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Geographic variation and genetic relationships in populations of the *Androniscus dentiger* complex from Central Italy (Isopoda, Oniscidea, Trichoniscidae)

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SUMMARY

Androniscus dentiger is a terrestrial isopod distributed from Great Britain to North Africa, inhabiting humid edafic environments, superficial underground compartments and both natural and artificial caves. In this study allozyme data have been used to investigate the geographic variation and the genetic relationships of several populations of *A.dentiger* from Central Italy, using as outgroups populations from four congeneric species, *A.calcivagus*, *A.*cfr.*subterraneus*, *A.spelaeorum*, and *A.degener*. Multivariate analysis of *A. dentiger* allele frequencies indicates the existence of a group of populations (group A) distributed in a wide geographic area which are genetically slightly differentiated, and several populations (arbitrarily defined as group B) which show differentiation levels comparable to those observed between the morphologically well differentiated species. The low valley of the river Tiber seems to act as an effective geographic barrier between the populations from group A and the remaining ones. The genetic divergence between populations within the group A seems to have a recent origin. This is suggested by the low genetic distances and heterozygosity values within the group A, and by the very low number of private alleles occurring in this group. The high degree of intraspecific and interspecific genetic differentiation is not consistent with the levels of morphological differentiation traditionally used to distinguish different species within this genus. On the whole, these data suggest that *A. dentiger* might be considered as a complex of cryptic/sibling species.

INTRODUCTION

The terrestrial isopod *Androniscus dentiger* inhabits, as other congeneric species, humid edafic environments, superficial underground compartments, and both natural and artificial caves. Usually, in Trichoniscidae, highly hygrophilic habits represent a strong constraint for dispersal. Evidence of this phenomenon is the high number of taxa (both at the species and genus level) which are geographically differentiated, and are also narrow endemics. *A.dentiger*, unlike other congeneric species and other Trichoniscidae, is widely distributed. It occurs in Great Britain, Central Europe, mainland Italy, Sicily, and North Africa and its range has been considered to be in a phase of active and passive (by man) expansion (Van-

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del, 1960). However, *A. dentiger* does not occur in Corsica (Taiti and Ferrara, 1996), Tuscanian archipelagos and in some localities along the Tuscanian coast (Taiti and Ferrara, 1980) where potentially colonizable environments occur. Moreover, this species is missing in many suitable caves within its range (Gentile, unpublished data).

In a previous study the levels of gene flow among populations of *A. dentiger* in Central Italy have been investigated using different *Nm* estimators. The very low levels of gene flow reported, even between neighboring populations, suggested that nearly all the populations studied have isolated gene pools (Gentile and Sbordoni, 1998). In this paper we discuss the geographic variation and the genetic relationships among the same *A. dentiger* populations, using as outgroups populations from four species belonging to the same genus.

MATERIAL AND METHODS

Twenty eight populations of *A. dentiger* from Central Italy have been studied, including cave and surface populations. Six populations taxonomically assigned to four different species (*A. calcivagus*, *A.* crf. *subterraneus*, *A. spelaeorum* and *A. degener*) were used as outgroups. These species are morphologically well differentiated (Vandel, 1960), and occur only in the North-Eastern Italian Prealpine mountains. Some of these species could also be found in syntopy. In these cases, no evidence of hybridization could be highlighted. In Table 1 the populations studied are reported. Cave and surface populations are indicated with a three letter symbol in upper and lower cases types, respectively.

Genetic variation was investigated using allozyme electrophoresis on cellulose acetate gels. The following enzymes were assayed: *Ada*, *Aldo*, *Ca*, *Dia*, *Me*, *Aph*, *Pgm*, β*Gal*, *G6pd*, *Gpi*, *Got*, *Idh*, *Mdh*, *Mpi*, *Pep*, *Pk*, for overall 19 gene loci scored. Details of the protocols used and allele frequencies are reported in Gentile and Sbordoni (1998).

Heterozygosity, Nei's (1972) and Reynolds' (Reynolds et. al., 1983) genetic distances were calculated using GEN-SURVEY (Vekemans and Lefebvre, 1997). We used the Nei's and Reynolds' indexes to provide more accurate dating of events of divergence in different evolutionary contexts. We used Nei's (1975) relationship $t=kD$, where t is the time of divergence, k (the substitution rate) is equal to $5*10^6$ and D is the Nei's distance. Reynolds' index, which assumes divergence to be caused only by genetic drift, was used in a context of short-term evolution. We applied the formula $t=D/2N$, where t is the time of divergence, N the effective population size and D is the Reynolds' coefficient. We estimated an average population size ranging from 500 to 5.000 individuals.

Table 1 – Sample sites

Species	Locality	Toponym	Altitude	Latitude	Longitude	Temp.(°C)	Label
A. dentiger	Crara, S.Lazzaro di Savena (BO)	Grotta della Spipola	135	44°25'47"	11°22'37"	6,6	SPI
"	Castelnuovo di Garfagnana (LU)	Tana di Magnano	673	44°10'36"	10°23'09"	12,7	MAG
"	Piteglio, S.Marcello Pistoiese (PT)	Lana Termini	340	44°02'27"	10°43'17"	11,8	TER
"	Vecchiano (PI)	La Cardinaccia	50	43°47'01"	10°23'52"	12,1	*can*
"	Onferno,Gemmano (FO)	Grotta di Onferno	304	43°52'19"	12°32'32"	13,2	ONF
"	Tosi (FI)	Buca delle Fate	465	43°48'00"	11°27'00"	11,5	TOS
"	Livorno Montenero (LI)	Cava del Santuario	193	43°29'00"	10°21'00"	14,6	*mon*
"	Picbbico, Monte Nerone (PS)	Grotta di Nerone	1025	43°34'16"	12°30'39"	7,1	NER
"	Vallombrosa (FI)	Fosso dei Bruciati	830	43°44'00"	11°34'00"	10,3	*val*
"	Tecchie, Cantiano (PS)	Bosco di Tecchie	650	43°26'00"	12°40'00"	11,7	*tec*
"	Genga, S.Vittore (AN)	Grotta del Mezzogiorno	350	43°24'08"	12°57'10"	12'5	MEZ
"	Gubbio, Monte Ingino (PG)	Grotta del Diavolo	660	43°21'20"	12°34'36"	11,3	DVL
"	Roccastrada, Belagaio (GR)	Tomba del Belagaio	250	43°00'48"	11°09'36"	13'8	TOM
"	Sassopiano, Assisi (PG)	Grotta del Subasio	1060	43°03'12"	12°39'30"	10,2	SUB
"	Sarteano (SI)	Grotta dell'Orso	516	42°59'59"	11°51'42"	14,2	ORS
"	La Roccaccia, Titignano (TR)	Pozzo della Piana	260	42°44'19"	12°19'29"	10,5	PIA
"	Ripe, Civitella del Tronto (TE)	Grotta S.Angelo	605	42°45'20"	13°37'30"	13,6	RIP
"	Fosso dell'Andreone, Spoleto (PG)	Grotta del Chiocchio	705	42°40'58"	12°40'03"	11,7	CHI
"	Castel S. Angelo (RI)	Rovine S.Vittorino	450	42°22'00"	13°00'00"	10,7	*vet*
"	Vignanello (VT)	Rovine etrusche	320	42°22'00"	12°19'00"	12,1	*vig*
"	Assergi (AQ)	Grotta a Male	950	42°25'46"	13°28'54"	9,9	*mal*
"	S.Demetrio nei Vestini (AQ)	Grotta di Stiffe	695	42°15'10"N	13°32'22"	10,4	STI
"	Poggio Moiano (RI)	Grotta La Pila	831	42°10'20"	12°55'49"	12,7	PIL
"	Pescorocchiano (RI)	Val di Varri	806	42°12'34"	13°09'05"	10,9	*var*
"	Popoli (AQ)	Sorgenti del Pescara	250	42°09'00"	13°19'00"	11	*pop*
"	Monti della Duchessa (RI)	Valle Fua	1050	42°10'00"	13°18'00"	12,1	*fua*
"	Anticoli Corrado (RM)	Rive dell'Aniene	350	42°01'00"	13°00'00"	10,5	*ant*
"	Trevi nel Lazio (FR)	Risorgenza del Trevi	600	41°51'18"	13°12'35"	9,8	TRV
A. calcivagus	Caprino Bergamasco (BG)	Büs del Böter	550	45°45'00"	09°29'00"	10,2	BOT
"	Villa di Serio (BG)	Miniere di Villa	310	45°43'00"	09°44'00"	13,6	MIN
"	Zandobbio (BG)	Lega Casina Melania	472	45°41'00"	09°55'00"	10,7	LAG
A. subterraneus	S.Anna d'Alfaedo, Veja (VR)	Grotta A Ponte di Veja	580	45°36'28"	10°58'06"	10,7	VJ2
A. spelaeorum	Roccolino, Rota d'Imagna (BG)	Tomba dei Polacchi	565	45°50'00"	09°30'00"	9,4	POL
A. degener	S.Anna d'Alfaedo, Veja (VR)	Grotta A Ponte di Veja	580	45°36'28"	10°58'06"	10,7	VJ1

Statistical significance of heterozygosity estimates and genetic distances between and within groups of populations was tested by 1000 bootstrap cycles over populations (Van Rossum et al., 1997; Vekemans and Lefebvre, 1997). The program GENETIX ver. 3.0 (Belkhir et al., 1996) was used to test the null hypothesis D=0 for each pair of populations.

An ordination of *A. dentiger* populations was carried out by means of the Factorial Correspondence Analysis carried out on allele frequencies (FCA, Benzecrì et al., 1973). A geographic contour map was obtained by interpolating the scores of the first axis of the Factorial Correspondence Analisys (Cavalli-Sforza et al., 1994).

The neighbor-joining (NJ tree, Saitou and Nei, 1987) method was applied to a matrix of genetic distances (Nei, 1972). Robustness of each node was evaluated by bootstrapping allele frequencies 1000 times, using the program SEQBOOT in PHYLIP 3.57 (Felsenstein, 1995).

We also carried out parsimony analyses on allozyme data. Allozymes were recoded considering a locus as a character, and a combination of alleles occurring at that locus as a state (Mabee and Humphries, 1993). In-

stead of ordering the character states and imposing a specific pathway, we considered all transformations to be possible. In a stepmatrix, a cost to every possible transformation was assigned by assuming that each gain or loss of an allele equals one evolutionary step. We used ASAP 1.5 (Thumfort and Sampson, personal communication) to recode allozyme data according to the procedure assessed in Mardulyn and Pasteels (1994). Most-parsimoniuos (MP) trees were derived by the heuristic search as implemented in PAUP 3.1.1 (Swofford, 1993). Ten random replicates of a heuristic search were performed. The options *random* and *tree-bisection-reconnection* (TBR) were used for stepwise addition and branch swapping procedures, respectively. The MP tree and the shortest trees supporting alternative phylogenetic hypotheses were compared using Templeton's (1983) test, as detailed in Larson (1994).

RESULTS

Figure 1 shows the results of the Factorial Correspondence Analysis carried out on allele frequencies of the 28 populations of A.dentiger. The first axis which explained 24.5% of variance allowed the discrimination between two major groups: group A, including populations distributed in a wide area ranging from the Apennines of Tuscany and Marches to the alluvial plains of Tuscany and Latium, and the group B including the remaining populations. The second axis (13%) clearly separates STI and PIA populations from all the others, while the third axis (10.2%) discriminated populations DVL, PIA, SUB and CHI. The second and third axis indicated that the Apennines populations do not form an homogeneous group.

Alternative alleles and a high number of private alleles occurred in most loci. In the Figure 2 the percentage of alleles which are shared by an increasing number of populations (represented by histograms) is reported together with their average frequency (represented by line). More than 40% of all alleles scored are shared by a maximum of three populations. These alleles showed an average frequency equal to 0.4.

Only two private alleles occurred in the group A, while 11 private alleles were found in the group B. Nearly all the alleles shared only by two and three populations were in group B.

Mean heterozygosity per population is reported in Table 2. Average heterozygosity estimates between and within groups A and B are reported in Tables 3a and 3b. We did not observe a statistically significant difference in mean heterozygosity between cave and surface populations. However, if groups A and B were analyzed separately, heterozygosity levels were statistically different between cave and surface populations within the group B.

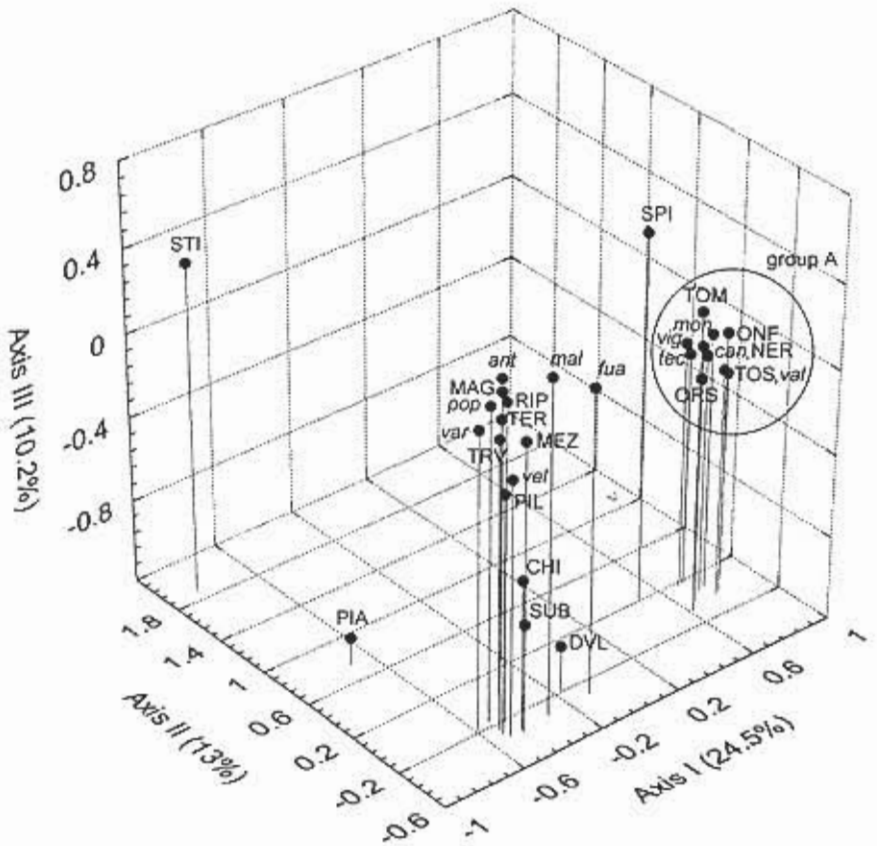

Fig. 1 – Factorial Correspondence Analysis on allele frequencies at 18 polymorphic loci in *A. dentiger*. The first three axis are represented.

Nei's genetic distances are reported in the Appendix. Genetic distances between and within groups A and B are summarized in Tables 3 and 4. Within *A. dentiger* intraspecific genetic distance values between populations are generally high, with an average of 0.493 ± 0.013. The genetic distance between group A and group B is very high (D=0.670; p=0.000).

Interspecific distances ranged from 0.3 to 1.539, with a mean of 0.749 ± 0.019. *A.* cfr. *subterraneus* has the smallest distance value from *A.dentiger* (D=0.578±0.027), whereas *A. degener* has the highest one (D=1.093±0.040). Average D values from *A. dentiger* and the two remaining species, *A. calcivagus* and *A. spelaeorum*, are 0.680±0.020 and 0.683±0.023, respectively.

The topology of the NJ tree obtained is shown in Fig.3a. The tree is arbitrarily rooted at *A. degener* (VJ1), the most differentiated species. *A. dentiger* splits into the groups A and B, being divided by *A. calcivagus*. *A. calcivagus* is the only robust cluster of the whole tree (bootstrap values > 70%). The remaining two species, *A.* cfr. *subterraneus* and *A. spelaeorum*, link together and are nested with group B.

Table 2 – Genetic variability at 19 loci in the 34 populations of *Androniscus*.

Population	Mean no. of alleles per locus	Percentage of loci polymorphic(*)	Mean heterozygosity	
			Observed	Expected
Androniscus dentiger				
SPI	1.6 ± 0.1	26,3	0.069 ± 0.024	0.069 ± 0.025
MAG	1.7 ± 0.1	42,1	0.123 ± 0.034	0.138 ± 0.038
TER	1.7 ± 0.1	26,3	0.147 ± 0.051	0.123 ± 0.040
can	1.4 ± 0.1	21,1	0.087 ± 0.035	0.090 ± 0.037
ONF	1.3 ± 0.1	15,8	0.072 ± 0.039	0.064 ± 0.034
TOS	1.5 ± 0.1	26,3	0.084 ± 0.032	0.083 ± 0.032
mon	1.5 ± 0.1	36,8	0.100 ± 0.037	0.104 ± 0.036
NER	1.6 ± 0.1	36,8	0.101 ± 0.037	0.108 ± 0.037
val	1.4 ± 0.1	42,1	0.094 ± 0.030	0.093 ± 0.031
tec	1.5 ± 0.1	36,8	0.101 ± 0.032	0.111 ± 0.036
MEZ	1.5 ± 0.1	26,3	0.088 ± 0.032	0.089 ± 0.033
DVL	1.4 ± 0.1	15,8	0.069 ± 0.030	0.066 ± 0.028
TOM	1.2 ± 0.1	15,8	0.062 ± 0.035	0.058 ± 0.033
SUB	1.7 ± 0.2	52,6	0.158 ± 0.043	0.169 ± 0.047
ORS	1.5 ± 0.1	31,6	0.089 ± 0.028	0.101 ± 0.034
PIA	1.5 ± 0.1	42,1	0.130 ± 0.037	0.151 ± 0.043
RIP	1.3 ± 0.1	21,1	0.072 ± 0.040	0.066 ± 0.031
CHI	1.3 ± 0.1	21,1	0.087 ± 0.040	0.083 ± 0.037
vel	1.9 ± 0.2	47,4	0.169 ± 0.042	0.169 ± 0.043
vig	1.5 ± 0.1	31,6	0.062 ± 0.018	0.062 ± 0.018
mal	1.5 ± 0.1	36,8	0.125 ± 0.039	0.119 ± 0.036
STI	1.3 ± 0.1	10,5	0.029 ± 0.018	0.027 ± 0.016
PIL	1.5 ± 0.1	26,3	0.062 ± 0.021	0.068 ± 0.023
var	1.7 ± 0.2	26,3	0.115 ± 0.040	0.113 ± 0.040
pop	1.5 ± 0.1	21,1	0.108 ± 0.044	0.104 ± 0.041
fua	1.6 ± 0.2	36,8	0.118 ± 0.040	0.127 ± 0.044
ant	1.7 ± 0.2	36,8	0.141 ± 0.043	0.158 ± 0.048
TRV	1.6 ± 0.2	36,8	0.120 ± 0.041	0.132 ± 0.043
Androniscus calcivagus				
BOT	1.5 ± 0.1	21,1	0.084 ± 0.040	0.074 ± 0.033
MIN	2.0 ± 0.2	47,4	0.151 ± 0.036	0.178 ± 0.045
LAG	1.6 ± 0.1	42,1	0.125 ± 0.038	0.127 ± 0.041
Androniscus* cfr. *subterraneus				
VJ2	1.4 ± 0.1	21,1	0.051 ± 0.021	0.060 ± 0.027
Androniscus spelaeorum				
POL	1.4 ± 0.1	26,3	0.095 ± 0.040	0.097 ± 0.037
Androniscus degener				
VJ1	1.5 ± 0.1	31,6	0.066 ± 0.020	0.062 ± 0.018

(*) A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

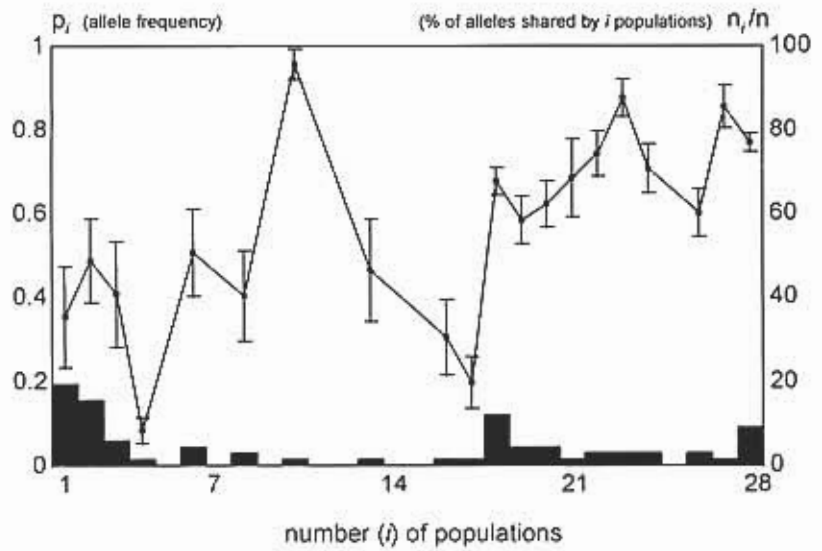

Fig. 2 – Genetic differentiation in *Androniscus dentiger*. Bars indicate the percentage (n_i/n) of alleles (n=67 alleles scored) shared by an increasing number of populations (*i*), up to 28. Whiskers represent standard error of average frequency (*pi*) of alleles shared by populations at each *i* classes.

The parsimony analysis produced 112 equally parsimonious trees (length=252). The MP tree is reported in Figure 3b. It is also rooted at *A. degener* (VJ1), and shows that *A. dentiger* splits into two different groups of populations, mainly corresponding to the groups A and B obtained by FCA, and the NJ tree. Groups A (plus SPI) and B are separated by the insertion *of A. calcivagus* and *A. spelaeorum*. *A.* cfr. *subterraneus* is nested with group B. We used Templeton's (1983) test to determine whether we could reject the hypothesis of the monophyletic origin of *A. dentiger*. We compared the MP tree to the most parsimonious tree obtained by forcing the monophyly of *A. dentiger*. The tree with *A. dentiger* monophyletic required 5 steps more than the MP tree. However, it was not significantly different from the MP tree.

DISCUSSION

Geographic variation and evolutionary patterns

Multivariate analysis (fca) of allele frequencies (Fig. 1) pointed out the existence of at least two groups of populations. The populations belonging to group A are strongly divergent from the remaining ones (group B). In the

South, the low valley of the river Tiber divides the two groups and might have represented a notable barrier to gene flow. Interestingly, the Tiber valley also represents a geographical barrier between populations belonging to the cave crickets *Dolichopoda laetitiae-geniculata* complex (Cesaroni et al., 1997).

Groups A and B show different geographical distribution an even very different evolutionary patterns. In group A the number of private alleles is low, suggesting that mutation did not play an important role in the evolutionary process within this group. Average genetic distances (Nei's index) within group A (Tables 3a,b; 4) suggest that the times of divergence between most of the *A. dentiger* populations within group A are very recent. The Reynolds' coefficient relates the divergence times within group A to a time-span ranging between 17,000 and 1,700 years ago. This estimate would correspond to the wide expansion in Central Italy of the mesophilic forests, which represent the main routes for dispersal of *A. dentiger*. This expansion started at the beginning of the Holocene (14,000 years ago) until the present (Magri and Follieri, 1992), after a long period (300,000 years) when mesophilic forest environments occurred in very few and short periods, and were limited to very narrow areas (Follieri et al., 1993). This scenario may explain the absence of *A. dentiger* in the whole of the tuscanian archipelagos and in the tuscanian coastal mountains (Taiti and Ferrara, 1980), which were connected with the mainland only in the last 10.000 years (Lanza, 1984).

Figure 4 shows a geographic representation of the genetic variation observed. The darkest area groups the most similar populations belonging to group A. It might be the possible area from which propagules from a limited number of populations started the colonization of the alluvial lands of Tuscany. Genetic drift might be responsible for the decreased genetic variability within new populations, where alleles which are rare in the source populations are less likely to be represented. Consistently with the hypothesis of a recent colonization, average genetic distance among surface populations within group A is comparable with the value obtained for cave ones (Tables 3a,b).

Mutation seems to be one of the main factors shaping the evolutionary pattern within group B. In this group in fact, the number of private alleles is high. The wide range of genetic distances within group B suggested that most of the splitting events within this group seem to have occurred in a wide time-span, which can be dated back to climatic shifts and marine transgressions during the Pliocene-Pleistocene glaciations. Extinctions and recolonizations during several glaciation episodes in the last Pliocene and during the Quaternary could explain both the observed lack of *A. dentiger* in many potentially colonizable habitats within its area and the varying degrees of genetic differentiation observed in the group B. Since these processes are much older than

the colonization by populations of group A, we would expect the populations from group B to have partly rebuilt their genetic variability. Indeed, we did observe a statistically significantly higher heterozygosity in the populations from group B than from group A (Mann-Whitney $Z=2.07$; $p<<0.05$ at two-tailed test). Furthermore, surface and cave populations of group B are also differentiated. Within group B, surface populations show a higher level of average heterozygosity than the value observed in the cave ones, which is in turn comparable to the value observed in group A (Tables 3a,b). So, in group B, increasing genetic variability occurred in surface populations only, while cave populations seem to be influenced by the effects of genetic drift or by some form of stabilizing selection. This scenario is consistent with the genetic distance values observed. In fact, group A and B are genetically distinct (Table 4), and in group B average genetic distance among cave populations is much higher than the value observed for surface ones (Tables 3a,b).

The geographical patterns of alleles shared by two and three populations might be interpreted as a trace of an ancestral polymorphism reduced by genetic drift due to extinction dynamics (Gentile and Sbordoni, 1998). In fact, the higher the number of populations sharing the same allele, the more unlikely it is that this allele arose by recurrent mutation in those populations. Populations sharing these alleles are separated by geographic distances up to 250 Km, suggesting that extinction events might have occurred over a wide geographic scale.

Table 3a – Level of population diversity within groups A and B: Average genetic distances (Nei_{72}) and observed heterozygosity within cave and surface populations.

	Cave (A)	Surface (A)	Cave (B)	Surface (B)
D (Nei_{72})	0.141	0.105	0.465	0.186
H_o	0.082	0.089	0.096	0.129

Table 3b – Differences (Δ) between levels of population diversity within groups A and B: The upper values is the triangular matrix are ΔD; the lower ones are ΔH_o.

	Cave (A)	Surface (A)	Cave (B)
Surface (A)	0.036 ns		
	0.008 ns		
Cave (B)	0.324 **	0.360 **	
	0.015 ns	0.007 ns	
Surface (B)	0.045 ns	0.081 *	0.279 **
	0.047 **	0.040 **	0.033 *

(*) $p[\Delta=0]<0.05$; (**) $p[\Delta=0]<0.01$

Table 4 – Level of population differentiation between groups A and B: Average genetic distances (Nei-$_{72}$) between cave and surface populations

	Cave (A)	Surface (A)	Cave (B)
Surface (A)	0.118 ns		
Cave (B)	0.710 **	0.671 **	0.371 *
Surface (B)	0.624 **	0.633 **	

(*) $p[D=0] < 0.05$; (**) $p[D=0] < 0.01$

Genetic relationships

The genetic distances between populations morphologically belonging to *A.dentiger* show a wide spectrum of values, including many values higher than 1. Thorpe (1983) suggested that genetic distance values higher than 0.163 between allopatric populations indicate that they belong to different species. If we accept this suggestion, most populations of *A. dentiger* are different species. As already pointed out (Lessios and Weinberg, 1994) there is no theoretical reason to consider the cut-off value indicated by Thorpe as an unambiguous threshold for speciation. The inclusion of species morphologically differentiated as outgroups allows us to calibrate the amount of genetic divergence that can be revealed by allozyme data, providing a "within taxon" standard which is useful to establish a threshold for speciation. Most of the average genetic distances between *Androniscus* species are of the same order of magnitude as many distances between populations of *A. dentiger*.

In contrast with the high degree of genetic divergence, morphological differentiation in *A.dentiger* does not show a degree of geographic variation useful to study the systematic relationships among populations (Vandel, 1960; Gentile, 1994).

In the last twenty years the number of cryptic/sibling species which have been claimed to occur in various taxa is greatly increased. Genetic, ecological and behavioral data are often used and sometimes combined to test the actual differentiation between putative species. In particular, most genetic studies of cave dwelling isopods, both aquatic and terrestrial, revealed the occurrence of high genetic distance values between morphologically indistinguishable populations as reported from studies on *Typhlocirolana* (Caccone et al., 1986), *Stenasellus* (Messana et al., 1995), *Oritoniscus* (Cobolli Sbordoni et al., 1995) and *Trichoniscus* (Cobolli Sbordoni et al., 1997). Since it has been possible to evidenciate that reproductive isolation may occur in allopatry as a by-product of a high degree of genetic differentiation (Coyne and Orr, 1989), it would appear reasonable that speciation events may occur more frequently than has been though. The high levels of genetic divergence we observed suggest that *A. dentiger* could be probably considered as a complex of cryp-

tic\sibling species. We could identify two genetically differentiated groups of populations (A and B). Additionally, in the group B, most of genetic distances observed between populations are much higher than the values reported for morphologically distinguishable species. However it remains to be assessed how many species *A. dentiger* complex might include. This appears to be a difficult task, since breeding experiments carried out on other Peracarids showed that the paradigm "high genetic distance - high degree of reproductive isolation" does not hold always (Scheepmaker, 1990).

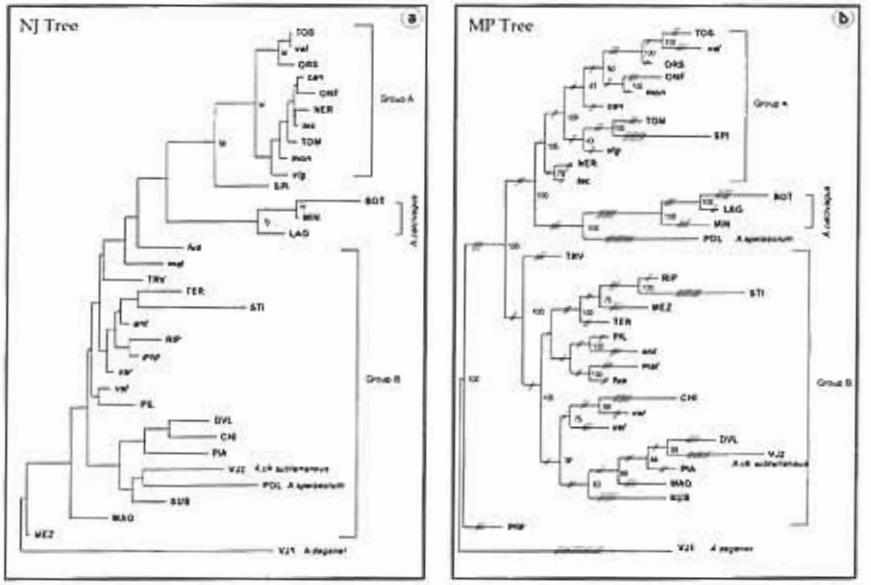

Fig. 3 – Genetic relationships between *Androniscus* species. a) Neigbbor-join (NJ) tree. The number at the nodes of NJ is the number of times the cluster at the right of the node occurs out of 100 bootstrap repetitions. Only bootstrap values higher than 50% are shown, b) Maximum parsimony tree (MP). The number at the nodes of MP is the number of times (percent) that the cluster at the right of the node occurs out of all most parsimonious trees found. Slashes represent the changes of character states between two contiguous nodes.

Both the NJ tree, and the parsimony analysis are in agreement with the multivariate analysis (FCA). However, neither NJ or MP tree (Fig. 3) is helpful to assess the genetic relationships between the different species of *Androniscus* studied. They suggest that *A. dentiger* is polyphyletic. However, bootstrapping and Templeton's test do not support the polyphyletic origin of *A. dentiger*, which indeed appears to be unreasonable even from a biogeographical point of view. In fact, among all the *Androniscus* (*Dentigero-*

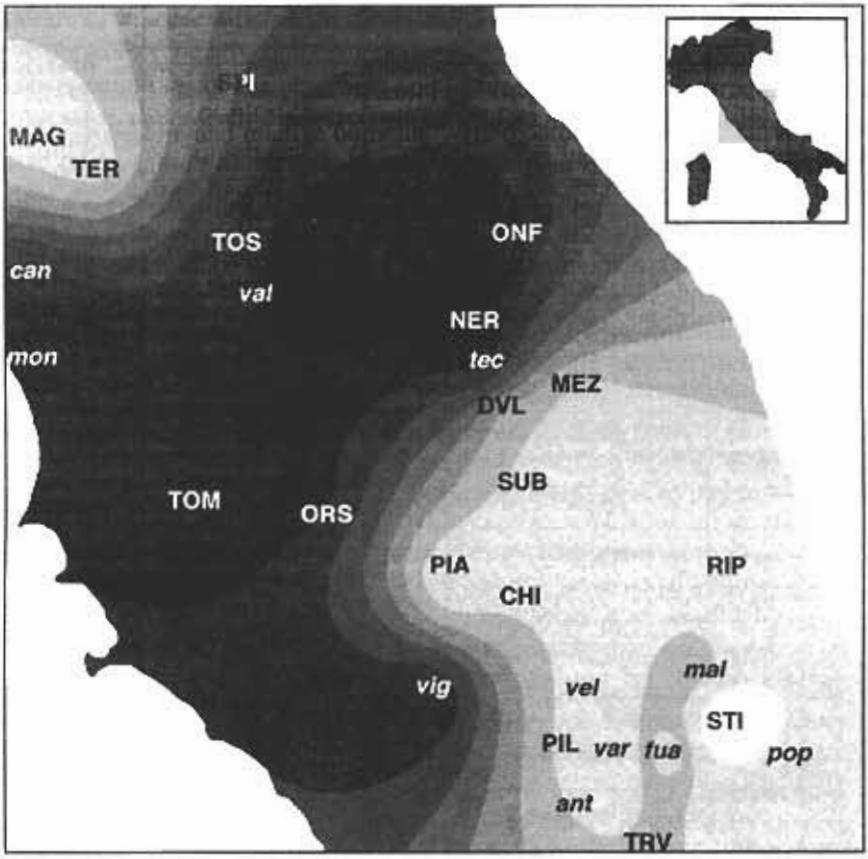

Fig. 4 – Geographic variation of allele frequencies in *A. dentiger* of Central Italy. The contour map has been obtained by interpolating the values of the coordinates on the first axis after a Factorial Correspondence Analysis. Each tone of color corresponds to an increment equal to 0.2 on the first axis. The dark area identifies the group A, while group B is represented by the white area (Redesigned from Gentile, 1998).

niscus) species, only *A. dentiger* occurs in Central Italy, the range of the other congeneric species being strictly limited to the North and North-Eastern Prealps. The difficulty to assess robust genetic relationships between populations and species of *Androniscus* might be explained by the high degree of genetic differentiation found.

Further investigations by using a better addressed genetic marker will probably be necessary to investigate the phylogenetic relationships among the species belonging to this genus.

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APPENDIX

	SPI	MAG	TER	*can*	ONF	TOS	*mon*	NER	*val*	*tec*	MEZ	DVL	TOM	SUB	ORS	PIA	RIP	CHI	*vel*	*vig*	*mal*	STI	PIL	*val*	*pop*	*fus*	*ant*	TRV	BOT	MIN	LAG	VJ2	POL	VJ1
SPI	*****	0.535	0.65	0.754	0.725	0.669	0.776	0.71	0.657	0.738	0.559	0.508	0.786	0.529	0.628	0.473	0.526	0.416	0.574	0.779	0.575	0.543	0.522	0.527	0.535	0.622	0.624	0.568	0.303	0.487	0.568	0.516	0.501	0.378
MAG	0.626	*****	0.662	0.481	0.474	0.532	0.531	0.458	0.521	0.48	0.765	0.633	0.533	0.621	0.451	0.595	0.649	0.646	0.802	0.488	0.739	0.625	0.721	0.826	0.773	0.696	0.758	0.781	0.563	0.523	0.569	0.561	0.454	0.442
TER	0.43	0.413	*****	0.506	0.501	0.44	0.528	0.474	0.448	0.518	0.764	0.559	0.548	0.721	0.435	0.628	0.767	0.552	0.757	0.54	0.648	0.668	0.7	0.766	0.745	0.609	0.795	0.667	0.397	0.445	0.462	0.584	0.672	0.417
can	0.282	0.733	0.682	*****	0.937	0.862	0.921	0.941	0.844	0.967	0.494	0.609	0.933	0.498	0.755	0.436	0.465	0.458	0.459	0.93	0.512	0.415	0.456	0.451	0.453	0.625	0.526	0.572	0.366	0.557	0.641	0.548	0.521	0.261
ONF	0.322	0.747	0.691	0.065	*****	0.924	0.966	0.903	0.91	0.928	0.472	0.554	0.94	0.448	0.81	0.379	0.438	0.399	0.466	0.884	0.503	0.403	0.44	0.445	0.436	0.619	0.513	0.518	0.402	0.582	0.68	0.481	0.457	0.248
TOS	0.402	0.632	0.822	0.148	0.079	*****	0.913	0.842	0.995	0.868	0.561	0.604	0.875	0.513	0.917	0.455	0.409	0.473	0.543	0.832	0.586	0.348	0.515	0.524	0.493	0.693	0.519	0.571	0.425	0.599	0.7	0.496	0.457	0.326
mon	0.254	0.633	0.639	0.082	0.035	0.091	*****	0.901	0.894	0.917	0.515	0.6	0.925	0.479	0.805	0.412	0.497	0.468	0.516	0.887	0.565	0.442	0.487	0.491	0.491	0.674	0.575	0.575	0.457	0.636	0.717	0.531	0.463	0.277
NER	0.343	0.781	0.746	0.061	0.102	0.172	0.104	*****	0.835	0.961	0.484	0.607	0.916	0.465	0.806	0.416	0.526	0.501	0.461	0.917	0.585	0.367	0.4	0.426	0.5	0.582	0.519	0.518	0.419	0.583	0.608	0.543	0.472	0.267
val	0.42	0.652	0.804	0.169	0.094	0.005	0.112	0.18	*****	0.864	0.573	0.6	0.86	0.521	0.936	0.468	0.424	0.472	0.553	0.827	0.594	0.347	0.516	0.538	0.497	0.685	0.529	0.57	0.424	0.6	0.699	0.479	0.459	0.317
tec	0.304	0.735	0.658	0.033	0.075	0.142	0.086	0.05	0.146	*****	0.504	0.609	0.926	0.517	0.781	0.43	0.463	0.461	0.5	0.659	0.535	0.422	0.439	0.459	0.449	0.622	0.533	0.585	0.372	0.564	0.663	0.529	0.538	0.273
MEZ	0.582	0.267	0.269	0.704	0.751	0.578	0.664	0.768	0.556	0.685	*****	0.637	0.524	0.781	0.585	0.686	0.704	0.527	0.813	0.546	0.667	0.516	0.765	0.819	0.76	0.668	0.806	0.69	0.468	0.522	0.509	0.686	0.669	0.524
DVL	0.676	0.458	0.561	0.497	0.59	0.504	0.51	0.5	0.511	0.496	0.451	*****	0.539	0.703	0.54	0.76	0.536	0.789	0.684	0.548	0.594	0.308	0.673	0.598	0.618	0.638	0.529	0.717	0.4	0.601	0.545	0.695	0.452	0.323
TOM	0.241	0.63	0.601	0.07	0.062	0.133	0.078	0.088	0.151	0.077	0.646	0.618	*****	0.441	0.767	0.379	0.497	0.391	0.517	0.892	0.565	0.452	0.499	0.496	0.491	0.67	0.578	0.52	0.352	0.539	0.629	0.48	0.456	0.325
SUB	0.636	0.476	0.327	0.698	0.802	0.668	0.736	0.766	0.653	0.66	0.247	0.353	0.819	*****	0.52	0.765	0.659	0.662	0.82	0.531	0.592	0.483	0.754	0.743	0.672	0.614	0.726	0.682	0.382	0.42	0.429	0.741	0.626	0.438
ORS	0.466	0.797	0.832	0.282	0.211	0.086	0.217	0.215	0.066	0.247	0.537	0.616	0.265	0.654	*****	0.51	0.52	0.467	0.541	0.801	0.633	0.339	0.531	0.542	0.563	0.609	0.563	0.545	0.427	0.573	0.624	0.492	0.449	0.286
PIA	0.749	0.518	0.466	0.829	0.97	0.787	0.887	0.877	0.76	0.844	0.377	0.274	0.971	0.268	0.673	*****	0.587	0.65	0.663	0.456	0.543	0.581	0.685	0.657	0.635	0.477	0.597	0.634	0.366	0.467	0.426	0.646	0.494	0.292
RIP	0.643	0.433	0.265	0.765	0.826	0.895	0.699	0.643	0.858	0.77	0.351	0.623	0.699	0.417	0.654	0.532	*****	0.584	0.796	0.499	0.779	0.633	0.8	0.839	0.893	0.63	0.903	0.703	0.544	0.485	0.411	0.572	0.483	0.328
CHI	0.878	0.437	0.595	0.782	0.92	0.75	0.76	0.691	0.75	0.773	0.641	0.237	0.938	0.412	0.76	0.431	0.537	*****	0.652	0.418	0.636	0.428	0.66	0.649	0.651	0.616	0.584	0.756	0.519	0.57	0.482	0.557	0.438	0.28
vel	0.555	0.221	0.278	0.778	0.764	0.611	0.661	0.775	0.592	0.693	0.205	0.379	0.659	0.199	0.614	0.411	0.229	0.428	*****	0.51	0.819	0.588	0.873	0.909	0.845	0.808	0.876	0.798	0.484	0.461	0.475	0.671	0.512	0.449
vig	0.25	0.718	0.616	0.072	0.124	0.184	0.12	0.087	0.19	0.042	0.605	0.601	0.114	0.634	0.222	0.785	0.695	0.873	0.673	*****	0.541	0.457	0.458	0.482	0.498	0.576	0.584	0.61	0.342	0.512	0.591	0.588	0.576	0.28
mal	0.553	0.302	0.434	0.669	0.688	0.534	0.57	0.537	0.52	0.626	0.405	0.521	0.571	0.524	0.457	0.61	0.25	0.453	0.2	0.615	*****	0.591	0.697	0.81	0.879	0.798	0.774	0.72	0.567	0.498	0.553	0.523	0.476	0.326
STI	0.612	0.471	0.404	0.881	0.909	1.057	0.816	1.002	1.059	0.863	0.662	1.177	0.794	0.728	1.083	0.542	0.458	0.848	0.531	0.782	0.526	*****	0.561	0.706	0.651	0.509	0.729	0.654	0.516	0.44	0.515	0.371	0.559	0.224
PIL	0.65	0.327	0.356	0.785	0.822	0.663	0.718	0.916	0.662	0.824	0.268	0.396	0.696	0.282	0.632	0.379	0.223	0.415	0.136	0.782	0.361	0.579	*****	0.853	0.8	0.745	0.816	0.794	0.391	0.453	0.458	0.581	0.458	0.384
val	0.641	0.191	0.266	0.796	0.811	0.647	0.712	0.854	0.619	0.778	0.2	0.514	0.703	0.297	0.612	0.42	0.176	0.432	0.095	0.729	0.21	0.348	0.16	*****	0.895	0.772	0.943	0.867	0.584	0.527	0.534	0.585	0.526	0.413
pop	0.625	0.258	0.294	0.792	0.83	0.708	0.711	0.693	0.699	0.8	0.274	0.481	0.711	0.398	0.575	0.455	0.114	0.429	0.169	0.697	0.129	0.43	0.223	0.111	*****	0.733	0.865	0.806	0.601	0.51	0.451	0.638	0.542	0.353
fus	0.475	0.362	0.496	0.47	0.479	0.367	0.394	0.541	0.379	0.474	0.404	0.449	0.4	0.488	0.496	0.741	0.462	0.484	0.214	0.552	0.226	0.675	0.295	0.259	0.31	*****	0.758	0.758	0.498	0.539	0.575	0.535	0.443	0.388
ant	0.472	0.277	0.23	0.642	0.667	0.656	0.553	0.656	0.636	0.629	0.216	0.637	0.547	0.321	0.575	0.516	0.102	0.538	0.133	0.539	0.257	0.317	0.204	0.059	0.145	0.277	*****	0.805	0.59	0.532	0.521	0.624	0.549	0.416
TRV	0.566	0.247	0.405	0.559	0.658	0.56	0.553	0.658	0.562	0.536	0.371	0.333	0.653	0.383	0.607	0.456	0.353	0.28	0.225	0.496	0.328	0.424	0.231	0.142	0.216	0.276	0.217	*****	0.565	0.625	0.619	0.608	0.552	0.352
BOT	1.193	0.575	0.925	1.005	0.91	0.856	0.783	0.869	0.857	0.989	0.759	0.916	1.045	0.962	0.852	1.004	0.609	0.656	0.725	1.073	0.568	0.662	0.94	0.537	0.51	0.698	0.528	0.57	*****	0.835	0.691	0.432	0.438	0.215
MIN	0.719	0.648	0.81	0.585	0.541	0.512	0.452	0.54	0.511	0.573	0.65	0.509	0.618	0.868	0.557	0.762	0.723	0.562	0.775	0.669	0.697	0.821	0.793	0.64	0.673	0.617	0.631	0.47	0.18	*****	0.85	0.491	0.481	0.261
LAG	0.566	0.563	0.772	0.444	0.386	0.357	0.333	0.497	0.358	0.411	0.676	0.608	0.463	0.845	0.472	0.852	0.889	0.73	0.744	0.52	0.593	0.665	0.781	0.628	0.797	0.553	0.652	0.48	0.37	0.163	*****	0.425	0.532	0.236
VJ2	0.662	0.577	0.538	0.602	0.732	0.702	0.633	0.611	0.736	0.636	0.377	0.363	0.733	0.3	0.708	0.436	0.559	0.586	0.4	0.531	0.649	0.992	0.543	0.536	0.449	0.625	0.471	0.498	0.84	0.71	0.856	*****	0.591	0.345
POL	0.691	0.791	0.397	0.651	0.784	0.784	0.77	0.75	0.779	0.619	0.402	0.795	0.785	0.468	0.802	0.704	0.727	0.825	0.669	0.552	0.743	0.582	0.781	0.643	0.612	0.814	0.6	0.594	0.826	0.732	0.631	0.525	*****	0.334
VJ1	0.972	0.816	0.875	1.344	1.392	1.122	1.284	1.322	1.147	1.299	0.646	1.131	1.125	0.826	1.252	1.23	1.114	1.273	0.8	1.272	1.121	1.494	0.967	0.883	1.041	0.945	0.877	1.043	1.539	1.343	1.443	1.063	1.095	*****

Genetic distance and genetic identity (Nei, 1972) below and above the diagonal, respectively.