenomenon of relatively little geographic variation also applies to the striking constancy of morphological features, especially in *Anergates* which is distributed throughout the western Palaearctic (Bolton, 1976). Again, this sharply contrasts with the troublesome morphological variability in their *Tetramorium* hosts.

Multiple origins of parasitism in the tetramoriine phylogeny could clearly be demonstrated to be independent of the kind of analysis, whereas relationships among *Tetramorium* species remained largely ambiguous. The latter finding was obtained by comparing many different methods and the use of resampling techniques. Four main species assemblages of *Tetramorium* were nonetheless grouped together very frequently (as an example see the consensus tree in Fig. 3), which are (*chefketi* + (*forte* + *marocanum*)), (*punctatum* + (*brevicorne* + *meridionale*), (*caespitum* + *impurum*) sometimes together with (*moravicum* + *rhenanum*), and (*diomedeum* + *semilaeve*) two times attached to *ferox*. The most straightforward reason for this lack of accuracy is that there are not enough informative characters to provide complete resolution of trees (Swofford et al., 1996). Computer simulations recently reported by Hillis (1996) suggest that including large numbers of taxa in an analysis may be another way to enhance phylogenetic accuracy. Additional taxa have the effect of shortening the branches (according to the problem of long branch attraction stated by Felsenstein, 1978) which makes the phylogenetic signal better distinguishable from the noise of homoplasy (Hillis, 1996; Purvis & Quicke, 1997).

The presently known host range of *Tetramorium* parasites in Europe and adjacent regions is summarized in Table 4. It shows a prevalence of *T. caespitum* and *T. impurum*, but there are still significant gaps in the documentation of host use which render the establishment of a complete list of host-parasite associations impossible. In addition, due to common misidentifications of *Tetramorium* species, several questionable records have been presented. A comprehensive revisionary work on the *Tetramorium* fauna of the Palaearctis is much needed since only a few partial studies of varying quality have been published (López 1991a; Radchenko 1992a, b; Sanetra et al., 1999). Many of the described taxa currently residing in species or subspecies rank (listed by Bolton, 1995) are likely to constitute no more than intraspecific forms. For instance, the proximity of the morphologically similar *T. forte* and *T. marocanum* in phylogenetic trees suggests their taxonomic synonymy. By contrast, the extensive survey of enzyme polymorphism in the genus *Tetramorium* led to the detection of some differentiated subpopulations (not included in this study) which might represent yet unrecognized, cryptic species (for examples see Sanetra et al., 1999).

Origin of social parasitism

The results of this study do not support Emery's rule in a strict sense because neither *Strongylognathus* nor *Anergates* nor *Teleutomyrmex* are most closely related to a single host species. *Strongylognathus* shows close phylogenetic affinities to the Palaearctic *Tetramorium* species, but exact sister group relationships are not well resolved. Yet, closer relations of *Strongylognathus* to *T. caespitum*, *T. moravicum* and *T. forte* (and some related species) are supported by their frequent occurrence in neighbouring branches. Recent DNA studies had already revealed *Anergates* and *Teleutomyrmex* to be relatively closely related to their tetramoriine hosts (Baur et al., 1996). In the present study they were placed as successive outgroups of the remaining Palaearctic species when distance methods and parsimony with independent allele coding were used. In contrast, parsimony analyses involving a hypothetical ancestor suggest relationships to *T. diomedeum* and *T. semilaeve*, which are not among their regular host species. These considerations, however, are complicated by the fact that tetramoriine parasites usually have two or more hosts (see Table 4). The results thus do not show if all host species of each parasite lineage are included in the respective sister groups.

In the Palaearctic Tetramoriini there exist at least two independent origins of social parasitism, which could have been the result of different types of speciation (e.g. Hölldobler & Wilson, 1990). The interspecific hypothesis of parasite evolution suggests colonization and exploitation of congeners that have arisen allopatrically (Wilson, 1971; Hölldobler & Wilson, 1990). In contrast, the intraspecific hypothesis assumes direct development of social parasites from their host stock, invoking the highly debated evolutionary process of sympatric speciation (Buschinger 1986, 1990; Bourke & Franks, 1991). An interesting synthesis of both these hypotheses is that intraspecific parasites could somehow start to use a different species as a host and subsequently attain reproductive isolation from its non-parasitic parent species in sympatry (Bourke & Franks, 1991; Heinze, 1998; see also West-Eberhard, 1986, 1990). This evolutionary concept bears much resemblance to sympatric speciation after host shifts in phytophagous insects (Bush 1975, 1994; Ridley, 1996). It should be noticed, however, that the interspecific hypothesis as originally proposed by Wilson (1971) relies on the most questionable premise that a species can switch to parasitism without phyletic change.

We now explicitly discuss the three above-mentioned hypotheses separately from the general considerations about phylogenetic relationships, because, as recently pointed out by Lowe & Crozier (1997), these relationships alone need not be decisive regarding the evolution of social parasitism. To paraphrase, whether or not Emery's rule applies in a strict sense does not necessarily indicate the underlying mode of speciation, as has been claimed by some authors (Carpenter et al., 1993; Choudhary et al., 1994). However, the intraspecific hypothesis requires the nearest non-parasitic outgroup of the parasite(s) to be a clade that includes the host species

(Buschinger, 1990; Ward, 1996). This relaxed interpretation of Emery's rule has recently become equated with the strict version of the rule (Ward, 1996; Heinze, 1998), but the desired unambiguous definition of Emery's rule throughout the literature is made very difficult by the synonymous usage of both versions. For clarity, it might be better to distinguish between sister species or sister groups in host-parasite relationships.

Because we cannot exclude the possibility that the tetramoriine host and parasite lineages are sister groups, there is no reason to reject the intraspecific hypothesis. The logic of Carpenter et al. (1993) and Choudhary et al. (1994) who state that monophyly of a group of parasites is inconsistent with sympatric speciation cannot be accepted (see also Lowe & Crozier, 1997). Particularly, the inquilines *Anergates* and *Teleutomyrmex* may have evolved from intraspecific parasites of their ancestral host species, since inbreeding, nest-mating and polygyny are expected under the sympatric speciation model (Buschinger, 1990). Bourke & Franks (1991) also concluded that intraspecific parasitism and sympatric speciation played an important role in the evolution of inquiline ants, but some cases of Emery's rule not applying (sister group of the parasite lacks the host species) have been proposed for extremely rare inquilines in the genera *Cataglyphis* (Agosti, 1994), *Pseudomyrmex* (Ward, 1989, 1996) and *Leptothorax* (Heinze, 1998). Though the data on host use of these parasites are very scant, they were taken as the basis for questioning the general occurrence of sympatric speciation in ants (Ward, 1996). However, host-parasite relationships not obeying Emery's rule can also be explained by host transfer with subsequent sympatric speciation (West-Eberhard, 1990; Bourke & Franks, 1991; Heinze, 1998). Yet this most realistic evolutionary model, which is a synthesis of both competing hypotheses, has received only little attention in recent discussions on the subject (e.g. Choudhary et al., 1994; Ward, 1996).

Tree building methods

Distance methods have long been used for inferring phylogenies from electrophoretic data (e.g. Buth, 1984; Berlocher, 1984; Heinze, 1998), but their efficiency at accomplishing this task has been questioned for a variety of reasons (e.g. Farris, 1981; Carpenter, 1990). We employed UPGMA, Neighbor-joining and least squares statistics, whose powers in phylogenetic reconstruction must be viewed differently. The UPGMA algorithm, better referred to as phenetic clustering, is unequivocally the least preferable of these methods (e.g. Saitou & Nei, 1987; Carpenter, 1990), because the underlying assumption of a molecular clock has been seriously undermined (e.g. Avise & Aquadro, 1982; Hillis et al., 1996). Neighbor-joining and least squares statistics do not rely on rate constancy (Felsenstein, 1984, 1997). The latter of these methods is considered the most effective at recovering simulated phylogenies (Kuhner & Felsenstein, 1994).

Several authors have stated that transforming character data to genetic distances results in a considerable loss of information (e.g. Farris, 1981; Patton & Avise, 1983; Swofford et al., 1996). Alternative methods of character analysis based directly on the distribution of electromorphs (e.g. Mickevich & Johnson, 1976; Mickevich & Mitter, 1981; Richardson et al., 1986) usually reconstruct trees under the criterion of maximum parsimony. Though conceptually simple, parsimony analyses belong to a group of theoretically more defensible techniques (e.g. Farris, 1981, 1983; Carpenter, 1990). However, these may be statistically inconsistent when parallel changes outnumber non-parallel ones (Felsenstein, 1978). For analysing electrophoretic data, the most serious problem for character-based methods is their high susceptibility to missing rare alleles by sampling error (e.g. Swofford & Berlocher, 1987; Swofford et al., 1996).

The results of this study may be viewed differently with respect to the above-mentioned principles of phylogenetic inference. Conclusions derived from distance methods could be misleading in the case of *Anergates* and *Teleutomyrmex*, because of considerable evolutionary rate variation within the group. The large amount of autapomorphic characters in these extreme inquilines has a strong influence on the resulting distance trees, but does not affect parsimony analyses. In turn, synapomorphies are of much lower consequence for distance analyses.

Coding of polymorphic characters, such as allozymes and ontogenetic variants, has always been problematic and has therefore become a major subject of debate (e.g. Mickevich & Mitter, 1981, 1983; Buth, 1984; Swofford & Berlocher, 1987; Mabee & Humphries, 1993; Murphy, 1993; Wiens, 1995). The frequency parsimony algorithm proposed by Swofford & Berlocher (1987) was not used in this study, because many objections have been put forward to the assumption that allele frequencies can be treated as heritable characters (see Crother, 1990; Murphy, 1993). We also did not use multistate locus coding, because it offered much ambiguity in similar applications (e.g. Carpenter et al., 1993; Wiens, 1995) and the assumption of non-additive transformation is unlikely for allelic arrays (e.g. Mabee & Humphries, 1993; Swofford et al., 1996). From the coding formats applied to our data set, independent allele coding led to several unresolved polytomies in the consensus tree, while in a comparable study of Carpenter et al. (1993) this coding method provided best resolution of the taxa examined. However, the characters are logically linked in their loci and inferred ancestors on the cladograms are assigned no alleles for some loci making this approach biologically unrealistic (e.g. Mickevich & Mitter, 1981; Swofford & Berlocher, 1987; Murphy, 1993). Minimum turnover coding (being similar to the "scaled" method of Mabee & Humphries, 1993 but here expanded according to Mardulyn & Pasteels, 1994) performed relatively best in terms of resolution of the consensus tree.

The main problem common to all the previously mentioned coding procedures is that they equate gains with losses in terms of evolutionary steps. Murphy (1993) proposed a coding method that considers only new mutation events as well as a stepwise evaluation procedure for analysing allozyme data. An important characteristic of the latter method is that clades can be divided into different

categories with respect to their reliability. In the order of reliability, branchings are derived from shared mutation events, shared losses of alleles or from unordered parsimony analyses. We used this conceptual framework in combination with all other evidence available, involving morphology and life-habits. It has been shown that the principle of using all relevant information in a combined analysis should be the strategy of choice, as opposed to comparing independently generated trees (total evidence versus taxonomic congruence; see Kluge, 1989, 1998; Nixon & Carpenter, 1996). The result based on simultaneous analysis of allozymes and non-molecular characters with Murphy's (1993) method is therefore a particularly interesting representation of the tetramoriine data, because the underlying assumptions are the most realistic in an evolutionary sense.

In this study various tree building methods were employed to analyse a single data set (see also e.g. Avise et al., 1994; Clabaugh et al., 1996; Van der Bank & Kramer, 1996). Given the controversial discussions about different tree building methods in the literature, we deem it insufficient to rely on a single method only (see also Hillis et al. 1996). To avoid this shortcoming we compared the results from alternative procedures in order to rule out analysis-dependent effects, and we regard phylogenetic arrangements consistently resolved by all methods as the most reliable. Even if conservative summaries of phylogenetic relationships as presented here often fail to resolve many of the taxa examined, such approaches should nevertheless become more frequently applied.

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REFERENCES

Acosta F.J. & Martínez M.D. 1982: Consideraciones sobre la dulosis en el género Strongylognathus Mayr, 1853 (Hym. Formicidae). *Boln Asoc. Esp. Entomol.* **6**: 121–124.

Agosti D. 1994: A new inquiline ant (Hymenoptera: Formicidae) in Cataglyphis and its phylogenetic relationship. *J. Nat. Hist.* **28**: 913–919.

Avise C.J. & Aquadro C.F. 1982: A comparative summary of genetic distances in the vertebrates. Patterns and correlations. *Evol. Biol.* **15**: 151–182.

Avise C.J., Nelso W.S. & Sibley C.G. 1994: Why one-kilobase sequences from mitochondrial DNA fail to solve the Hoatzin phylogenetic enigma. *Mol. Phylog. Evol.* **3**: 175–184.

Barnes P.T. & Laurie-Ahlberg C.C. 1986: Genetic variability of flight metabolism in Drosophila melanogaster. III. Effects of GPDH allozymes and environmental temperature on power output. *Genetics* **112**: 267–294.

Baroni Urbani C. 1962: Studi sulla mirmecofauna d'Italia. I. *Redia* **47**: 129–138.

Baroni Urbani C. 1969: Gli Strongylognathus del gruppo huberi nell'Europa occidentale: saggio di una revisione basata sulla casta operaia (Hymenoptera Formicidae). *Boll. Soc. Entomol. It.* **99–101**: 132–168.

Baur A., Chalwatzis N., Buschinger A. & Zimmermann F.K. 1995: Mitochondrial DNA sequences reveal close relationships between social parasitic ants and their host species. *Curr. Genet.* **28**: 242–247.

Baur A., Sanetra M., Chalwatzis N., Buschinger A. & Zimmermann F.K. 1996: Sequence comparisons of the internal transcribed spacer region of ribosomal genes support close relationships between parasitic ants and their respective host species (Hymenoptera: Formicidae). *Insectes Soc.* **43**: 53–67.

Baverstock P.R., Adams M. & Beveridge I. 1985: Biochemical differentiation in bile duct cestodes and their marsupial hosts. *Mol. Biol. Evol.* **2**: 321–337.

Baverstock P.R., Cole S.R., Richardson B.J. & Watts C.H.S. 1979: Electrophoresis and cladistics. *Syst. Zool.* **28**: 214–219.

Berlocher S.H. 1984: Insect molecular systematics. *Annu. Rev. Entomol.* **29**: 403–433.

Bolton B. 1976: The ant tribe Tetramoriini (Hymenoptera: Formicidae). Constituent genera, review of smaller genera and revision of Triglyphothrix Forel. *Bull. Br. Mus. Nat. Hist. (Entomol.)* **34**: 281–379.

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. (Entomol.)* **36**: 67–151.

Bolton B. 1979: The ant tribe Tetramoriini (Hymenoptera: Formicidae). The genus Tetramorium Mayr in the Malagasy Region and in the New World. *Bull. Br. Mus. Nat. Hist. (Entomol.)* **38**: 129–181.

Bolton B. 1980: The ant tribe Tetramoriini (Hymenoptera: Formicidae). The genus Tetramorium Mayr in the Ethiopian zoogeographical region. *Bull. Br. Mus. Nat. Hist. (Entomol.)* **40**: 193–384.

Bolton B. 1995: *A New General Catalogue of the Ants of the World.* Harvard University Press, Cambridge, MA, 504 pp.

Bourke A.F.G. & Franks N.R. 1991: Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants. *Biol. J. Linn. Soc.* **43**: 157–178.

Bremer K. 1994: Branch support and tree stability. *Cladistics* **10**: 295–304.

Brooks D.R. 1979: Testing the context and extent of host-parasite coevolution. *Syst. Zool.* **28**: 299–307.

Buschinger A. 1985: New records of rare parasitic ants (Hym., Form.) in the French Alps. *Insectes Soc.* **32**: 321–324.

Buschinger A. 1986: Evolution of social parasitism in ants. *Trends Ecol. Evol.* **1**: 155–160.

Buschinger A. 1987: Teleutomyrmex schneideri Kutter 1950 and other parasitic ants found in the Pyrenees (Hymenoptera, Formicidae). *Spixiana* **10**: 81–83.

Buschinger A. 1989: Evolution, speciation, and inbreeding in the parasitic ant genus Epimyrma (Hymenoptera, Formicidae). *J. Evol. Biol.* **2**: 265–283.

Buschinger A. 1990: Sympatric speciation and radiative evolution of socially parasitic ants – heretic hypotheses and their factual background. *Z. Zool. Syst. Evol.-Forsch.* **28**: 241–260.

Buschinger A. 1995: Nicht am Ende: Die "Endameise" Teleutomyrmex schneideri. *Ameisenschutz Aktuell* **9**: 1–7.

Buschinger A. & Douwes P. 1993: Socially parasitic ants of Greece. *Biol. Gall. Hell.* **20**: 183–189.

Bush G.L. 1975: Modes of animal speciation. *Annu. Rev. Ecol. Syst.* **6**: 339–364.

Bush G.L. 1994: Sympatric speciation in animals: new wine in old bottles. *Trends Ecol. Evol.* **9**: 285–288.

Buth D.G. 1984: The application of electrophoretic data in systematic studies. *Annu. Rev. Ecol. Syst.* **15**: 501–522.

Carpenter J.M. 1988: Choosing among multiple equally parsimonious cladograms. *Cladistics* **4**: 291–296.

Carpenter J.M. 1990: Of genetic distances and social wasps. *Syst. Zool.* **39**: 391–397.

Carpenter J.M., Strassmann J.E., Turillazzi S., Hughes C.R., Solís C.R. & Cervo R. 1993: Phylogenetic relationships among paper wasp social parasites and their hosts (Hymenoptera: Vespidae; Polistinae). *Cladistics* **9**: 129–146.

Cavalli-Sforza L.L. & Edwards A.W.F. 1967: Phylogenetic analysis: models and estimation procedures. *Evolution* **21**: 550–570.

Choudhary M., Strassmann J.E., Queller D.C., Turillazzi S. & Cervo R. 1994: Social parasites in polistine wasps are monophyletic: implications for sympatric speciation. *Proc. R. Soc. Lond. (B)* **257**: 31–35.

Clabaugh J.P., Knott K.E., Wood R.M. & Mayden R.L. 1996: Systematics and biogeography of subnose darters, genus Etheostoma (Teleostei: Percidae) from the Black Warrior River System, Alabama. *Biochem. Syst. Ecol.* **24**: 119–134.

Collingwood C.A. 1956: A rare parasitic ant (Hym., Formicidae) in France. *Entomol. Mon. Mag.* **92**: 197.

Crother B.I. 1990: Is "some better than none" or do allele frequencies contain phylogenetically useful information? *Cladistics* **6**: 277–281.

Douwes P. 1990: Morphology of the parasitic myrmicine ant. In Veeresh G.K., Mallik B. & Viraktamath C.A. (eds): *Social Insects and the Environment.* Oxford & Ibh Publishing Co., New Dehli, pp. 147–148.

Emery C. 1909: Über den Ursprung der dulotischen, parasitischen und myrmekophilen Ameisen. *Biol. Zbl.* **29**: 352–362.

Fain A. 1994: Adaption, specifity and host-parasite coevolution in mites (Acari). *Int. J. Parasitol.* **24**: 1273–1283.

Farris J.S. 1969: A successive approximations approach to character weighting. *Syst. Zool.* **18**: 374–385.

Farris J.S. 1981: Distance data in phylogenetic analysis. In Funk V.A. & Brooks D.R. (eds): *Advances in Cladistics. Vol. 1.* New York Botanical Garden, New York, pp. 3–23.

Farris J.S. 1982: Outgroups and parsimony. *Syst. Zool.* **31**: 328–334.

Farris J.S. 1983: The logical basis of phylogenetic analysis. In Platnick N.I & Funk V.A. (eds): *Advances in Cladistics. Vol. 2.* Columbia University Press, New York, pp. 7–35.

Farris J.S. 1988: Hennig86: PC-DOS computer program and documentation for phylogenetic systematics (version 1.5). Port Jefferson Station, New York.

Farris J.S. 1989: The retention index and the rescaled consistency index. *Cladistics* **5**: 417–419.

Felsenstein J. 1978: Cases in which parsimony and compatibility methods will be positively misleading. *Syst. Zool.* **27**: 401–410.

Felsenstein J. 1984: Distance methods for inferring phylogenies: a justification. *Evolution* **38**: 16–24.

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

Felsenstein J. 1995: Phylip: Phylogeny Inference Package (version 3.572c for Windows). University of Washington, Seattle.

Felsenstein J. 1997: An alternating least squares approach to inferring phylogenies from pairwise distances. *Syst. Biol.* **46**: 101–111.

Fitch W.M. & Margoliash E. 1967: Construction of phylogenetic trees. *Science (Washington)* **155**: 279–284.

Forel A. 1905: Sklaverei, Symbiose und Schmarotzertum bei den Ameisen. *Mitt. Schweiz. Entomol. Ges.* **11**: 85–90.

Hafner M.S., Sudman P.D., Villablanca F.X., Spradling T.A., Demastes J.W. & Nadler S.A. 1994: Disparate rates of molecular evolution of cospeciating hosts and parasites. *Science (Washington)* **265**: 1087–1090.

Heinze J. 1991: Biochemical studies on the relationship between socially parasitic ants and their hosts. *Biochem. Syst. Ecol.* **19**: 195–206.

Heinze J. 1998: The origin of workerless parasites in Leptothorax (s. str.) (Hymenoptera: Formicidae). *Psyche* **102**: 195–214.

Hillis D.M. 1996: Inferring complex phylogenies. *Nature (London)* **383**: 130–131.

Hillis D.M., Mable K.M. & Moritz C. 1996: Applications of molecular systematics: the state of the field and a look to the future. In Hillis D.M., Moritz C. & Mable B.K. (eds): *Molecular Systematics.* Sinauer, Sunderland, MA, pp. 515–543.

Hölldobler B. & Wilson E.O. 1990: *The Ants.* Harvard University Press, Cambridge, MA, 732 pp.

Keller L. & Ross K.G. 1993: Phenotypic basis of reproductive success in a social insect: genetic and social determinants. *Science (Washington)* **260**: 1107–1110.

Kluge A.G. 1989: A concern for evidence and a phylogenetic hypothesis of relationships among Epicrates (Boidae, Serpentes). *Syst. Zool.* **38**: 7–25.

Kluge A.G. 1998: Total evidence or taxonomic congruence: cladistics or consensus classification. *Cladistics* **14**: 151–158.

Kluge A.G. & Farris S. 1969: Qualtitative phylogenetics and the evolution of anurans. *Syst. Zool.* **18**: 1–32.

Kratochvíl K. 1940: Beiträge zur Kenntnis der Ameise Strongylognathus kratochvili Šilhavý. *Věst. Čsl. Spol. Zool.* **8**: 24–46.

Kuhner M.K. & Felsenstein J. 1994: A simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates. *Mol. Biol. Evol.* **11**: 459–468.

Kutter H. 1920: Strongylognathus Huberi For. r. alpinus Wh. eine Sklaven raubende Ameise. *Biol. Zbl.* **40**: 528–538.

Kutter H. 1950: Über eine neue, extrem parasitische Ameise (Teleutomyrmex schneideri Kutter). 1. Mitteilung. *Mitt. Schweiz. Entomol. Ges.* **23**: 81–94.

Kutter H. 1969: Die sozialparasitischen Ameisen der Schweiz. *Naturforsch. Ges. Zürich (Neujahrsblatt)* No. 171, 62 pp.

López F. 1991a: Estudio morfológico y taxonómico de los grupos de especies ibéricas del género Tetramorium Mayr, 1855 (Hym.: Formicidae). *Boln Asoc. Esp. Entomol.* **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. Entomol.* **15**: 65–78.

Lowe R. & Crozier R.H. 1997: The phylogeny of bees of the socially parasitic Australian genus Inquilina and their Exoneura hosts (Hymenoptera, Anthophoridae). *Insectes Soc.* **44**: 409–414.

Mabee P.M. & Humphries J. 1993: Coding polymorphic data: examples from allozymes and ontogeny. *Syst. Biol.* **42**: 166–181.

Maddison W.P., Donoghue M.J. & Maddison D.R. 1984: Outgroup analysis and parsimony. *Syst. Zool.* **33**: 83–103.

MARDULYN P. & PASTEELS J.M. 1994: Coding allozyme data using step matrices: defining new original states for ancestral taxa. *Syst. Zool.* **43**: 567–572.

MENOZZI C. 1921: Formiche dei dintorni di Sambiase di Calabria. *Boll. Lab. Zool. Gen. Agr. Portici* **15**: 24–32.

MICKEVICH M.F. & JOHNSON M.S. 1976: Congruence between morphological and allozyme data in evolutionary inference and character evolution. *Syst. Zool.* **25**: 260–270.

MICKEVICH M.F. & MITTER C. 1981: Treating polymorphic characters in systematics: a phylogenetic treatment of electrophoretic data. In Funk V.A. & Brooks D.R. (eds): *Advances in Cladistics. Vol. 1.* New York Botanical Garden, New York, pp. 45–58.

MICKEVICH M.F. & MITTER C. 1983: Evolutionary patterns in allozyme data: a systematic approach. In Platnick N.I. & Funk V.A. (eds): *Advances in Cladistics. Vol. 2.* Columbia University Press, New York, pp. 169–176.

MICKEVICH M.F. & WELLER S.J. 1990: Evolutionary character analysis: tracing character change on a cladogram. *Cladistics* **6**: 137–170.

MORAN G.F., BELL J.C. & PROBER S. 1990: The utility of isozymes in the systematics of some Australian tree groups. *Aust. Syst. Bot.* **3**: 47–57.

MURPHY R.W. 1993: The phylogenetic analysis of allozyme data: invalidity of coding alleles by presence/absence and recommended procedures. *Biochem. Syst. Ecol.* **21**: 25–38.

MURPHY R.W., SITES J.W. JR., BUTH D.G. & HAUFLER C.H. 1996: Proteins: isozyme electrophoresis. In Hillis D.M., Moritz C. & Mable B.K. (eds): *Molecular Systematics.* Sinauer, Sunderland, MA, pp. 51–120.

NEI M. 1972: Genetic distance between populations. *Am. Nat.* **106**: 283–292.

NIXON K.C. 1998: Clados: Computer program and documentation for cladogram character analysis (version 1.6.1.). Cornell University, Ithaca, New York.

NIXON K.C. & CARPENTER J.M. 1993: On outgroups. *Cladistics* **9**: 413–426.

NIXON K.C. & CARPENTER J.M. 1996: On simultaneous analysis. *Cladistics* **12**: 221–241.

NOVÁK V. & SADIL J. 1941: Klíč k určování mravenců střední Evropy. [Identification key to ants of central Europe.] *Entomol. Listy* **4**: 65–115 (in Czech).

PATTON J.C. & AVISE J.C. 1983: An empirical evaluation of qualitative Hennigian analyses of protein electrophoretic data. *J. Mol. Evol.* **19**: 244–254.

PEDERSEN B.V. 1996: A phylogenetic analysis of cuckoo bumblebees (Psithyrus, Lepeletier) and bumblebees (Bombus, Latreille) inferred from sequences of the mitochondrial gene cytochrome oxidase I. *Mol. Phylog. Evol.* **5**: 289–297.

PISARSKI B. 1966: Étude sur les fourmis du genre Strongylognathus Mayr (Hym.: Formicidae). *Ann. Zool.* **23**: 509–523.

PLATNICK N.I. 1989: An empirical comparison of microcomputer parsimony programs, II. *Cladistics* **5**: 145–161.

PREVOSTI A., OCANA J. & ALONSO G. 1975: Distances between populations of Drosophila subobscura based on chromosome arrangement frequencies. *Theor. Appl. Genet.* **45**: 231–241.

PURVIS P. & QUICKE D.L.J. 1997: Building phylogenies: are the big easy? *Trends Ecol. Evol.* **12**: 49–50.

RADCHENKO A.G. 1991: Ants of the genus Strongylognathus (Hymenoptera, Formicidae) of the USSR. *Zool. Zh.* **70**: 84–90 (in Russian).

RADCHENKO A.G. 1992a: Ants of the genus Tetramorium (Hymenoptera, Formicidae) in the fauna of the USSR. Part 1. *Zool. Zh.* **71**: 39–49 (in Russian).

RADCHENKO A.G. 1992b: Ants of the genus Tetramorium (Hymenoptera, Formicidae) in the fauna of the USSR. Part 2. *Zool. Zh.* **71**: 50–58 (in Russian).

RICHARDSON B.J., BAVERSTOCK P.R. & ADAMS M. 1986: *Allozyme Electrophoresis. A Handbook for Animal Systematics and Population Structure.* Academic Press, Sydney, 410 pp.

RIDLEY M. 1996: *Evolution. 2nd ed.* Blackwell Science Inc., Cambridge, MA, 719 pp.

ROHLF F.J. 1990: Ntsys-pc: Computer program for numerical taxonomy and multivariate analysis (version 1.60). Exceter, New York.

ROGERS J.S. 1972: Measures of genetic similarity and genetic distance. *Stud. Genet. VII. Univ. Texas Publ.* No. 7213, pp. 145–153.

SAITOU N. & NEI M. 1987: The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.

SANDERSON M.J. 1989: Confidence limits on phylogenies: the bootstrap revisited. *Cladistics* **5**: 113–129.

SANETRA M. & BUSCHINGER A. 1996: Slave raiding behaviour in socially parasitic Strongylognathus ants (Hymenoptera: Formicidae). *Proc. XX. Int. Congr. Entomol., Firenze, 1996.* Firenze, p. 399.

SANETRA M., GÜSTEN R. & SCHULZ A. 1999: On the taxonomy and distribution of Italian Tetramorium species and their social parasites (Hymenoptera: Formicidae). *Mem. Soc. Entomol. It.* **77**: 317–357.

SANETRA M., HEINZE J. & BUSCHINGER A. 1994: Enzyme polymorphism in the ant genus Tetramorium Mayr and its social parasites (Hymenoptera: Formicidae). *Biochem. Syst. Ecol.* **22**: 753–759.

SEIFERT B. 1996: *Ameisen beobachten, bestimmen.* Naturbuch Verlag, Augsburg, 352 pp.

SHOEMAKER D.D., COSTA J.T. & ROSS K.G. 1992: Estimates of heterozygosity in two social insects using a large number of electrophoretic markers. *Heredity* **69**: 573–582.

SIDDALL M.E. 1994: Random Cladistics: Computer program and documentation for testing tree stability. Available from ftp://zoo.edu.toronto/pub/random.zip.

SIDDALL M.E. 1995: Another monophyly index: revisiting the jackknife. *Cladistics* **11**: 33–56.

SNEATH P.H.A. & SOKAL R.R. 1973: *Numerical Taxonomy.* W.H. Freeman & Co., San Francisco, 573 pp.

STUMPER R. 1951: Teleutomyrmex schneideri Kutter (Hymenoptera: Formicidae). 2. Mitteilung über die Lebensweise der neuen Schmarotzerameise. *Mitt. Schweiz. Entomol. Ges.* **24**: 129–152.

SWOFFORD D.L. & BERLOCHER S.H. 1987: Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. *Syst. Zool.* **36**: 293–325.

SWOFFORD D.L., OLSEN G.J., WADDELL P.J. & HILLIS D.M. 1996: Phylogenetic inference. In Hillis D.M., Moritz C. & Mable B.K. (eds): *Molecular Systematics.* Sinauer, Sunderland, MA, pp. 407–514.

THOMPSON J.N. 1989: Concepts of coevolution. *Trends Ecol. Evol.* **4**: 179–183.

THOMPSON J.N. 1994: *The Coevolutionary Process.* University of Chicago Press, Chicago, 376 pp.

VAN DER BANK F.H. & KRAMER B. 1996: Phylogenetic relationships between eight African species of mormyriform fish (Teleostei, Osteichthyes): resolution of a cryptic species, and reinstatement of Cyphomyrus Myers, 1960. *Biochem. Syst. Ecol.* **24**: 275–290.

WARD P.S. 1989: Genetic and social changes associated with ant speciation. In Breed M.D. & Page R.E. (eds): *The Genetics of Social Evolution.* Westview Press, Boulder, CO, pp. 123–148.

WARD P.S. 1996: A new workerless parasite in the ant genus Pseudomyrmex (Hymenoptera: Formicidae), with a discussion of the origin of social parasitism in ants. *Syst. Entomol.* **21**: 253–263.

WASMANN E. 1905: Ursprung und Entwickelung der Sklaverei bei den Ameisen. *Biol. Zbl.* **25**: 273–292.

WASMANN E. 1909: Über den Ursprung des sozialen Parasitismus, der Sklaverei und der Myrmekophilie bei den Ameisen. *Biol. Zbl.* **29**: 619–637, 651–663.

WATROUS L.E. & WHEELER Q.D. 1981: The out-group comparison method of character analysis. *Syst. Zool.* **30**: 1–11.

WATT W.B., CARTER P.A. & DONOHUE K. 1986: Females' choice of "good genotypes" as mates is promoted by an insect mating system. *Science (Washington)* **233**: 1187–1190.

WHEELER W.M. 1910: *Ants: Their Structure, Development, and Behaviour.* Columbia University Press, New York, 663 pp.

WEST-EBERHARD M.J. 1986: Alternative adaptations, speciation, and phylogeny. A review. *Proc. Natn. Acad. Sci. USA* **83**: 1388–1392.

WEST-EBERHARD M.J. 1990: Parallel size-related alternatives in males and females and the question of sympatric speciation in Hymenopteran social parasites. In Veeresh G.K., Mallik B. & Viraktamath C.A. (eds): *Social Insects and the Environment.* Oxford & Ibh Publishing Co., New Dehli, pp. 162–163.

WIENS J.J. 1995: Polymorphic characters in phylogenetic systematics. *Syst. Biol.* **44**: 482–500.

WILSON E.O. 1971: *The Insect Societies.* Harvard University Press, Cambridge, MA, 548 pp.

WILSON E.O. 1984: Tropical social parasites in the ant genus Pheidole, with an analysis of the anatomical inquiline syndrome (Hymenoptera: Formicidae). *Insectes Soc.* **31**: 316–334.

Appendix 1. Material examined in this study

Abbreviation codes in parentheses following each locality refer to colony numbers in our collection.

Independent species: ***Tetramorium lanuginosum*** (n = 1). Thailand: Nakhon Si Thammarat, Ban Nai Plao, 10 km S Khanom, 100 m (T 553), leg. Schulz & Vock. – ***T.* cf. *centum*** (n = 1). Thailand: Prov. Satun, Thale Ban National Park, 20–30 km E Satun, 200–400 m (T 552), leg. Schulz & Vock. – ***T. kheperra*** (n = 1). Thailand: Prov. Nakhon Si Thammarat, Phromm Lok Waterfall, 25 km NW Nakhon, 100–300 m (T 554), leg. Schulz & Vock. – ***T. acutisetum*** (n = 2). Morocco: Rif, Rd S 603, 9 rkm dir. Ksar-el-Kebir, ca. 10 km SW Chefchaouen, ca. 600 m (T 473), Forét de la Mamora, ca. 2 rkm N Ain-Johra, ca. 100 m (T 474), leg. Aßmuth, Sanetra & Schulz. – ***T. caldarium*** (n = 1). United States: Caribbean Sea, Barbados (T 517), leg. J. Heinze. – ***T. simillimum*** (n = 1). Côte d'Ivoire, Lomoc National Park (T 431). – ***T. insolens*** (n = 2). Malaysia: Sarawak-Borneo, Bako Park (T 145), leg. Buschinger. Germany: Düsseldorf, Aqua-Zoo (T 237), leg. Hauser. – ***T. hispidum*** (n = 2). United States: Arizona, Paradise, Cochise Co., 1650 m (T 235–236), leg. J. Heinze. – ***T. caespitum*** (n = 57). Germany: Bavaria, Homburg nr Marktheidenfeld (T 172–175), Würzburg (T 508), leg. Buschinger. Hesse, Rhine river, 2 km N Lorchhausen, ca. 100 m (T 188–189, 510); Seeheim-Jugenheim (T 371); Rheinland-Pfalz, Cochem/Mosel (T 514), leg. Sanetra. Austria: Niederösterreich, ca. 3 km NE St. Pölten, 250 m (T 200); Staatz, 250 m (T 209, 211–212); Purgstall, 300 m (T 214–215), leg. Dietrich & Sanetra. Hungary: Komárom, 4 km W Süttö, 200–300 m (T 217); Bács-Kiskun, ca. 3 km SW Jakabszallas (T 225–226); ca. 1 km N Bócsa, nr Kis-Molnar Tanya (T 227–228), leg. Sanetra. Italy: Prov. Udine, M. Simeone NW Gemona Friuli, nr Bordano, 300–600 m (T 39, 564); Prov. Cuneo, Val Gesso, nr Valdieri, 600 m (T 81–83); Sicily, Prov. Messina, Nebrodi, N. 289 Cesaro-S. Fratello, P. Femmina Morta, 1500 m (T 316–318), leg. Sanetra. Calabria, Prov. Reggio di Calabria, 2 km W Melito di Porto Salvo, ca. 5 m (T 320); Aspromonte, Prov. Reggio di Calabria, Montalto, 1950 m (T 324–326); Sila Grande, Prov. Cosenza, Lago Arvo, ca. 1200 m (T 331); M. Botte Donato, ca. 1800 m (T 333), leg. Güsten, Sanetra & Schulz. Greece: Peloponnesos, Killini northern slope, ca. 45 km W Korinthos, 1600–1800 m (T 364–365), leg. Schulz & Vock. France: Dep. Alpes de Hautes-Provence, ca. 6 km NNE Jausiers, ca. 1300 m (T 405), leg. Buschinger, Güsten, Sanetra & Schumann. Spain: Prov. Jaén, Sierra de Cazorla, Rio Guadalquivir, ca. 25 rkm S Tranco, ca. 800 m (T 486); Prov. Cuenca, Rio Jucar, ca. 10 rkm NE San Lorenzo de la Parilla, ca. 600 m (T 490); Prov. Teruel, Rio Guadalaviar, 5 rkm NE Albarracin, ca. 1200 m (T 499); Rio de la Fuente del Berro, 4 rkm E Calomarde, SW Albarracin, ca. 1200 m (T 501–502); Prov. Huesca, Puerto de Monrepós, ca. 30 km N Huesca, ca. 1300 m (T 503–505); Embalse de Búbal, nr El Pueyo de Jaca, N Biescas, ca. 1100 m (T 506), leg. Aßmuth & Sanetra. Ukraine: Crimea, Nykyta, National Reserve, ca. 100 m (T 520–521); SW Bakchysaraj, Manhup-Kale, 300–500 m (T 523, 525); S Simferopol', nr Zalis's'a, 300–500 m (T 526, 531–533, 535–536), leg. Buschinger, Kipyatkov, Radchenko & Sanetra. – ***T. impurum*** (n = 52). Germany: Bavaria, nr Leidersbach, ca. 300 m (T 176); Hesse, Rhine river, 2 km N Lorchhausen, ca. 100 m (T 186), leg. Sanetra. Switzerland: Valais, Saas Fee, 1800 m (T 98, 103); St. Luc, Tignousa, 1600–2200 m (T 108–110); Lötschen valley, nr Eisten, 1600–2000 m (T 116–119); Moosalp, nr Visp, 2000–2100 m (T 128), leg. Buschinger, Güsten & Sanetra. France: Pyrenees, Dep. Basses-Pyrénées, Val d'Osseau, Lac de Fabrèges – Cabane du Lurien, 1600–1700 m (T 131–133), leg. Sanetra; Cevennes, Dep. Lozère, Mont Lozère, Pont du Tarn, 1300 m (T 136); Causse Méjeau, Nimes-le-Vieux (Chaos), 1100 m (T 137), leg. Buschinger; Dep. Hautes-Alpes, NW Briançon, Col de Granon, 2000-2100 m (T 380–384); S Col d'Izoard, nr Arvieux, 1700–1800 m (T 401–402); Dep. Alpes de Hautes-Provence, ca. 6 km NNE Jausiers, ca. 1300 m (T 407), leg. Buschinger, Güsten, Sanetra & Schumann. Hungary: Pest, Donau, 3 km E Dömös (T 223–224); Somogy, 4 km W Nagybajom (T 229), leg. Sanetra. Italy: Calabria, M. Pollino, Prov. Cosenza, ca. 8 km E Morianno, 1200–1300 m (T 344); 4 km NW Morano Calabro, 1000–1100 m (T 347–348); Basilicata, M. Pollino, Prov. Potenza, nr Rif. di Gasperi, ca. 8 km SE Rotonda, 1600 m (T 345); Abruzzi, Gran Sasso, Prov. L'Aquila, ca. 6 km NE Castel del Monte, 1600 m (T 357–359), leg. Güsten, Sanetra & Schulz. Spain: Prov. Granada, Sierra Nevada, Rd GR 420 ca. 24 km SE Granada, ca. 1700 m (T 475); Rd GR 420, 2 rkm E Rd dir. Mirador de Canales, 2200 m (T 478–481); Puerto de la Ragua, 2000–2100 m (T 482–483), leg. Aßmuth & Sanetra. Ukraine: Crimea, SW Bachcysaraj, Manhup-Kale, 300–500 m (T 522); S Simferopol', nr Zalis's'a, 300–500 m (T 534); Catyr-Dah, nr pec Cholodna, ca. 1000 m (T 537–539); Aj-Petri, SW Jalta, ca. 1200 m (T 545–549), leg. Buschinger, Kipyatkov, Radchenko & Sanetra. – ***T. moravicum*** (n = 19). Czech Republic: Bohemia, nr Nebrich (T 195), leg. Buschinger; Moravia, Mohelensko hadcová step, nr Mohelno, 300 m (T 201–202, 204–207), leg. Dietrich & Sanetra. Austria: Niederösterreich, Staatz, 250 m (T 208, 210, 213), leg. Dietrich & Sanetra. Hungary: Pest, Visegrad, 200–300 m (T 219–222), leg. Sanetra. Ukraine: Crimea, SW Bakchysaraj, Manhup-Kale, 300–500 m (T 524); S Simferopol', nr Zalis's'a, 300–500 m (T 527–528); Catyr-Dah, nr pec Cholodna, ca. 1000 m (T 540); Kara-Dah, E Kurortne, 100–500 m (T 543), leg. Buschinger, Kipyatkov, Radchenko & Sanetra. – ***T. rhenanum*** (n = 6). Germany: Hesse, Rhine river, 2 km N Lorchhausen, ca. 100 m (T 182–184, 509, 511–512), leg. Sanetra & Schulz. France: Dep. Alpes

Maritimes, nr Maurioun, ca. 6 km N Breil-sur-Roya, 450–700 m (T 409–410), leg. Schulz. – ***T. forte*** (n = 8). Spain: Prov. Granada, Sierra Nevada, Rd GR 420, ca. 3 rkm NW Sierra Nevada, ca. 1900 m (T 476–477); Prov. Jaén, SE Desfiladero de Despeñaperros, Pto. de los Jardines, 870 m (T 487–489); Prov. Cuenca, 2 rkm E Villalba de la Sierra, ca. 20 km N Cuenca, ca. 1200 m (T 491); 2 rkm N Rd Beamud-Buenache dir. Embalse de la Toba, NE Cuenca, ca. 1400 m (T 493); Prov. Teruel, Rio Guadalaviar, 5 rkm NE Albarracin, ca. 1200 m (T 498), leg. Aßmuth & Sanetra. – ***T. marocanum*** (n = 2). Morocco: Middle Atlas, Rd 3325, 6 rkm N Rd S 309, 6 km SE Ifrane, ca. 1800 m (T 444); Middle Atlas, Rd S 303, ca. 24 rkm S Ain Leuh, ca. 1300 m (T 465), leg. Aßmuth, Güsten, Sanetra, Schulz & Schumann. – ***T. chefketi*** (n = 3). Greece: Prov. Pieriá, 4 km E Litóhoro, 50 m (T 571); Stená Pétras, 45 km WSW Katerini, 300–500 m (T 574–575), leg. Schulz & Vock. – ***T. biskrense*** (n = 5). Tunisia: Djebel Bou Hedma, ca. 25 km SW Mezzouna, 200–300 m (T 146–149); Medjerda, 20 km SW Jendouba, 400–500 m (T 152), leg. Sanetra. – ***T. brevicorne*** (n = 5). Italy: Sardinia, Prov. Sassari, Monte Limbara, 700–800 m (T 285–286); Monte Limbara, 1000–1100 m (T 289); Prov. Nuoro, N. 125 Dorgali-Baunei, ca. 13 km NW Genna Coggina, 800–900 m (T 293–294); N. 198 Seui-Ussassai, Cant. Arcueri, 980 m (T 301–302), leg. Sanetra. – ***T. semilaeve*** (n = 84). France: Dpt. Pyrenées orientales, 4 rkm NW Banyuls sur Mer, ca. 100 m (T 507), leg. Aßmuth & Sanetra. Spain: Prov. Cuenca, ca. 2 rkm E Villalba de la Sierra, ca. 20 km N Cuenca, ca. 1200 m (T 492); Prov. Jaén, Sierra de Cazorla, 1 Rkm NW Tiscar, ca. 900 m (T 484); Rd Cazorla-Tranco, ca. 14 rkm NE Burunchel, ca. 800 m (T 485), leg. Aßmuth & Sanetra; Gran Canaria, Tejeda, Roque Bentaiga, ca. 1400 m (T 240–241); Santa Brigida, Calde de Bandama, ca. 500 m (T 242); San Bartolome, Morro de las Vacas, ca. 1200 m (T 243–244); Artenara, Altavista, ca. 1300 m (T 245–246); Artenara, Caldera Pinar de Galdar (T 247); San Bartolome, Cruz de San Antonio, ca. 900 m (T 248); Valleseco, Balcón de Zamora, ca. 800 m (T 249), leg. Buschinger. Italy: Sardinia, Prov. Sassari, Fiume Coghinas, ca. 8 km NE Perfugas, ca. 100 m (T 281–282); Prov. Nuoro, ca. 5 km S Bitti, ca. 600 m (T 291); N. 125 Dorgali-Baunei, ca. 13 km NW Genna Coggina, 800–900 m (T 292); Lago alto del Flumendosa, ca. 800 m (T 297); Sicily, Prov. Catania, ca. 5 km W Ramacca, ca. 400 m (T 305); Prov. Syrakus, ca. 5 km NE Floridia, ca. 100 m (T 310); 5 km NE Canicattini Bagni, 300 m (T 312); Prov. Catania, Etna, ca. 5 km N Ragalna, 1000–1200 m (T 313), leg. Sanetra; Campania, Prov. Salerno, 2 km E Montecorvino, ca. 350 m (T 319); Calabria, Prov. Reggio di Calabria, ca. 4 km N Bova, ca. 1100 m (T 321); Prov. Catanzaro, Terme Caronte, ca. 2 km NW Sambiase di Calabria, 200–300 m (T 327–330); 3 km E Savelli, ca. 700 m (T 334–335); 2 km NW Umbriatico, ca. 350 m (T 340, 342), leg. Güsten, Sanetra & Schulz. Malta: Ghar Lapsi, ca. 5 km SW Siggiewi, ca. 50 m (T 432); Delimara-Peninsula, ca. 2 km SE Marsaxlokk (T 433); Mistra Valley, ca. 2 km SE Mellieha, ca. 50 m (T 434–436); Isola Gozo, ca. 2 km NW Zebbug, nr Forna Point, ca. 20 m (438–439); Isola Comino, ca. 20 m (T 440), leg. Sanetra. Tunisia: Tabarka, ca. 50 m (T 153–154); Mogod, nr Ain El Kefma, ca. 20 km W Mateur, ca. 200 m (T 155–156), leg. Sanetra. Morocco: Rif, 4 km W Chefchaouen, ca. 500 m (T 443); High Atlas, Tizi-n-Talrhemt, ca. 25 rkm SE Midelt, ca. 1800 m (T 445); Gorges du Todra, ca. 15 rkm N Tinerhir, ca. 1500 m (T 446); 2 rkm N Tizi-n-Test, ca. 2000 m (T 450–451); 1–2 rkm N Imlil, ca. 1700 m (T 453); Oued R'Mat nr Chouiter, ca. 20 rkm SE Marrakech, ca. 500 m (T 454); High Atlas, ca. 3 rkm N Tizi-n-Tichka, 2000–2100 m (T 456–457); Middle Atlas, ca. 15 rkm W Azilal, ca. 1300 m (T 458); Prov. Beni-Mellal, Rd 1805A, ca. 10 rkm N Souk-el-Arba-Ouakbli, ca. 1300 m (T 461); Rd 1805A, 1 rkm S Rd 1901, 23 rkm S El-Ksiba ca. 1300 m (T 462–463); Jbel Tazzeka, ca. 1800 m (T 466); Rif, Prov. Chefchaouen, ca. 5–15 rkm NW Zoumi, 600–900 m (T 469–470); Jbel Tisirene, ca. 5 km NW Bab-Berret, 1600–1900 m (T 471); Rd S 603, 9 Rkm dir. Ksar-el-Kebir, ca. 10 km SW Chefchaouen, ca. 600 m (T 472), leg. Aßmuth, Güsten, Sanetra, Schulz & Schumann. Greece: Peloponnesos, nr Pteri, 15 km S Egion, ca. 30 km E Patras, ca. 1100 m (T 367–370), leg. Schulz & Vock; Crete, Prov. Rethimnon, ca. 3 km N Kouroutes, ca. 800 m (T 65); 3 km E Hordaki, ca. 200–300 m (T 70); nr Koxare, 200–300 m (T 72), leg. Sanetra & Schulz; Cyprus, Prov. Paphos, ca. 2 km NW Simou, ca. 500 m (T 250); ca. 1 km W Kritou Marottou, ca. 500 m (T 252–255); nr Nikoklia, Lake Asprokremmos, ca. 200 m (T 257); Arminou, nr Ayios Ioannis, ca. 800 m (T 258–260); ca. 2 km E Pano Panayia, ca. 800 m (T 261–262); Prov. Limassol, ca. 4 km NE Omodhos, ca. 800 m (T 264); Prov. Paphos, ca. 2 km SW Kedhares, ca. 800 m (T 265), leg. Sanetra. Turkey: Prov. Antalya, Finike/Limyra (T 159); Fethiye-Ölüdeniz (T 160), leg. Buschinger. – ***T. punctatum*** (n = 8). Italy: Sicily, Prov. Messina, Francavilla di Sicilia, 300 m (T 166); Prov. Catania, ca. 5 km W Ramacca, ca. 400 m (T 303–304); nr Carlentini, ca. 200 m (T 306), leg. Sanetra; Calabria, Prov. Reggio di Calabria, ca. 4 km N Bova, ca. 1100 m (T 322–323); Prov. Catanzaro, 3 km E Savelli, ca. 700 m (T 338); 2 km NW Umbriatico, ca. 350 m (T 339), leg. Güsten, Sanetra & Schulz. – ***T. meridionale*** (n = 5). Italy: Sardinia, Prov. Sassari, Lago Coghinas, ca. 10 km NW Oschiri, ca. 200 m (T 283); Prov. Nuoro, ca. 5 km S Bitti, ca. 600 m (T 290); Lago alto del Flumendosa, ca. 800 m (T 295–296, 298), leg. Sanetra. – ***T. ferox*** (n = 1). Czech Republic: Moravia, Mohelenská hadcová step, nr Mohelno, 300 m (T 203), leg. Dietrich & Sanetra. – ***T. diomedeum*** (n = 10). Italy: Sicily, Prov. Syrakus, ca. 5 km NE Floridia, ca. 100 m (T 307–309); Prov. Catania, Etna, ca. 5 km N Ragalna, 1000–1200 m (T 314), leg. Sanetra; Calabria, Prov. Catanzaro, Terme Caronte, ca. 2 km NW Sambiase di Calabria, 200–300 m (T 329); Prov. Catanzaro, 3 km E Savelli, ca. 700 m (T 336–337); 2 km NW Umbriatico, ca. 350 m (T 341); M. Pollino, Prov. Cosenza, 4 km N Morano Calabro, ca. 800 m (T 351), leg. Güsten, Sanetra & Schulz. Malta: Isola Gozo, Dwejra Bay, nr Quara Tower, ca. 20 m (T 437), leg. Sanetra.

Social Parasites: ***Strongylognathus huberi*** (n = 2) – Italy: Puglia, Gargano, Prov. Foggia, 2 rkm NE inters. to Carpino (N. 528), 700 m (S 355), leg. Güsten & Sanetra. Spain: Sierra Nevada, Prov. Granada, Rd GR 420, 2 Rkm E Rd dir. Mirador de Canales, 2200 m (S 480), leg. Aßmuth & Sanetra. – ***S. christophi*** (n = 10). Ukraine: Crimea, SW Bakhchysaraj, Manhup-Kale, 300–500 m (S 525); S Simferopol', nr Zalis's'a, 300–500 m (S 531–532); Catyr-Dah, nr pec Cholodna, 1000 m (S 538–539); Aj-Petri, SW Jalta, 1200 m (S 545–549), leg. Buschinger, Kipyatkov, Radchenko & Sanetra. – ***S. kratochvili*** (n = 1). Czech Republic, Mohelenská hadcová step, nr Mohelno, 300 m (S 207), leg. Dietrich & Sanetra. – ***S. alpinus*** (n = 13). Switzerland: Valais, Lötschen valley, nr Eisten, 1600–2000 m (S 116–119), leg. Buschinger, Güsten & Sanetra; Italy: Sicily, Prov. Messina, Nebrodi, Cesaró-S. Fratello (N. 289), P. Femmina Morta, 1500 m (S 316–318), leg. Sanetra; Calabria, Aspromonte, Prov. Reggio di Calabria, Montalto summit, 1950 m (S 325–326), leg. Güsten, Sanetra & Schulz. France: Dep. Hautes Alpes, NW Briancon, Col de Granon, 1770 m (S 390); S Col d'Izoard, nr Arvieux, 1700–1800 m (S 401–403), leg. Buschinger, Güsten, Sanetra & Schumann. – ***S. italicus*** (n = 1). Italy: Elba, Monte Maolo, ca. 200 m dir. M. Capanne, 750 m (S 560), leg. Sanetra. – ***S. destefanii*** (n = 6). Italy: Sicily, Prov. Syrakus, 5 rkm NE Floridia, 300 m (S 162); 5 rkm NE Floridia, 100 m (S 310–311); Calabria, Prov. Catanzaro, 3 rkm E Savelli, 700 m (S 335); M. Pollino, Prov. Cosenza, 1 rkm NW Frasci-

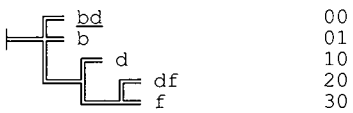
neto, 4 rkm E Castrovillari, 500 m (S 349); Puglia, Gargano, Prov. Foggia, Str. M. San Angelo-Carpino, 1.5 rkm NW inters. to Vico, 700 m (S 356). – ***S. silvestrii*** (n = 7). Greece: Crete, Prov. Rethimnon, 1 rkm NW Melambes, 500 m (S 63); Psiloritis-Oros, 10 rkm S Anogia, 1400 m (S 69); Prov. Chania, 1 rkm S Anisaraki, 700–800 m (S 74–75), leg. Sanetra & Schulz; Peloponnesos, Taygetos Oros, Profitis Ilias, 1800–2000 m (S 361); Prov. Préveza, 3 rkm NE Kamarina, (Zalongo), 400–600 m (S 580–581), leg. Schulz & Vock. – ***S. testaceus*** (n = 20). Italy: Prov. Cuneo, Val Gesso, nr Valdieri, 600 m (S 81); Sardinia, Prov. Sassari, M. Limbara, 1000–1100 m (S 289); Prov. Nuoro, N. 125 Dorgali-Baunei, 13 rkm NW Genna Coggina 800–900 m (S 294), leg. Sanetra; Calabria, Sila Grande, Prov. Cosenza, Lago Arvo, 1200 m (S 331); M. Pollino, Prov. Cosenza, 4 km NW Morano Calabro, 1000–1100 m (S 348), leg. Güsten, Sanetra & Schulz. France: Pyrenees, Dep. Basses-Pyrénées, Val d'Osseau, Lac de Fabrèges-Cabane du Lurien, 1600–1700 m (S 132–133, 141–142), leg. Buschinger, Douwes & Sanetra. Germany: Bavaria, Homburg nr Marktheidenfeld (S 170–172), leg. Buschinger; Hesse, Rhine river, 2 rkm N Lorchhausen, 100 m (S 189), leg. Güsten, Sanetra & Schulz; Saxony, Gohrischer Heide, 3 km NW Zeithain (S 378), leg. Güsten & Schulz. Spain: Prov. Granada, Sierra Nevada, Puerto de la Ragua, 2000–2100 m (S 482); Prov. Cuenca, 8.5 rkm SW Guadalaviar, 200 m SW Rd dir. Orea, NE Cuenca, 1500 m (S 496); Prov. Teruel, 10 Rkm S Orihuela del Tremedal, NW Albarracin, 1700 m (S 497); Rio de la Fuente del Berro, 4 rkm E Calomarde, SW Albarracin, 1200 m (S 500), leg. Aßmuth & Sanetra. Greece: Prov. Magnissia, Pilion, 7 rkm SW Portaria, 500–700 m (576–577), leg. Schulz & Vock. – ***S. karawajewi*** (n = 4). Ukraine: Crimea, S Simferopol', nr Zalis's'a, 300–500 m (S 533–536), leg. Buschinger, Kipyatkov, Radchenko & Sanetra. – ***A. atratulus*** (n = 11). Switzerland: Valais, Saas Fee, Wildi, 1800 m (Aa 96); St. Luc, Tignousa, 1600–2200 m (Aa 105); Lötschen valley, nr Eisten, 1600–2000 m (Aa 120, 122); Moosalp, nr Visp, 2000–2100 m (Aa 126), leg. Buschinger, Güsten & Sanetra. Italy: Basilicata, M. Pollino, Prov. Potenza, nr Rif. di Gasperi, 8 rkm SE Rotonda, 1600 m (Aa 345), leg. Güsten, Sanetra & Schulz. France: Dep. Hautes-Alpes, NW Briançon, Col de Granon, 2000–2100 m (Aa 385); Dep. Alpes de Hautes-Provence; 6 rkm NNE Jausiers, 1300 m (Aa 405), leg. Buschinger, Güsten, Sanetra & Schumann. Spain: Prov. Granada, Sierra Nevada, Rd GR 420, 2 rkm E Rd dir. Mirador de Canales, 2200 m (Aa 481), leg. Aßmuth & Sanetra. Germany: Hesse, Rhine river, 2 rkm N Lorchhausen, 100 m (Aa 510), leg. Sanetra. Ukraine: Crimea, Catyr-Dah, nr pec Cholodna, 1000 m (Aa 541), leg. Buschinger, Kipyatkov, Radchenko & Sanetra. – ***T. schneideri*** (n = 9) – France: Pyrénées, Dep. Basses-Pyrénées, Val d'Osseau, Lac de Fabrèges-Cabane du Lurien, 1600–1700 m (Tel 134), leg. Sanetra; Dep. Hautes-Alpes, NW Briançon, Col de Granon, 2000–2100 m (Tel 380–384); Col de Granon, Chemin de Roy, 2000–2200 m (Tel 396–398), leg. Buschinger, Güsten, Sanetra & Schumann.

Appendix 2. Coding and transformation series

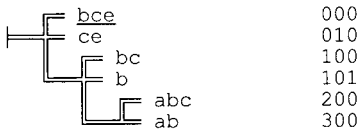
Minimum turnover coding

Branching character state trees, with ordinal coding applied. Presumed ancestral states are underlined.

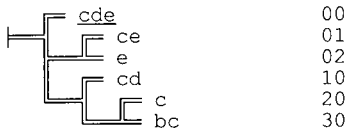
Acoh-1
L = 4



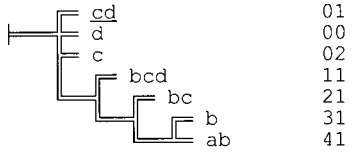
Ark
L = 5



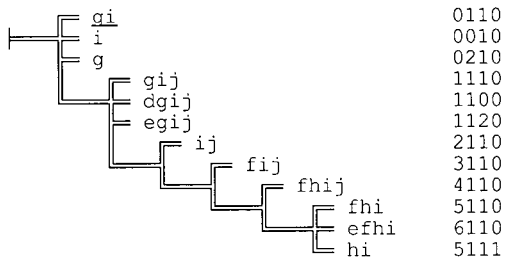
Ao
L = 5



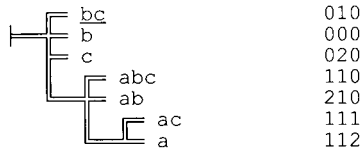
G3pdh-1
L = 6



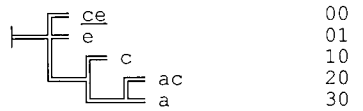
Gpi
L = 10



Hk-1
L = 6

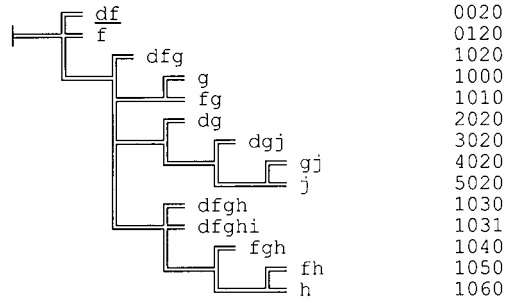


Hk-2
L = 4



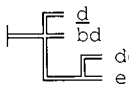
b = non-additive

Idh
L = 13



Mdh-1

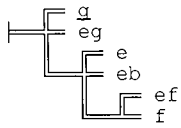
L = 3



00
01
10
20

Mdhp

L = 5

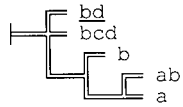


02
12
22
21
32
42

d and c = non-additive

Pgdh

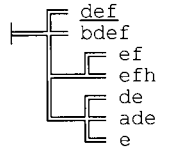
L = 4



00
01
10
20
30

Pgm-1

L = 6

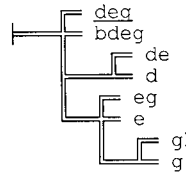


011
001
111
211
021
022
020

g = non-additive

Pgm-2

L = 7



010
000
210
030
110
210
112
111

c = non-additive

Ark, Pk non-additive

Sod: b=0, a=1

Xdh: d=0, b/c=1

Mutation coding for the "quadraphenic evaluation procedure" (Murphy, 1993)

Inferences of order among character states resulted from the first and second evaluation step by using taxonomic and functional outgroup analysis, respectively. Some unreliable loci were discarded based on successive character weighting (Farris, 1989). State number zero is the ancestral condition.

Acoh-1: bd=0, f=1
Ak: bce=0, a=1
G3pdh-1: cd=0, b=1
G3pdh-2: a=0, b=1
Gpi: gi=0, j=1, e=2, h=3, f=4
Hk-2: a=0, b=1
Mdh-1: d=0, e=1
Pgdh: bd=0, a=1
Pgm-2: deg=0, h=1

Idh

L = 3

00
10
20
11

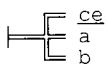
Mdhp

L = 3

00
10
20
11

Hk-2

L = 2



00
10
01

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