# PHYLOGEOGRAPHIC STRUCTURE OF *AUSTROPOTAMOBIUS PALLIPES* POPULATIONS IN SWITZERLAND.

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## ABSTRACT

Due to the wide-spread occurrence of *Austropotamobius pallipes* in different drainage systems of Europe, a phylogeographic structuring reflecting these subdivisions can be hypothesized. Five major drainage systems take their origin in the alpine region, *i.e.* Rhine, Rhone, Pô, Adige and Danube. We have analysed population samples of *A. pallipes* from Switzerland, France and Italy that represent four of these drainages. The methods employed were enzyme electrophoresis and morphological analysis. Our findings are discussed in the light of the present knowledge on intraspecific taxonomy of *A. pallipes*, and we point out the implications for the conservation of genetic diversity of this species.

**Key-words :** crayfish, genetic population structure, enzyme electrophoresis, allozymes, phylogeography, *Austropotamobius pallipes*, *Austropotamobius berndhauseri*, conservation genetics.

# STRUCTURE PHYLOGÉOGRAPHIQUE DES POPULATIONS D'*AUSTROPOTAMOBIUS PALLIPES* EN SUISSE.

## RÉSUMÉ

Du fait de la présence d'*Austropotamobius pallipes* dans les systèmes hydrographiques principaux de l'Europe, une structure phylogéographique peut être suspectée. Cinq bassins hydrographiques majeurs ont leur origine dans la région alpine : ceux du Rhin, du Rhône, du Pô, de l'Adige et du Danube. Afin d'analyser la structure des populations de cette écrevisse, nous avons échantillonné en Suisse, en France et en Italie, représentant quatre de ces bassins. Les données ont été recueillies par analyse des caractères morphologiques et électrophorèse enzymatique. Les résultats sont discutés en tenant compte de la connaissance actuelle de la taxonomie intraspécifique d'*Austropotamobius pallipes,* et des conclusions sont également tirées en ce qui concerne la variabilité génétique de cette espèce et le problème de sa conservation.

**Mots-clés :** écrevisse, structure génétique des populations, électrophorèse enzymatique, allozymes, biogéographie, *Austropotamobius pallipes, Austropotamobius berndhauseri*, conservation.

## INTRODUCTION

The white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) occurs naturally in France, Switzerland, northern Italy and north-west Jugoslavia. In Spain, England, Ireland and Scotland, the species was presumably introduced (LAURENT, 1988). Morphological geographic variation has already been detected but the taxonomic implications of this variation have been the cause of conflicting interpretations (BOTT, 1950, 1972 ; ALBRECHT, 1982). Recently, two studies using the variation of RFLP in mitochondrial DNA have revealed genetic differences between populations from four distant european regions but very little variation within those regions (GRANDJEAN *et al.*, 1997 a, b).

All over its distribution range, populations of *A. pallipes* have severely declined, following the destruction of favourable crayfish habitats, the introduction of the crayfish plague from the American continent in the 1870's, and increasing water pollution (HOLDICH and LOWERY, 1988 ; HOFMANN, 1980). The maintenance of genetic diversity of a species is the basis for its evolutionary potential to adapt to a changing environment and depends critically on the knowledge of the genetic architecture of that species to be protected (AVISE, 1994 ; AVISE and HAMRICK, 1996). In most animal and plant species, at least some degree of genetic differentiation among geographic regions can be observed (EHRLICH and RAVEN, 1969 ; NEVO, 1978 ; AVISE, 1994 ; RODERICK, 1996). The use of molecular genetic markers to characterize the population subdivision has significantly contributed to the definition of phylogeographic entities (AVISE and HAMRICK, 1996) and, quite often, unexpected structures, which were not recognized by traditional morphological analysis, have appeared (AVISE, 1992, 1994).

In this study, we combine the efforts of our two laboratories and contrast morphological variation of *A. pallipes* and genetic variation as revealed by allozyme electrophoresis. In particular, we address the questions to what extent the alpine populations of *A. pallipes* show genetic differentiation, what implications these results have for conserving the genetic variability in this species and how the variation observed compares with current taxonomy existing in the literature.

## MATERIAL AND METHODS

### Sampling

19 samples of *A. pallipes* were collected from sites representing four main drainage systems of the alpine region, *i.e.* Rhine, Rhone, Pô and Adige (Fig. 1). The names of the water

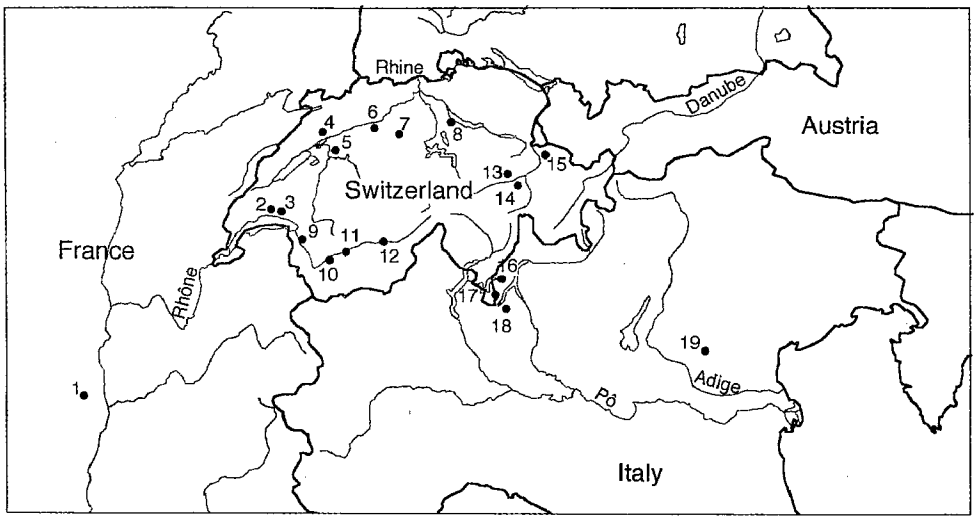


**Figure 1**

**Location of population samples (Numbers as in Table I).**

**Figure 1**

**Situation des sites échantillonnés (Les numéros correspondent à ceux du Tableau I).**

bodies where samples were taken, the drainage systems they belong to, the sample sizes and the numbers of samples used in Figures 1 and 2 are given in Table I. Sampled individuals were transported alive to the laboratory, killed through freezing and subsequently dissected at 4°C. For electrophoretic analysis, tail muscle tissue was used. The extracted pieces of tail muscle were stored in cryotubes (Nunc©) in a deep freezer at – 80°C until used for electrophoretic separation. From all individuals sampled, the carapace as well as the gonopods of males were retained for morphological analysis.

**Table I**

**Number and name of locations where samples were taken, abbreviations used in Figure 2, drainage system, political community and number of individuals sampled (N).**

**Tableau I**

**Numéros et sites d'échantillonnage, abréviations utilisées dans la Figure 2, bassin hydrographique, communauté politique et nombre d'individus échantillonnés (N).**

| No. | Water body | Abbrev. | River system | Community | N |
|---|---|---|---|---|---|
| 1 | Eyrieux/Doux | Rô1 | Rhône | Lamastre (Ardèche, F) | 15 |
| 2 | Paudèze | Rô2 | Rhône | Les Fiolettes | 10 |
| 3 | Curbit | Rô3 | Rhône | La Persevérance | 12 |
| 4 | Orvine | Ri1 | Aare (Rhine) | Petit Moulin | 12 |
| 5 | Gräntschelbach | Ri2 | Aare (Rhine) | Lyss | 13 |
| 6 | Steinbach | Ri3 | Aare (Rhine) | Seeberg | 10 |
| 7 | Moosbach | Ri4 | Aare (Rhine) | Ursenbach | 10 |
| 8 | Lindenbach | Ri5 | Limmat (Rhine) | Obfelden | 9 |
| 9 | Sous le Cex | Rô4 | Rhône | Massongez | 8 |
| 10 | Pied des Champs | Rô5 | Rhône | Ardon | 16 |
| 11 | Canal Blancherie | Rô6 | Rhône | Les Poujes | 15 |
| 12 | Nordkanal | Rô7 | Rhône | Raron | 8 |
| 13 | Lac Grond | Ri6 | Rhine | Laax | 12 |
| 14 | Stradabach | Ri7 | Rhine | Ilanz | 6 |
| 15 | Gumpen | Ri8 | Rhine | Zizers | 10 |
| 16 | Falloppia | Pô1 | Ticino (Pô) | Seseglio | 4 |
| 17 | Piave Murin | Pô2 | Ticino (Pô) | Ligornetto | 14 |
| 18 | V. del Lanza | Pô3 | Pô | Como | 8 |
| 19 | Monti Berici | Ad1 | Adige | Vicenza | 8 |

## Morphology

All individuals sampled were analysed for the morphological characters that BOTT (1972) used to distinguish the taxa *A. p. pallipes*, *A. p. italicus* and *A. berndhauseri*, respectively : number of postcervical spines, shape of rostrum (position and characteristics of the side spines) and shape of male gonopods I (symmetrical or asymmetrical lobes). The characteristics for the three taxa are compiled in Table II. All populations were then tentatively assigned to either *A. p. pallipes*, *A. p. italicus* or *A. berndhauseri* according to these characteristics.

**Table II**

**Morphological characters of the taxa *A. p. pallipes*, *A. p. italicus* and *A. berndhauseri* as described by BOTT (1972).**

**Tableau II**

**Rappel concernant les caractères morphologiques des groupes taxonomiques *A. p. pallipes*, *A. p. italicus* et *A. berndhauseri* décrits par BOTT (1972).**

| | *A. p. pallipes* | *A. p. italicus* | *A. berndhauseri* |
|---|---|---|---|
| Number of post-cervical spines | more than 2 | 1-2 | less than 3 |
| Rostrum | | | |
| - position of side spines | 1/5 of rostrum length (short tip) | 1/3 of rostrum length (longer tip) | 1/5 of rostrum length (short tip) |
| - shape of side spines | distinct, but small | long and pointed | small or missing |
| Shape of lobes of gonopod I (male) | symmetrical tip | asymmetrical tip | asymmetrical tip |

### Electrophoresis

Vertical starch gel electrophoresis of individual homogenates followed the methods as described in CLALÜNA (1996). 17 enzyme systems were scored, yielding 20 individual loci. The enzyme systems, their abbreviations, E.C. numbers, number of isozyme loci and buffers used for routine analysis of population samples are given in Table III. Staining of gels proceeded according to standard methods used in the laboratory (SCHOLL *et al.*, 1978 ; GEIGER and SCHOLL, 1985).

**Table III**

**List of enzymes, their abbreviations, E.C. number, number of loci scored and buffers used for routine electrophoresis.**

**Tableau III**

**Liste des enzymes analysées, leurs abréviations, numéro E.C., nombre de loci analysés et de tampons utilisés pour l'électrophorèse.**

| Enzyme | Abbreviation | EC Nr. | Number of loci scored | Buffer |
|---|---|---|---|---|
| Glycerol-3-phosphate dehydrogenase | G3PDH | 1.1.1.8. | 1 | TC |
| Malat dehydrogenase | MDH | 1.1.1.37. | 1 | EBT |
| Malic enzyme | MEP | 1.1.1.40. | 1 | EBT |
| Isocitrate dehydrogenase | IDHP | 1.1.1.42. | 1 | EBT |
| Glyceraldehyde-3-phosphate dehydrogenase | GAPDH | 1.2.1.12. | 1 | TBE |
| Superoxide dismutase | SOD | 1.15.1.1. | 2 | EBT |
| Aspartate aminotransferase | AAT | 2.6.1.1. | 2 | AC |
| Alanine aminotransferase | ALAT | 2.6.1.2. | 1 | TBE |
| Adenylate kinase | APK | 2.7.4.3. | 1 | AC |
| Esterase | EST | 3.1.1./. | 2 | TBE |
| Dipeptidase | PEPA | 3.4.-.-. | 1 | TBE |
| Fructose bisphosphate aldolase | FBA | 4.1.2.13 | 1 | TBE |
| Fumarate hydratase | FH | 4.2.1.2 | 1 | TC |
| Aconitate hydratase | ACOH | 4.2.1.3. | 1 | TBE |
| Glucose-6-phosphate isomerase | GPI | 5.3.1.9. | 1 | TC |
| Mannose-6-phosphate isomerase | MPI | 5.3.1.8. | 1 | TBE |
| Phosphoglucomutase | PGM | 5.4.2.2. | 1 | TC |

### Genetic data analysis

Genotypic frequencies for polymorphic loci in all samples were tested for goodness of fit to Hardy-Weinberg (HW) expectations performing an exact HW-test (HALDANE, 1954 ; WEIR, 1990). With no more than five alleles per locus, the complete enumeration method by LOUIS and DEMPSTER (1987) was used. To test the hypothesis that the allelic distribution is identical between populations, a probability test on a contingency table for each locus between all population samples was performed according to RAYMOND and ROUSSET (1995).

Genetic distance between all pairwise combinations of population samples was estimated using NEI's genetic distance (NEI, 1972). As an outgroup, genotypic data from a population sample from the sister species *Austropotamobius torrentium* (Schrank, 1803) from eastern Switzerland (Ellighausen, canton Thurgau) were added to the data set (CLALÜNA, 1996). Based on the calculated estimates, graphic representations of the matrices were carried out using the neighbour-joining (SAITOU and NEI, 1987) and the UPGMA (SNEATH and SOKAL, 1973) methods. The reliability of the inferred trees was tested by a bootstrapping analysis (1000 data sets) and a subsequent construction of a strict consensus tree. The percentage a given node appeared in the 1000 bootstrapped data sets was then given for each node. The above-mentioned analyses were carried out using the program packages Genepop (RAYMOND and ROUSSET, 1995) and Phylip (FELSENSTEIN, 1995).

To estimate the degree of genetic subdivision between populations and groups of populations, WRIGHT's F-Statistics were applied to the data (WRIGHT, 1951, 1978). For the hierarchical analysis of population differentiation, two different hierarchies were used. The first hierarchy had the levels : single population samples, drainage systems, total. The second hierarchy followed the levels : single population samples - distinct clusters - total. The clusters were the groups of population samples resulting from the cluster analysis. Analysis of F-statistics was carried out using the program Biosys-1 (SWOFFORD and SELANDER, 1989).

## RESULTS

### Morphological data

Based on a combination of the characters listed in Table II, the population sample from France (site 1) and those from north-western Switzerland (sites 2-8) were assigned to *A. p. pallipes*. However, the number of postcervical spines and, in a few cases, the shape of the rostrum spines showed more variability than it was expected (Table IV). According to BOTT (1972), more than two postcervical spines are typical of *A. p. pallipes*. In populations 2-8, many of the individuals analysed had only one or two spines. Two specimens from site 8 had very short side spines at their rostra, as it is typical for *A. berndhauseri*. In addition, the populations 2 and 3 (Curbit and Paudèze) could only tentatively be assigned to *A. p. pallipes*. Several specimens of these samples showed atypical characteristics of *A. p. pallipes* (low number of postcervical spines ; unequivocal shape of side spines ; shape of claws rather as in *A. berndhauseri*).

The population of Sous le Cex (site 9) had intermediate characters between *A. berndhauseri* and *A. p. italicus*. The rostrum, which is the main morphological criterion that separates these taxa, is subdivided as in *A. berndhauseri* (short tip), but has long spines and a pointed tip as in *A. p. italicus*.

The population samples from the southern part of Switzerland (sites 10-17) clearly exhibited the morphological characteristics of *A. berndhauseri* (Table IV). These samples include the populations from the upper Rhone (sites 10-12), the upper Rhine (sites 13-15) and the Ticino (sites 16-17). Also, the Italian population from site 18 (V. del Lanza) showed typical characters of *A. bernhauseri*. However, in the lake Grond sample (site 13) and the Nordkanal sample (site 12), a few individuals had three postcervical spines in contrast with the lower number that is typical of *A. berndhauseri*.

**Table IV**

**Distribution of morphological characters found in population samples. The number of specimens exhibiting the morphological characters described by BOTT (1972) is given for each sample. p = *A. p. pallipes* ; i = *A. p. italicus* ; b = *A. berndhauseri* ; { } = several individuals with atypical characteristics ; sym. = symmetrical.**

**Tableau IV**

**Distribution des caractères morphologiques trouvés dans les échantillons des populations analysées. Le nombre de spécimens montrant les caractères morphologiques décrits par BOTT (1972) est donné pour chaque échantillon. p = *A. p. pallipes* ; i = *A. p. italicus* ; b = *A. berndhauseri* ; { } = échantillons dont certains individus ont des caractères atypiques ; sym. = symétrique.**

| No. | Water body | Postcervical spines | | Shape of side spines | Shape of lobes of gonopod I | | |
|---|---|---|---|---|---|---|---|
| | | 1-2 | 3-4 | | sym. | not sym. | ? |
| 1 | Eyrieux/Doux | 5 | 10 | p | 15 | 0 | 0 |
| 2 | Paudèze | 9 | 8 | p | 6 | 0 | 3 |
| 3 | Curbit | 5 | 5 | {p} | 1 | 0 | 4 |
| 4 | Orvine | 0 | 11 | p | 2 | 0 | 5 |
| 5 | Gräntschelbach | 7 | 8 | p | 7 | 1 | 1 |
| 6 | Steinbach | 3 | 7 | p | 1 | 0 | 6 |
| 7 | Moosbach | 7 | 10 | p | 5 | 0 | 6 |
| 8 | Lindenbach | 9 | 5 | p | 5 | 0 | 0 |
| 9 | Sous le Cex | 14 | 0 | b/i | 0 | 10 | 1 |
| 10 | Pied des Champs | 17 | 1 | b | 2 | 10 | 0 |
| 11 | Canal Blancherie | 11 | 0 | b | 1 | 8 | 0 |
| 12 | Nordkanal | 7 | 5 | {b} | 0 | 8 | 0 |
| 13 | Lac Grond | 11 | 2 | {b} | 0 | 11 | 1 |
| 14 | Stradabach | 5 | 0 | b | 0 | 3 | 0 |
| 15 | Gumpen | 20 | 0 | b | 0 | 18 | 1 |
| 16 | Falloppia | 5 | 0 | b | 0 | 0 | 2 |
| 17 | Piave Murin | 15 | 0 | b | 0 | 3 | 4 |
| 18 | V. del Lanza | 11 | 0 | b | 0 | 8 | 0 |
| 19 | Monti Berici | 17 | 0 | b/p/i | 0 | 6 | 0 |

Most specimens of the Monti Berici sample (site 19) from Vicenza showed a strange combination of characters of *A. berndhauseri, A. p. pallipes* and *A. p. italicus*. The number of postcervical spines and the shape of the gonopods fit the characters of *A. berndhauseri* and *A. p. italicus*, but several specimens had a rostrum with intermediate characters of all three taxa.

### Genetic data

At 14 out of 20 loci, the samples analysed were fixed for the same allele. Allelic frequencies of the six loci that showed intraspecific variability are given in Table V. The polymorphic loci within population samples were PEPA, ALAT, G3PDH, AAT-2, and MDH. At the SOD-2 locus, samples were fixed for alternative alleles. Consequently, estimates of genetic variability were low, with estimates of heterozygosity ranging from 0.00 to 0.044 with an average of 0.006 (Table VI) and the percentages of polymorphic loci varied from 0 to 15 %.

In the analysis of the distribution of genotypic frequencies for congruence with Hardy Weinberg's expectations, two out of twelve tests performed showed significant deviations at $p<0.05$. Both concerned samples from the upper Rhone at the locus G3PDH. No heterozygote genotype could be observed at this locus, which may cast doubt on the interpretation of the zymograms in question. However, an independent re-examination of zymograms reached the same results.

**Table V**

**Allele frequencies of polymorphic loci, taxonomic groups according to morphological characters described by BOTT (1972). b = *A. berndhauseri* ; p = *A. p. pallipes* ; i = *A. p. italicus* ; { } = several individuals with atypical characters.**

**Tableau V**

**Fréquences alléliques des loci polymorphes, groupements taxonomiques suivant les caractères morphologiques décrits par BOTT (1972). b = *A. berndhauseri* ; p = *A. p. pallipes* ; i = *A. p. italicus* ; { } = échantillons dont certains individus ont des caractères atypiques.**

| Water Body (with Number) | 1 Eyrieux/Doux | 2 Paudèze | 3 Curbit | 4 Orvine | 5 Gräntschelbach | 6 Steinbach | 7 Moosbach | 8 Lindenbach | 9 Sous le Cex | 10 Pied des Champs | 11 Canal Blancherie | 12 Nordkanal | 13 Lake Grond | 14 Stradabach | 15 Gumpen | 16 Falloppia | 17 Piave Murin | 18 V. del Lanza | 19 Monti Berici |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Abbreviation | Rô1 | Rô2 | Rô3 | Ri1 | Ri2 | Ri3 | Ri4 | Ri5 | Rô4 | Rô5 | Rô6 | Rô7 | Ri6 | Ri7 | Ri8 | Pô1 | Pô2 | Pô3 | Ad1 |
| Morphology | p | {p} | {p} | p | p | p | p | p | b/i | b | b | b | b | b | b | b | b | b | {b}/? |
| Enzymes | | | | | | | | | | | | | | | | | | | |
| AAT-2 | | | | | | | | | | | | | | | | | | | |
| 98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | | | | | | | | | 0.19 |
| 100 | | | | | | | | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.81 |
| ALAT | | | | | | | | | | | | | | | | | | | |
| 89 | | | 0.03 | | | | | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.25 |
| 94 | 1.00 | 1.00 | 0.97 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | | | | | | | | | 0.75 |
| SOD-2 | | | | | | | | | | | | | | | | | | | |
| 95 | 1.00 | 1.00 | 1.00 | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 98 | | | | 1.00 | | | | | | | | | | | | | | | |
| MDH | | | | | | | | | | | | | | | | | | | |
| 78 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.13 |
| 94 | | | | | | | | | | | | | | | | | | | 0.87 |
| G3PDH | | | | | | | | | | | | | | | | | | | |
| 98 | | | | | | | | | | 0.87 | 0.81 | | | | | | | | |
| 100 | | | | | | | | | | | | | | | | | | | |
| 101 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.13 | 0.19 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| PEPA | | | | | | | | | | | | | | | | | | | |
| 89 | | 0.12 | 0.19 | | 0.77 | 0.60 | 0.10 | | | | | | | | | | | | |
| 103 | 1.00 | 0.88 | 0.81 | 1.00 | 0.23 | 0.40 | 0.90 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | |
| 111 | | | | | | | | | | | | | | | | | | | 1.00 |

**Table VI**

**Measures of genetic variability detected in the population samples. No. = number of water body, N = average number of individuals analysed per locus, X = average number of alleles per locus, P = percentage of polymorphic loci, H (obs) = average observed heterozygosity, H (exp) = average heterozygosity expected under Hardy-Weinberg equilibrium.**

**Tableau VI**

**Mesures de la variabilité génétique des échantillons analysés. No. = numéro du cours d'eau (voir Tableau I), N = moyenne du nombre d'individus analysés par population, X = nombre moyen d'allèles par locus, P = pourcentage de loci polymorphes, H (obs) = moyenne d'hétérozygotie observée, H (exp) = moyenne d'hétérozygotie attendue sous l'hypothèse d'un équilibre de Hardy-Weinberg.**

| Water body | No | N | X | P | H (obs) | H (exp) |
|---|---|---|---|---|---|---|
| Eyrieux/Doux | 1 | 14.7 | 1.0 | 4.5 | 0.003 | 0.003 |
| Paudèze | 2 | 9.8 | 1.0 | 5 | 0.015 | 0.013 |
| Curbit | 3 | 9.0 | 1.1 | 10 | 0.025 | 0.022 |
| Orvine | 4 | 11.6 | 1.0 | 0 | 0.000 | 0.000 |
| Gräntschelbach | 5 | 12.3 | 1.0 | 5 | 0.015 | 0.018 |
| Steinbach | 6 | 9.6 | 1.0 | 5 | 0.010 | 0.025 |
| Moosbach | 7 | 10.0 | 1.0 | 5 | 0.010 | 0.009 |
| Lindenbach | 8 | 8.9 | 1.0 | 0 | 0.000 | 0.000 |
| Sous le Cex | 9 | 7.4 | 1.0 | 0 | 0.000 | 0.000 |
| Pied des Champs | 10 | 16.0 | 1.0 | 5 | 0.000 | 0.016 |
| Canal Blancherie | 11 | 15.0 | 1.0 | 5 | 0.000 | 0.012 |
| Nordkanal | 12 | 7.4 | 1.0 | 0 | 0.000 | 0.000 |
| Lac Grond | 13 | 12.0 | 1.0 | 0.0 | 0.000 | 0.000 |
| Stradabach | 14 | 6.0 | 1.0 | 0.0 | 0.000 | 0.000 |
| Gumpen | 15 | 10.0 | 1.0 | 0.0 | 0.000 | 0.000 |
| Falloppia | 16 | 3.7 | 1.0 | 0 | 0.000 | 0.000 |
| Piave Murin | 17 | 13.3 | 1.0 | 0 | 0.000 | 0.000 |
| V. del Lanza | 18 | 7.2 | 1.0 | 0 | 0.000 | 0.000 |
| Monti Berici | 19 | 7.4 | 1.1 | 15 | 0.044 | 0.048 |
| Average | | | | | 0.0064 | 0.0087 |

### Cluster analysis

The cluster analysis of NEI's genetic distance measure (NEI, 1972) using the neighbor-joining and the UPGMA technique revealed nearly identical tree topologies. In Figure 2, the UPGMA-consensus tree computed for 1000 bootstrapped data sets is shown. The samples from the river drainages of the lower Rhine and lower Rhone (sites 1-8, "northern cluster") form one cluster which occurred in 87 % of the bootstrapped data sets. A cluster composed of the samples from the rivers Ticino, upper Rhone and upper Rhine (sites 9-18,"southern cluster") was found in 89 % of the 1000 data sets, and the sample from the river Adige (site 19) had an isolated position in 74 % of the bootstrapped data sets. As it is documented in Table V, this differentiation into three distinct groups of populations is mainly due to the two loci AAT-2 and ALAT for the differentiation between the northern and southern population groups and the loci PEPA and MDH for the differentiation of the Adige population. At the locus AAT-2, the two population groups are fixed for alternative alleles and, at the locus ALAT, they are nearly fixed. The Adige sample is fixed for an alternative allele at the locus PEPA, and nearly fixed for an alternative allele at the locus

MDH. At the locus ALAT, both allelic variants that separate the northern and southern population groups were detected in this sample. In the northern cluster, the samples were joined at a low level of divergence but most nodes were poorly supported by the bootstrapping analyses. However, the sample from the Swiss Jura mountains (site 4) was fixed for an alternative allele at the locus SOD-2 (Table V).

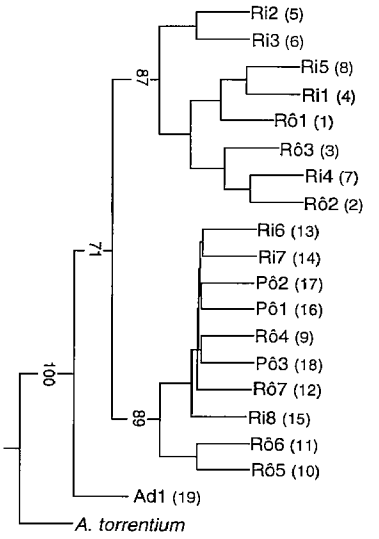


**Figure 2**

**Rooted strict consensus UPGMA tree based on a bootstrapping analysis (1000 data sets) using NEI's distance D (NEI, 1972). Rooting was conducted using data from the sister species *A. torrentium*. Numbers at the nodes represent the percentage a given node appeared in the 1000 bootstrapped data sets. Only values above 70 % are given. Abbreviations as in Table I.**

**Figure 2**

**Arbre de consensus strict raciné, élaboré par la méthode UPGMA et basé sur une analyse de bootstrap (1000 sets de données) utilisant le coefficient de distance génétique de NEI (1972). La racine est donnée par l'espèce-sœur *A. torrentium*. Les numéros aux nœuds correspondent aux pourcentages pour ces nœuds dans les mille sets de données bootstrappés. Seules les valeurs au-dessus de 70 % sont incluses. Les abréviations sont les mêmes que pour le Tableau I.**

### Genetic differentiation

An overall very strong differentiation between population samples was found (Fst=0.883). Due to the fixed differences observed at several loci, this is not surprising. In the analysis of the hierarchical structure with samples of common drainage systems grouped in one hierarchy level, practically all the differentiation is due to differences between samples within river systems (Gsg(t)Å0.883). In the scenario with the clusters of Figure 2 as basis for the hierarchy, the main part of differentiation was found between clusters (Ggt=0.831) and considerably less within clusters (Gsg(t)=0.052). However, all values observed indicated from moderate to strong differentiation.

## DISCUSSION

### Taxonomy

Using morphological characters, the taxonomy of *A. pallipes* in the alpine region has been contradictory according to several authors (BOTT, 1950, 1972 ; KARAMAN, 1963 ; ALBRECHT, 1982). KARAMAN named two species : *A. pallipes* and *A. italicus*, subdivided the latter into three

subspecies of which only *A. i. italicus* occurs in the alpine region. BOTT named three taxa for the studied area : the subspecies *A. p. pallipes* for France and in Switzerland for the Central plateau and the canton Graubünden, *A. p. italicus* for northern Italy, southern Switzerland and Dalmatia, and a separate species *A. berndhauseri* for southern Switzerland (canton Tessin and southern valleys of the canton Graubünden). In contrast, ALBRECHT (1982) proposed one species : *A. pallipes,* with several varieties. In the alpine region, he lists the varieties *pallipes*, *lombardicus* and *carnthiacus*.

The structure detected in the present study cannot fully resolve itself between these propositions. According to the present morphological analysis, three more or less distinct groups, *i.e. A. p. pallipes* and *A. berndhauseri sensu* BOTT (1972), and a sample from Vicenza which represents a mixture of characteristics of *A. p. pallipes*, *A. p. italicus* and *A. berndhauseri* can be distinguished. In several samples, intermediate or atypical individuals were observed, and the variability of some of the morphological characters proposed by BOTT (1972) is high. In particular, the samples from Graubünden, which BOTT attributed to *A. p. pallipes*, exhibited morphological characters of *A. berndhauseri*. This discrepancy may have come about through a replacement of *A. p. pallipes* stocks at these sites in recent years. Alternatively, the relatively few individuals studied by BOTT may have prevented the detection of the variability inherent to these populations.

ALBRECHT (1982) named a form *lombardicus*, which he considered to be autochthonous from northern Italy (Lombardia) and the Tessin and introduced by man into the canton Graubünden. The present study showed that samples from the canton Tessin, northern Italy and the canton Graubünden effectively form one genetic entity. However, ALBRECHT (1982) and BOTT (1972) both did not mention *A. pallipes* from the canton Wallis, where we found populations that clearly belong to the southern population group. However, because samples from northern Italy and the Apennines that would represent the taxon *A. p. italicus* could not be obtained in due time, further taxonomic considerations should be postponed until additional material is available.

**Genetic population structure**

The present analysis of the genetic population structure of *A. pallipes* in Switzerland and the adjacent parts of northern Italy and France revealed three differentiated groups, representing three tentative phylogeographic entities according to AVISE *et al.* (1987). However, the structure does not encompass expected geographic entities, because samples from the same river systems are not found within the same groups. Several non-exclusive hypotheses can be formulated to explain this pattern. The two groups represent 1) natural entities resulting from genetic drift in isolated small populations, 2) natural entities resulting from long-term migration effects, and 3) artificial entities resulting from recent human influences.

The first hypothesis is rather unlikely to explain the separation into the two main groups ("southern" and "northern" clusters) because it would involve concerted effects of genetic drift in several adjacent populations. The second explanation requires the separation of three distinct gene pools and their differentiation in isolation. This implies the migration of a southern population group back into the alpine valleys and over at least two passes. However, *A. pallipes* does not occur in alpine environments today which makes crossing passes by *A. pallipes* rather unlikely. If human influence is included in the scenario at the moment the crayfish have reached the alpine valleys, the picture becomes more realistic. Since at least the Middle Ages, crayfish have been an important food source for man (CUZERKIS, 1988), and even though *Astacus astacus* is considerably more valued as a commercial food than *A. pallipes* (CUZERKIS, 1988 ; LAURENT, 1988), in certain regions of Europe *A. pallipes* still serves as a local food (MAIO, pers. comm.). This makes transplantations of crayfish stocks from the Pô river system over the Alps into the upper Rhone and upper Rhine by man a realistic hypothesis that could explain the present day distribution patterns (SPITZY, 1979 ; ALBRECHT, 1982).

With respect to the incorporation of population samples from the Rhine and the Rhone basin into the "northern" cluster, a historical explanation is possible. There is evidence that there has been a connection between Lake Geneva and the Rhine basin at the end of the Würm Glaciation (STEINMANN, 1951). One branch of the Rhone glacier expanded west into France and the other north-east crossing the low watershed into the Aare, and therefore, via Rhine into the

Atlantic drainage system. According to STEINMANN's hypothesis, whitefish (*Coregonus* sp.) followed the retreating Atlantic branch of the glacier and thus colonized Lake Geneva. LARGIADER *et al.* (1996) suggested the same pathway of recolonization for Arctic char (*Salvelinus alpinus*) and the brown trout (*Salmo trutta*). For crayfish which are known to be able to migrate over the land, this watershed most likely did not constitute an efficient barrier to dispersal, and movements in both directions seem possible.

For the third genetically distinct branch, which is composed of only one sample from near Vicenza, the data need further comments. At the loci that differentiate the northern and the southern population groups, this sample takes an intermediate position. In addition, it is fixed for alternative alleles at two other loci. Whether this population represents an ancestral stock which is indicated by its basal position near to the outgroup species *A. torrentium,* or whether it is a mixture of several taxa will only be interpretable if additional samples from this region and sites in northern Italy that represent *A. p. italicus* are available for analysis.

**Conclusion for conservation**

The distribution patterns detected in the present study have important implications for nature conservation. In the effort to save Earth's biodiversity, the maintenance of genetically unique entities has great importance (WILSON, 1988 ; AVISE, 1994 ; AVISE and HAMRICK, 1996). Recent studies on geographical variation of mitochondrial DNA in *A. pallipes* have demonstrated differentiation between samples from distant European regions, but negligible differentiation was detected among samples at a regional scale (GRANDJEAN *et al.*, 1997 a, b). Following common knowledge and the results of these studies, the build-up of stocks for reintroduction schemes would rely on the reasoning that populations from the same river system should be genetically rather similar. In the present case, as far as the samples from Switzerland are concerned, this would be wrong, and a mixing rather than a conservation of phylogeographically and genetically distinct entities would be the consequence. As to the choice of methods to reveal genetic structuring of this endangered species, no preference can be stated at the moment, as no parallel study using both approaches has been carried out to date.

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