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Genetic differentiation in *Gammarus fossarum* and *G. caparti* (Crustacea, Amphipoda) with reference to *G. pulex pulex* in northwestern Europe

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Abstract

Genetic differentiation among *G. fossarum* Koch, 1835 from different stations in Germany, Switzerland, Belgium and northern France, and the closely related Belgian form *G. caparti* Pètre-Stroobants, 1980 was investigated by electrophoresis at 20 enzyme loci. Although morphologically variable, geographically distant populations of *G.* cf. *fossarum* were hitherto considered conspecific. In the present study, populations of *G.* cf. *fossarum* and *G. caparti* were examined with reference to *G. pulex pulex* as an estimate for genetic differentiation at the species level.

With *G. p. pulex* as a standard, genetic differentiation among geographically distant populations of *G.* cf. *fossarum* is occasionally observed at species level. The populations of *G. caparti* studied were shown to be genetically very similar to certain populations of *G. fossarum*. The taxonomic status of *G. caparti* and the genetically distinct forms of *G. fossarum* is discussed.

Résumé

La différenciation génétique de *G. fossarum* Koch, 1835, provenant de divers stations d'Allemagne, de Suisse, de Belgique et du nord de la France, et de *G. caparti* Pètre-Stroobants, 1980 (forme étroitement apparentée provenant de Belgique) a été étudiée par électrophorèse à 20 loci d'enzymes. Bien que morphologiquement variables, des populations géographiquement éloignées de *G.* cf. *fossarum* ont été considérées jusqu'à présent comme conspécifiques. Dans la présente étude, des populations de *G.* cf. *fossarum* et *G. caparti* ont été étudié par rapport à *G. pulex pulex*, utilisé comme estimation de la différenciation génétique au niveau spécifique.

Avec *G. p. pulex* comme référence, la différenciation génétique entre des populations géographiquement éloignées de *G.* cf. *fossarum* atteint occasionnellement le niveau spécifique. Les populations de *G. caparti* étudiées se sont montrées génétiquement très semblables à certaines populations de *G. fossarum*. Le statut taxonomique de *G. caparti* et celui des formes génétiquement distinctes de *G. fossarum* est discuté.

Introduction

Local populations of *Gammarus fossarum* Koch, 1836 have often been described as separate species (Karaman & Pinkster, 1977; Pètre-Stroobants, 1980, 1981). The species is widely distributed in northwestern Europe (Karaman & Pinkster, 1977), and exhibits morphologically significant geographic variation (Goedmakers, 1972). Cross-breeding experiments among samples of morphologically distinct populations of the *G. pulex*-group (Pinkster, 1983; Scheepmaker, 1987) indicate complete interfertility and genetic homogeneity and these populations are therefore considered conspecific. Similarly, cross-breeding experiments among morphologically distinct allopatric samples of *G. fossarum* (Goedmakers, 1972; Scheepmaker, unpubl. data) and experiments run by Scheepmaker (unpubl. data), between *G. caparti* and *G. fossarum* also suggest complete interfertility and thus conspecificity.

The present study aims to provide an estimate of intra-specific genetic differentiation and a genetic base for the reconsideration of the conspecificity of allopatric populations of *G. fossarum*. Secondly, the taxonomic status of *G. caparti* is treated. Assessment of the species level of genetic differentia-

Table 1. Sampling localities and species distribution.

Station no.	Area in fig. 1A	Species	Country	Prov /dept	Drainage system	Locality description	Sampling date
1	D	*G. fossarum*	Germany	Regensburg	Donau	Brook along road between Thalmassing and Dünzling, 4km W. of road 15	15-V-'87
2	D	*G. fossarum*	Germany	Regensburg	Donau	Brook draining in Gr. Laaber at Pinkofen, 3 km E. of road 15	15-V-'87
3	E	*G. fossarum*	Germany	Regensburg	Donau	Brook at Wutzdorf, 1 km S. of road 20, 24 km N.N.E. of Regensburg	15-V-'87
4	F	*G. fossarum*	Switzerland	Oberaargau	Langete	Stream draining in Langete at Kleindietwill, 3km N. E. of road 23	17-V-'87
5	F	*G. fossarum* *G. p.pulex*	Switzerland	Bern	Aare	Stream draining into Aare, at Oberthal, 4 km N. of road 30	17-V-'87
6	F	*G. fossarum*	Switzerland	Bern	Aare	Brook drainig into Aare, near Frieswill, 8 km S. E. of road 22 at Aarberg	17-V-'87
7	C	*G. caparti*	Belgium	Luxembourg	Meuse	Lomme, at the Mirwart fish farm, 1 km S. E. of road N46	20-VI-87
8	C	*G. caparti*	Belgium	Liège	Meuse	Samson, at the crossing with road N42, 1 km N. of Faulx-les-Tombes	20-VI-87
9	C	*G. fossarum* *G. p. pulex*	Belgium	Namur	Escaut	Source/brooklet draining into the Dyle, near N21, 3.5 km N. W. of Corroy-le-Chateau	
10	C	*G. fossarum*	Belgium	Brabant	IJzer	Rivulet in woodland along road N75 draining into the Kemmelbeek, 2 km S. E. of Westouter	22-VI-'87
11	B	*G. fossarum*	France	Pas-de-Calais	Liane	Brooklet in the Fôret de Boulogne draining into the Liane, near road D254, 9 km E. of Boulogne-sur-Mer	21-VI-'87
12	B	*G. fossarum.*	France	Pas-de-Calais	Wimereux	Upper reach of the Wimereux, along road 251e, 1 km S. S. E. of Boursin	21-VI-'87
13	B	*G. fossarum*	France	Pas-de-Calais	Slack	Country road at Héronval, near road D127, 3 km S. E. E. of Réty	21-VI-'87
14	B	*G. fossarum* *G. p. pulex*	France	Pas -de-Calias	Slack	Upper reach/source of the Slack, along road 251e, 2.5 km N. E. of Boursin	21-VI-'87
15	-	*G. p. pulex*	Holland	Gelderland	Hierdense beek	Hulshorst, 0.2 km N. N. W. of the railroad station and road E35	2-I-'88

tion is based on 4 geographic samples of *G. pulex pulex* (Linnaeus, 1758). *G. p. pulex* is morphologically well-defined, often found sympatric with *G. fossarum*, but maintains its integrity in these areas (Wautier & Roux, 1959; Roux, 1971; Meijering, 1972).

In this study of samples from northwestern Europe, genetic variation is investigated using starch gel enzyme electrophoresis.

Materials and methods

Sampling and collection sites

Sampling for electrophoretic studies has been carried out based on the procedures of Scheepmaker, 1987. The collection sites and sampling dates along with the species collected are listed in table 1 and fig. 1A–F. Four sampling areas in northern France, Belgium, Switzerland, and southern Germany were selected as exhibiting extremes in morphology. The morphological variation recorded (following criteria of Goedmakers, 1972) is summarized in table 2 and fig. 2. *G. caparti* (table 1, fig. 1A, C: stations 7, 8) differs from *G. fossarum* primarily by the presence of a seta on the first segment of the right mandibular palp in most (but not all) individuals (fig. 2E). *G. fossarum* from stations 10–14 in northern France and Belgium (table 1; fig. 1A, B and C) differs from individuals of all other populations by the short setation of the A2 and P4 (fig. 2B, G). Individuals from Swiss populations of *G. fossarum* (table 1; fig. 1A, F) are characterized by much longer setae on the flagellum (fig. 2A). The presence or absence of calceoli (fig. 2B) is usually constant within populations. In German populations this character can be variable; in populations of *G. fossarum* from the Regensburg area

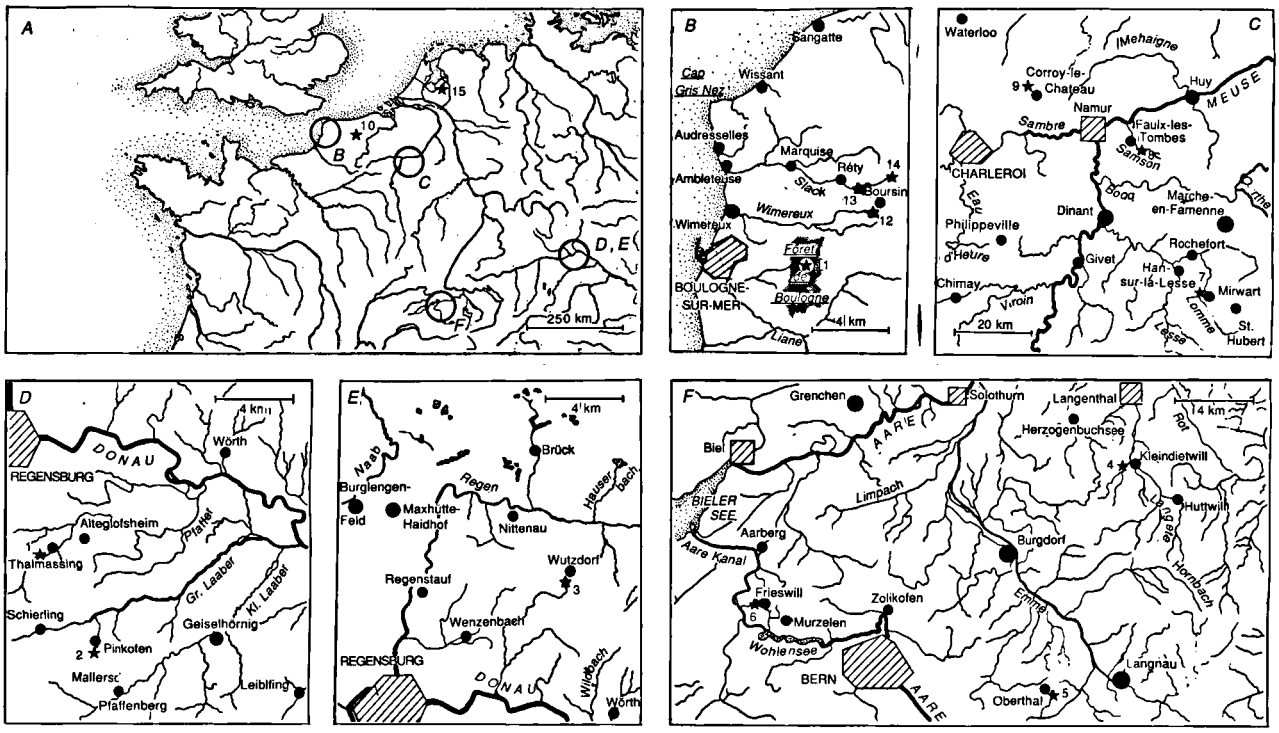
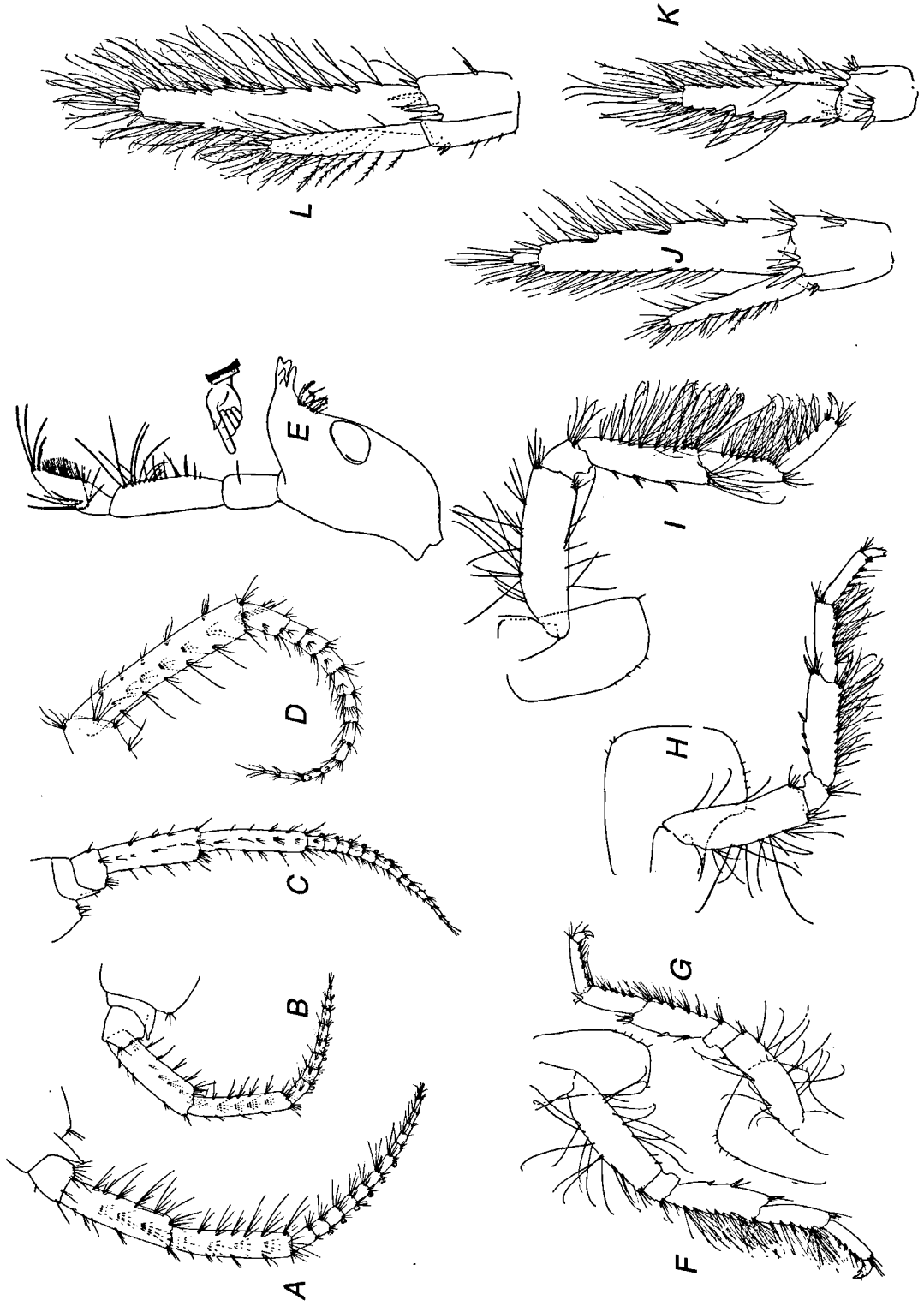


Fig. 1A–F. Study areas and sampling localities (cf. table 1).

Table 2. Morphological differentiation of some characters in *G. fossarum* and *G. caparti*. For legend see fig. 2.

Station no	A2: Length of the setae with reference to the diameter of the segments on which they are inplanted					Density setation				Setation P3 curly	Calceoli present	seta on first segment mandibular palp
	peduncle segment		flagellum									
	4	5		P3	P4	A2	P3	P4	U3			
1	=	>=	=	>>	=	-	++	+	++	+	- /+	-
2	=	>=	=	>>	=	-	++	+	++	+	- /+	-
3	=	>=	=	>>	>=	-	++	+	+	+	- /+	-
4	=	>=	>>=	>>	>=	+	++	+	+	-	+	-
5	>=	>>=	>>=	>	>=	+	+	+	+	+	+	-
6	=	>=	>>=	>>	>=	+	++	+	-	+	+	-
7	=	>=	=	>>	>=	- /+	+	-	+	-	+	- /+
8	=	>=	=	>>	>=	- /+	+	-	+	-	+	- /+
9	<	<	<	>>	>	- /+	++	+	++	+	+	-
10	<=	<=	<=	>>	=	-	++	-	+	+	+	-
11	<=	<=	<=	>	=	-	+	-	+	+	+	-
12	<=	<=	<=	>	=	-	-	-	- /+	-	+	-
13	<=	<=	<=	>	<=	-	+	-	- /+	-	+	-
14	<=	<=	<=	>	=	-	+	-	- /+	-	+	-


K
L
J
E
I
D
H
C
G
B
F
A

some individuals lack calceoli. However, the variation of most morphological characters overlaps among the populations studied. In each area 3 samples were taken to evaluate local genetic variation. The sampling localities in southern Germany are from the surrounding area of the former type locality of *G. fossarum* in the Regensburg area. The sampling localities in Belgium include the type locality of *G. caparti* (station 7); however, at 2 previously recorded sampling localities of *G. caparti* (Pètre-Stroobants, 1980) only *G. fossarum* was found (stations 9, 10).

Electrophoresis

Electrophoresis and staining procedures were identical to those of Scheepmaker et al. (1988). The following enzyme systems were assayed: ADA – Adenosine deaminase, E.C. No. 3.5.4.4; ALP – Alkaline phosphatase, E.C. No. 3.1.3.1; APK – Arginine phosphate kinase, E.C. No. 2.7.3.3; EST – Esterase, E.C. No. 3.1.1.1; GDH – Glutamate dehydrogenase, E.C. No. 1.1.1.47; GOT – Glutamic oxaloacetic transaminase, E.C. No. 2.6.1.1; GPI – Glucose phosphate isomerase, E.C. No. 5.3.1.11; HK – Hexokinase, E.C. No. 2.7.1.1; LAP – Leucine aminopeptidase, E.C. No. 3.4.1.1; MDH – Malate dehydrogenase, E.C. No. 1.1.1.37; ME – Malic enzyme, E.C. No. 1.1.1.40; MPI – Mannosephosphate isomerase, E.C. No. 5.3.1.8; PEP – Peptidase, E.C. No. 3.4.11/13; 6PGD – 6 Phosphogluconate dehydrogenase, E.C. No. 1.1.1.44; PK – Pyruvate kinase, E.C. No. 2.7.1.40.

Analysis of allozyme variation

The genetic interpretation of the variation was inferential. Electromorph frequencies and matrices of genetic identity (*I*) and (*D*) according to Nei (1972) and Rogers' (1972) genetic distance were calculated with the computer program BIOSYS-1 (Swofford & Selander, 1981). From these data, an UPGMA dendrogram (Sneath & Sokal, 1973) and a distance Wagner network (Farris, 1972) were constructed.

Fig. 2. Morphological differentiation of selected variable characters in *G. fossarum* and *G. caparti*. In parentheses: symbols used in table 2.

A: A2, bearing calceoli, setation of peduncle segments 4, 5 and flagellum much longer than or equal to the diameter of the segments on which they are implanted (> > =);

B: A2, bearing calceoli, setation of peduncle segments 4, 5 and flagellum predominantly equal to or shorter than the diameter of the segments on which they are implanted (< =);

C: A2, bearing calceoli, setation of the peduncle segments 4, 5 and flagellum much shorter than the diameter of the segments on which they are implanted (<);

D: A2, without calceoli, setation of the peduncle segments 4, 5 and flagellum equal to or longer than the diameter of the segments on which they are implanted (> =);

E: right mandibular palp of *G. caparti* with a seta implanted on the first segment;

F: P3, setae longer than or equal to the diameter of the segment on which they are implanted, setosity moderate ("> =" and "+", respectively), setae not curly;

G: P4, setae shorter than or equal to the diameter of the segment on which they are implanted, setosity poor ("< =" and "–", respectively);

H: idem, setae longer than or equal to the diameter of the segment on which they are implanted, setosity moderate ("> =" and "+", respectively);

I: P3, setae much longer than the diameter of the segment on which they are implanted, setosity dense ("> >" and "+ +", respectively), setae curly;

J: Uropod 3, poor setation ("–");

K: idem, moderate setation ("+");

L: idem, rich setation ("+ +");

(Drawings from: Goedmakers, 1972; Karaman & Pinkster, 1977; Pètre-Stroobants, 1980).

Table 3. Electromorph frequencies at 13 presumptive gene loci.

station		1	2	3	4	5	6	7	8	9	10	11	12	13	14	5	9	14	15
		f	*f*	*f*	*f*	*f*	*f*	*c*	*c*	*f*	*f*	*f*	*f*	*f*	*f*	*p*	*p*	*p*	*p*
locus	*electromorph*																		
Apk	(N)	38*	35	66	66	41	50	40	43	37	43	39	35	43	22	6	35	28	30
	b	1.00	1.00	0.80	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
	c	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	h	-	-	*0.18*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Est-1	(N)	40	47	44	29	35	52	42	61	37	29	27	44	29	19	3	26	21	20
(b)	c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00
(c)	d	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	0.00	0.00	0.00	0.00
Got-1	(N)	41	62	70	68*	39	59	56	30	39	39	33	33	36	21	3	35	50	33
	a	0.79	0.88	0.82	0.46	1.00	0.23	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(a)	b	0.00	0.10	0.12	0.47	0.00	0.77	0.88	1.00	0.99	0.97	0.98	0.98	0.93	0.98	0.00	0.00	0.00	0.00
(c)	d	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	e	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	f	0.00	0.01	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.03	0.02	0.02	0.07	0.02	1.00	1.00	1.00	1.00
	g	0.21	0.01	0.05	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	h	*0.31*	*0.24*	*0.12*	*0.26*	-	*0.19*	*0.20*	-	*0.03*	*0.05*	*0.03*	*0.03*	*0.12*	*0.05*	-	-	-	-
Got-2	(N)	39	62	69	56	36	48	56	30	39	39	33	33	36	21	3	35	50	33
(a)	b	0.80	0.48	0.35	0.14	0.88	0.63	0.00	0.02	0.00	0.01	0.00	0.09	0.08	0.00	0.00	0.00	0.02	0.00
(b)	c	0.00	0.00	0.00	0.85	0.12	0.37	1.00	0.96	1.00	0.99	1.00	0.89	0.92	1.00	1.00	1.00	0.98	1.00
(c)	d	0.20	0.52	0.65	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
	h	*0.26*	*0.42*	*0.44*	*0.16*	*0.03*	*0.33*	-	*0.70*	*0.02*	*0.03*	-	*0.15*	*0.11*	-	-	-	*0.04*	-
Gpi	(N)	76	107	59	80	20	80	61	54	52	51	30	53	45	45	9	40	32	37
	a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	1.00	0.99	0.96	0.98	0.00	0.00	0.00	0.00
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	c	0.00	0.00	0.00	0.65	0.00	0.60	0.93	0.96	0.00	0.44	0.00	0.01	0.03	0.02	1.00	0.95	1.00	0.83
	d	0.00	0.00	0.00	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(d)	e	0.99	0.76	0.56	0.18	0.97	0.37	0.03	0.01	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14
(e)	g	0.01	0.06	0.02	0.14	0.03	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.03
(h)	m	0.00	0.10	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	p	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	h	*0.26*	*0.35*	*0.50*	*0.53*	*0.05*	*0.39*	*0.13*	*0.07*	*0.02*	*0.41*	-	*0.02*	*0.09*	*0.04*	-	*0.10*	-	*0.27*
Ilk	(N)	34	53	63	36	39	39	37	40	35	36	34	37	36	15	39	35	25	16
	a	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	b	0.00	0.00	0.00	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
Mdh-1	(N)	41	61	76	70	28	64	58	30	39	41	33	33	46	32	3	34	38	19
	a	0.06	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
(a)	b	0.94	1.00	1.00	0.59	0.07	0.71	0.82	1.00	1.00	0.98	1.00	1.00	0.98	0.97	1.00	0.93	0.99	0.45
	d	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
(b)	e	0.00	0.00	0.00	0.10	0.93	0.23	0.02	0.00	0.00	0.02	0.00	0.00	0.01	0.03	0.00	0.00	0.01	0.55
(c)	f	0.00	0.00	0.00	0.30	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(e)	h	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	h	*0.12*	-	-	*0.51*	*0.14*	*0.38*	*0.22*	-	-	*0.05*	-	-	*0.04*	*0.06*	-	*0.15*	*0.03*	*0.26*
Mdh-2	(N)	34	57	76	66	28	62	58	29	39	30	33	27	41	32	3	34	38	33
	b	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	c	1.00	0.98	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	d	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	h	-	*0.03*	-	-	-	-	*0.03*	-	-	-	-	-	-	-	-	-	-	-
Me	(N)	36	78	67	54	43	60	45	49	51	46	42	36	46	14	3	43	20	11
	b	1.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	c	0.00	0.00	0.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 3. Continued.

station		1	2	3	4	5	6	7	8	9	10	11	12	13	14	5	9	14	15
locus		*f*	*f*	*f*	*f*	*f*	*f*	*c*	*c*	*f*	*f*	*f*	*f*	*f*	*f*	*p*	*p*	*p*	*p*
electromorph																			
Mpi	(N)	47	58	44	63	61	54*	40	49	34*	48	30	42	33	32	6	37	33	34
	d	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.93	0.20	0.44
(b)	f	0.00	0.00	0.00	0.02	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(c)	g	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.80	0.56
	j	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(f)	k	0.86	0.16	0.60	0.01	0.01	0.15	0.01	0.00	0.41	0.00	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00
	l	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(g)	m	0.09	0.84	0.39	0.97	0.00	0.76	0.93	1.00	0.59	0.87	0.67	1.00	0.70	0.66	0.00	0.00	0.00	0.00
	n	0.01	0.00	0.01	0.00	0.00	0.00	0.06	0.00	0.00	0.13	0.33	0.00	0.04	0.34	0.00	0.00	0.00	0.00
	h	*0.17*	*0.19*	*0.36*	*0.06*	*0.02*	*0.11*	*0.10*	-	*0.18*	*0.06*	*0.33*	-	*0.30*	*0.38*	-	*0.13*	*0.21*	*0.29*
Pep-2	(N)	43*	46*	32	33	42	40	59	41	46	41	34	29	53	10	5	47	30	26
	c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.95	1.00
(e)	f	0.45	0.46	0.70	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
	h	0.51	0.53	0.27	0.00	1.00	0.00	0.00	0.00	0.33	0.01	0.00	0.00	0.00	0.05	0.00	0.00	0.03	0.00
	i	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	j	0.00	0.01	0.03	1.00	0.00	1.00	1.00	1.00	0.00	0.99	1.00	1.00	1.00	0.95	0.00	0.00	0.00	0.00
	h	*0.28*	*0.24*	*0.22*	-	-	-	-	-	-	*0.02*	-	-	-	*0.10*	-	-	*0.10*	-
Pep-4	(N)	41	43	42	41	43	45	57	42	45	41	36	29	53	10	5	48	30	26
	a	0.00	0.00	0.00	0.01	0.00	0.07	0.56	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.00	0.00
(a)	c	0.00	0.00	0.00	0.95	0.00	0.82	0.39	0.98	1.00	1.00	0.99	1.00	1.00	1.00	1.00	0.53	1.00	1.00
	e	0.99	1.00	1.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
	f	0.01	0.00	0.00	0.04	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	g	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	h	*0.02*	-	-	*0.10*	-	*0.27*	*0.32*	*0.05*	-	-	*0.03*	-	-	-	-	*0.40*	-	-
Pk	(N)	40	61	63	65	41	70	45	40	47	45	35	35	45	15	2	40	35	31
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.87	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00
	c	0.88	0.80	0.89	0.89	1.00	0.81	1.00	1.00	0.13	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00
	d	0.13	0.20	0.11	0.12	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	h	*0.20*	*0.36*	*0.22*	*0.23*	-	*0.29*	-	-	*0.21*	-	-	-	-	-	-	-	-	-
	H	*0.07*	*0.03*	*0.04*	*0.04*	*0.01*	*0.33*	*0.02*	*0.01*	*0.02*	*0.03*	*0.02*	*0.01*	*0.03*	*0.03*	-	*0.02*	*0.02*	*0.04*

f = *G. fossarum*; *c* = *G. caparti*; *p* = *G. p. pulex*; N = sample size; *h* = heterozygosity per locus (direct count);
H = mean heterozygosity over all (including monomorphic) loci;
* = significant departure from Hardy-Weinberg distribution ($P < 0.05$); electromorph designation from fastest to slowest in alphabetical order, in accordance with Scheepmaker (in prep.); in parentheses: alternative allelic designation employed by Scheepmaker et al., 1988.

Results

Twenty presumptive gene loci from 17 enzyme systems were scorable in all populations. Electromorph frequencies and relative mobilities of the enzymes coded for by these loci are listed in table 3.

The *Ada, Alp-1, Alp-2, Est-2, Gdh, 6Pgd* and *Lap* loci were monomorphic for all populations. The number of loci and subunit structures resolved were consistent with the interpretation of Scheepmaker et al. (1988).

Table 4. Number of diagnostic loci (0.99 criterion; Ayala & Powell, 1972) among samples of the populations studied.

station no.	species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	5	9	14	15
1	*G. fossarum*	-																	
2	*G. fossarum*	-	-																
3	*G. fossarum*	-	-	-															
4	*G. fossarum*	3	3	3	-														
5	*G. fossarum*	2	2	2	3	-													
6	*G. fossarum*	4	3	3	-	4	-												
7	*G. caparti*	3	2	4	-	4	-	-											
8	*G. caparti*	5	3	3	-	4	-	-	-										
9	*G. fossarum*	3	3	3	1	3	2	2	2	-									
10	*G. fossarum*	5	4	4	-	5	-	-	-	2	-								
11	*G. fossarum*	6	5	5	1	6	1	1	2	3	-	-							
12	*G. fossarum*	6	4	4	-	6	-	-	-	3	-	-	-						
13	*G. fossarum*	6	4	4	-	5	-	-	-	3	-	-	-	-					
14	*G. fossarum*	5	5	5	-	5	-	-	-	2	-	-	-	-	-				
5	*G. p. pulex*	8	9	10	5	7	5	4	4	6	4	5	4	4	4	-			
9	*G. p. pulex*	5	7	7	5	7	5	3	6	5	4	5	4	4	4	-	-		
14	*G. p. pulex*	7	8	8	5	7	5	4	6	5	4	4	4	4	2	-	-	-	
15	*G. p. pulex*	7	9	8	5	7	5	4	6	5	4	4	4	4	3	-	1	-	-

Table 5. Matrix of similarity and distance coefficients. Below diagonal: Nei's (1972) genetic distance. Above diagonal: Nei's (1972) genetic identity.

station no.	species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	5	9	14	15
1	*G. fossarum*	-	0.96	0.97	0.67	0.79	0.71	0.64	0.63	0.75	0.64	0.63	0.63	0.65	0.63	0.52	0.53	0.53	0.52
2	*G. fossarum*	0.04	-	0.98	0.72	0.78	0.74	0.69	0.68	0.77	0.69	0.67	0.68	0.68	0.67	0.53	0.54	0.54	0.52
3	*G. fossarum*	0.03	0.02	-	0.70	0.75	0.72	0.67	0.66	0.76	0.67	0.66	0.66	0.68	0.66	0.53	0.54	0.54	0.52
4	*G. fossarum*	0.39	0.33	0.36	-	0.69	0.98	0.96	0.98	0.81	0.97	0.94	0.94	0.94	0.94	0.75	0.74	0.75	0.75
5	*G. fossarum*	0.24	0.26	0.29	0.37	-	0.72	0.64	0.62	0.71	0.63	0.62	0.62	0.63	0.63	0.52	0.53	0.52	0.56
6	*G. fossarum*	0.34	0.30	0.33	0.02	0.33	-	0.95	0.97	0.81	0.96	0.93	0.94	0.94	0.93	0.73	0.72	0.74	0.74
7	*G. caparti*	0.44	0.37	0.39	0.04	0.44	0.05	-	0.98	0.79	0.97	0.96	0.93	0.93	0.93	0.74	0.74	0.74	0.73
8	*G. caparti*	0.46	0.38	0.41	0.024	0.48	0.04	0.02	-	0.81	0.98	0.95	0.95	0.96	0.95	0.75	0.74	0.76	0.74
9	*G. fossarum*	0.28	0.27	0.28	0.21	0.34	0.20	0.24	0.21	-	0.82	0.81	0.81	0.82	0.81	0.72	0.71	0.72	0.72
10	*G. fossarum*	0.45	0.38	0.40	0.32	0.46	0.04	0.03	0.02	0.20	-	0.99	0.99	0.99	0.99	0.74	0.73	0.75	0.73
11	*G. fossarum*	0.46	0.40	0.42	0.07	0.47	0.08	0.07	0.06	0.21	0.01	-	0.99	1,00	1,00	0.71	0.99	0.97	0.97
12	*G. fossarum*	0.46	0.38	0.41	0.06	0.47	0.07	0.07	0.05	0.21	0.01	0.01	-	1,00	1,00	0.70	0.69	0.71	0.70
13	*G. fossarum*	0.43	0.39	0.39	0.06	0.46	0.06	0.07	0.05	0.20	0.01	0.00	0.00	-	1,00	0.72	0.70	0.72	0.71
14	*G. fossarum*	0.45	0.40	0.41	0.06	0.46	0.07	0.07	0.05	0.21	0.01	0.00	0.01	0.00	-	0.71	0.70	0.72	0.71
5	*G. p. pulex*	0.65	0.63	0.64	0.29	0.66	0.32	0.31	0.29	0.33	0.30	0.34	0.36	0.33	0.34	-	0.99	0.97	0.97
9	*G. p. pulex*	0.63	0.62	0.62	0.31	0.64	0.33	0.31	0.31	0.35	0.20	0.21	0.21	0.20	0.20	0.01	-	0.96	0.96
14	*G. p. pulex*	0.64	0.62	0.63	0.28	0.64	0.30	0.30	0.28	0.32	0.29	0.33	0.35	0.32	0.33	0.33	0.04	-	0.98
15	*G. p. pulex*	0.66	0.65	0.66	0.28	0.57	0.31	0.32	0.30	0.33	0.31	0.35	0.36	0.34	0.34	0.03	0.04	0.02	-

Genetic variation among populations

Differences in electromorph distribution among the samples studied are shown in table 3. *G. p. pulex* populations are characterized by the predominating *Est-1*^{a}, *Mpi*^{d} and *Pep-2*^{c} electromorphs. Except for *G. fossarum* from station 9 (table 1, 3; fig. 1A, C), the *Pk*^{b} electromorph is likewise restricted to populations of *G. p. pulex*.

The unique occurrence of the *Got-2*^{d} and *Hk-1*^{a} electromorphs in *G. fossarum* samples from the Regensburg area (stations 1, 2 and 3; table 1, 3; fig. 1A, D and E) discriminates them from nearly all other samples of the species studied.

The *Me*^{b} electromorph was found in samples of *G. fossarum* from the Regensburg area (stations 1, 2 and 3) and stations 9 and 5 (Belgium and Switzerland respectively; see Tables 1, 3 and fig. 1C and F). The *Got-1*^{a} electromorph is predominant in samples of *G. fossarum* from the Regensburg area and

Table 6. Matrix of Rogers' (1972) distance.

station no.	species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	5	9	14	15
1	*G. fossarum*	-																	
2	*G. fossarum*	0.08	-																
3	*G. fossarum*	0.09	0.07	-															
4	*G. fossarum*	0.34	0.31	0.34	-														
5	*G. fossarum*	0.24	0.26	0.30	0.34	-													
6	*G. fossarum*	0.32	0.29	0.32	0.07	0.31	-												
7	*G. caparti*	0.38	0.34	0.36	0.09	0.37	0.11	-											
8	*G. caparti*	0.38	0.34	0.37	0.07	0.39	0.10	0.05	-										
9	*G. fossarum*	0.27	0.26	0.27	0.24	0.31	0.24	0.25	0.21	-									
10	*G. fossarum*	0.37	0.33	0.36	0.09	0.38	0.10	0.07	0.04	0.20	-								
11	*G. fossarum*	0.38	0.35	0.36	0.12	0.38	0.13	0.10	0.07	0.21	0.04	-							
12	*G. fossarum*	0.38	0.34	0.36	0.10	0.38	0.12	0.10	0.05	0.21	0.03	0.02	-						
13	*G. fossarum*	0.37	0.34	0.35	0.11	0.38	0.11	0.10	0.07	0.20	0.04	0.02	0.02	-					
14	*G. fossarum*	0.38	0.35	0.36	0.12	0.38	0.13	0.11	0.07	0.21	0.03	0.01	0.03	0.02	-				
5	*G. p. pulex*	0.48	0.47	0.48	0.28	0.49	0.31	0.29	0.25	0.29	0.27	0.29	0.30	0.29	0.29	-			
9	*G. p. pulex*	0.47	0.46	0.47	0.30	0.48	0.31	0.28	0.28	0.32	0.30	0.32	0.33	0.32	0.31	0.03	-		
14	*G. p. pulex*	0.47	0.46	0.47	0.27	0.48	0.30	0.28	0.25	0.29	0.27	0.29	0.30	0.29	0.29	0.04	0.07	-	
15	*G. p. pulex*	0.49	0.48	0.49	0.27	0.45	0.29	0.30	0.28	0.31	0.29	0.31	0.32	0.31	0.30	0.06	0.08	0.05	-

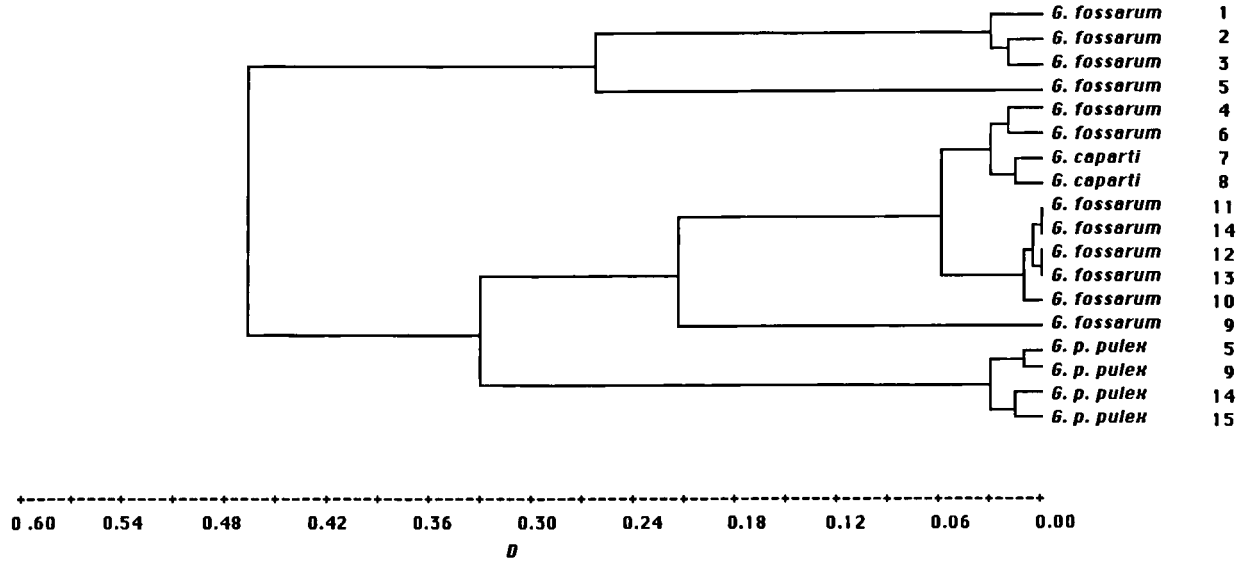


Fig. 3. UPGMA dendrogram of Nei's (1972) genetic distance (*D*) based upon 20 enzyme loci.

station 5, but occurs also in the *G. fossarum* population from station 6 (table 1, fig. 1F).

A half matrix of diagnostic loci (0.99 criterion; Ayala & Powell, 1972) is summarized in table 4. From the data in tables 5 and 6, respectively, an UPGMA dendrogram and a distance Wagner network were generated (figs. 3 and 4).

The most conspicuous level of divergence is noted between samples of *G. fossarum* populations from the Regensburg area (stations 1, 2 and 3) plus station 5, and samples of the remaining stations; this level is even beyond the divergence level of *G. pulex* samples (stations 5, 9, 14 and 15; tables 1, 3, fig. 1A, B, C, E) with regard to samples of all the *G.* cf. *fossarum* populations involved (figs. 3, 4). Another cluster is formed by the samples of *G. fossarum* and *G. caparti* from the Channel coast (stations 10–14; tables 1, 3; fig. 1A and B), Switzer-

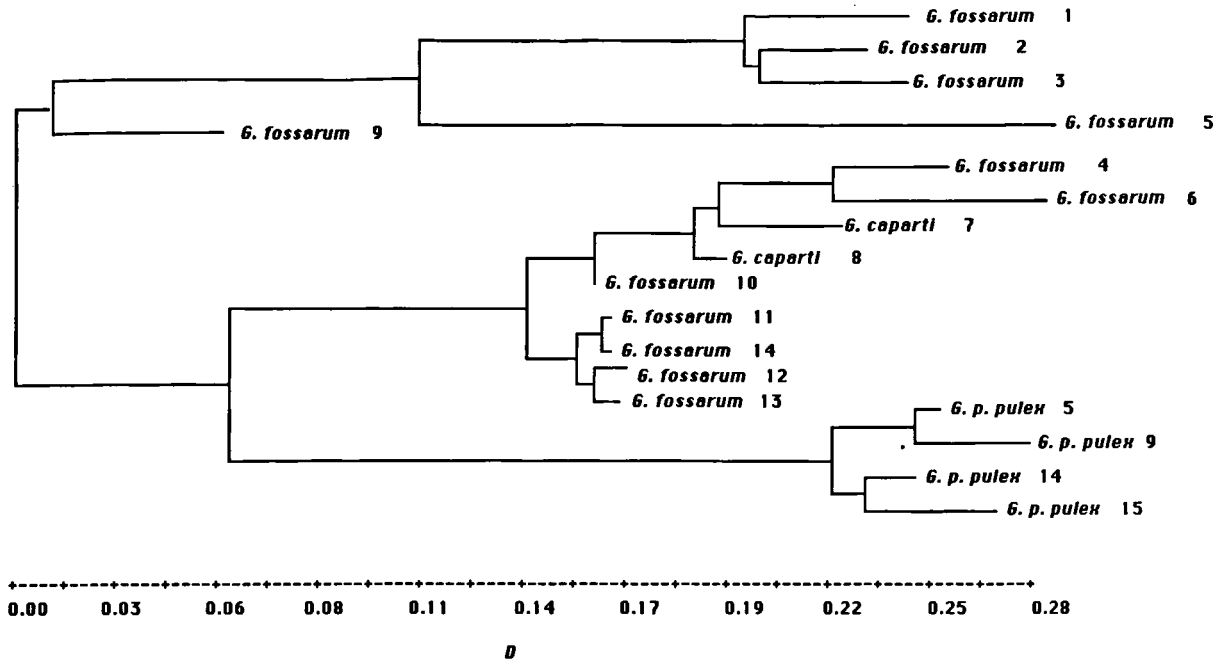


Fig. 4. Distance Wagner network of Rogers' (1972) genetic distance rooted at the midpoint of the longest path (Farris, 1972) based upon 20 enzyme loci.

Table 7. Mean intra- and inter-cluster values of the genetic identity (*I*; Nei, 1972), averaged over species, based upon 20 enzyme loci. Range $I_{min} - I_{max}$ in italics.

Station no.	*G. fossarum* 4-6-11-12 13-14	*G. fossarum* 9	*G. fossarum* 1-2-3	*G. fossarum* 5	*G. p. pulex* 5-9-14-15	*G. caparti* 7-8
4-6-11-12 13-14	0.97 *0.93-1.00*					
9	0.81 *0.81-0.82*					
1-2-3	0.67 *0.63-0.74*	0.76 *0.75-0.77*	0.97 *0.96-0.98*			
5	0.65 *0.62-0.72*	0.71 *0.71-0.71*	0.77 *0.74-0.79*			
5-9-14-15	0.72 *0.69-0.75*	0.71 *0.70-0.72*	0.53 *0.52-0.54*	0.53 *0.51-0.56*	0.97 *0.96-0.99*	
7-8	0.95 *0.93-0.98*	0.80 *0.79-0.81*	0.66 *0.63-0.69*	0.63 *0.62-0.64*	0.74 *0.73-0.76*	0.98 *0.98-0.98*

land (stations 4 and 6; tables 1, 3; fig. 1F), and Belgium (stations 7 and 8; tables 1, 3; fig. 1A, C). This cluster can be subdivided into two smaller clusters. The first one consists of the Swiss samples of *G. fossarum* from stations 4 and 6, and *G. caparti* from stations 7 and 8. The second is formed by the genetically uniform samples of *G. fossarum* from the Channel coast area (stations 10–14). The *G. fossarum* samples from stations 5 (Switzerland; tables 1, 3; fig. 1F) and 9 (Belgium; tables 1, 3; fig. 1C) deviate genetically from the general pattern. From the data in table 5 mean inter- and intra-cluster values of *I* can be calculated. These values (shown in table 7) suggest that there is no overlap of inter- and intra-cluster values of *I*, indicating (figs. 3, 4) stable clusters formed by: (a) *G. fossarum* from the Regensburg area (stations 1–3), (b) *G. pulex* (stations 5, 9, 14 and 15) and (c) *G. fossarum* + *G. caparti* from the Belgian stations 7 and 8 (*G. caparti*), the Swiss stations 4, 6, the French stations 11–14 and the Belgian station 10 (all *G. fossarum*). Intra-specific values of *I* among *G. fossarum* samples from this cluster and inter-specific values of *I* among samples of *G. fossarum* and *G. caparti* overlap almost entirely and do not differ significantly in a Mann-Whittney U-test ($U = 98.5$; $p = 0.48$). Thus, based on the allozyme data, samples attributed to *G. caparti* do not differ significantly from samples of *G. fossarum* in the same cluster (figs. 3, 4).

The topology of the UPGMA dendrogram (fig. 3) and the distance Wagner dendrogram (fig. 4) is almost the same. However, one conspicuous dif-

ference is the position of the "deviating" samples of *G. fossarum* population from station 9. In the distance Wagner network, this population clusters with *G. fossarum* samples from the Regensburg area (stations 1–3) and station 5.

Discussion and conclusions

The genetic differentiation pattern of samples of the species examined (shown in figs. 3 and 4) is unexpected. Among the main clusters (a) (stations 1–3: *G. fossarum*), (b) (stations 5, 9, 14 and 15: *G. p. pulex*) and (c) (*G. caparti* from stations 7–8 and *G. fossarum* from stations 4, 6 and 10–14) discussed above, *I*-values between cluster (b) and the presumed conspecific samples from cluster (c) range from 0.63–0.74. Inter-specific values of *I* between *G. p. pulex* (cluster b) and *G. fossarum* including *G. caparti* (clusters a and c) range from 0.52–0.54 and 0.69–0.75, respectively, which is of about the same order. With *G. p. pulex* (cluster b) as standard for genetic differentiation at the species level, the samples of *G. fossarum* examined warrant division into two different species: *G. fossarum*, including the samples from the Regensburg area (cluster b: stations 1–3) and possibly the samples from stations 5 and 9 (fig. 4); and a second species, including the samples from stations 4 and 6, 10–14, and 7–8 (cluster c). According to rules of taxonomic nomenclature, the latter species should be named *G. caparti* Pètre-Stroobants, 1980.

The results of this study do not provide genetic evidence for recognition of *G. caparti* as a distinct species. Moreover, based upon the criteria of Pètre-Stroobants (1980), only part of the individuals sampled at the type locality could be attributed to *G. caparti*, whereas others had to be attributed to *G. fossarum* (Pinkster, pers. comm.). Electrophoretically, however, the population sample seems perfectly homogeneous (table 3). Consequently, it must be concluded, that the samples of the Belgian stations 7 and 8 (*G. caparti*), the Swiss stations 4, 6, the French and Belgian stations 10–14 (all *G. fossarum*) are likely to be conspecific. Cross-breeding experiments between several morphologically distinct population samples of *G. fossarum* (including samples of *G. caparti*) yielded positive results (Goedmakers, 1972; Scheepmaker, unpubl. data). Most of these samples have been screened electrophoretically (Scheepmaker, unpubl. data) and proved to be genetically very similar. One could argue, that "true" *G. fossarum* population samples from the Regensburg area in the neighbourhood of the former type-locality (cluster b) were not involved in any of the cross-breeding experiments cited above. However, cross-breeding experiments involving species of *Gammarus* do not necessarily solve the taxonomic status among populations. For instance, in experiments by Scheepmaker et al. (1988), populations yielding *I*-values as low as 0.56 proved to be still partly interfertile.

In an attempt to avoid more confusion, rather than contributing to it, we would propose the term *G. fossarum sensu lato* for all populations hitherto identified as *G. fossarum* (including *G. caparti*), and reserve *G. fossarum sensu stricto* for the populations from the surroundings of its former type-locality in Regensburg (cluster b).

An explanation for the considerable amount of genetic divergence of *G. fossarum s. str.* with regard to the other population samples studied is probably provided by its distribution in the Danubian basin, which is characterized by a variety of species limited to this area (e.g. Thienemann, 1950). It is interesting to note that Siegismund (pers. comm.), who investigated *G.* cf. *fossarum* populations from brooks in the Rhine basin, did not find greater differences between these two areas than could be found within the Danube basin. However, these brooks in the Rhine basin were close to brooks belonging to the Danube drainage system.

In fig. 3, Swiss and Belgian populations samples of *G. fossarum s.l.* belonging to the Rhine- and Meuse drainage systems (stations 4 and 6–8) are differentiated from the Channel coast population samples (stations 10–14). These 2 subclusters may have a common descent from an ancestor inhabiting the upper reaches of streams of unglaciated lowlands during one or more glaciations. Differentiation may have originated by populations following the withdrawing ice. This hypothesis seems in agreement with the configuration of *G. fossarum*

s.l. populations from stations 10, 8, 7 and 4, forming a monophyletic group in fig. 4.

The Swiss stations 4–6 are situated in the same, rather limited geographical area (fig. 1A, F). According to Thienemann (1950), stations 4 and 6 are located in a part of this area covered with ice during the Würm glacial. Station 5, however, is located in a tongue of land beyond the maximum extension of the Würm glaciers. This circumstance might explain the deviating character of the *G. fossarum s.l.* population sample from station 5. This population possibly represents the original form, whereas the area once covered with ice was recolonized by populations from a different ancestor. An explanation for the other "deviating" population, *G. fossarum s.l.* from station 9, is not readily at hand.

The genetic differentiation of geographically distant population samples of *G. p. pulex* is homogeneous with reference to *G. fossarum s.l.* (figs. 3, 4). This is probably because *G. p. pulex* inhabits middle and lower reaches of rivers, allowing genetic exchange among drainage systems, whereas *G. fossarum s.l.* generally inhabits the upper reaches. As the upper reaches are generally separated from each other by lower reaches, *G. fossarum s.l.* populations are therefore more subjected to genetic isolation.

Although there is a considerable overlap in morphological variation among the populations studied, the genetic variation recorded in *G. fossarum s.l.* seems to be paralleled by some morphological differentiation (table 2). For instance, all population samples from the Channel coast area (stations 10–14; fig. 1A, B), forming a cluster in fig. 3, are characterized by a short setation of the A2 and P3; individuals from Swiss populations all have relatively long setae on the flagellum of the A2; individuals from station 1–3 do not always bear calceoli; individuals from the "deviating" population 9 differ from all other population samples by the longer setation of the peduncle segment 5 of A2, and the short setation of P3, etc. (table 2). However, one should be extremely careful: none of these characters are singly diagnostic, and only particular combinations of characters may designate a group of populations. Moreover, many of these characters are subject to allometric growth and seasonal variation.

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