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THE VALUE OF MORPHOLOGICAL CHARACTERS IN THE TAXONOMY OF *GAMMARUS*

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ABSTRACT

In the Hercynian Massif, a geologically old area in Southern France, various morphologically distinct populations of the *Gammarus pulex*-group were found. When applying the morphological criteria postulated by Karaman & Pinkster (1977), at least three species can be distinguished. However, in hybridization experiments these populations proved to be interfertile, and thus conspecific. Likewise it was proved that this species cannot be identical with *G. fossarum* or *G. ibericus*, it is considered a new species: *G. stupendus*. Because of its polymorphic nature, it is difficult to describe the species and to find discriminating characters with *G. fossarum* and *G. ibericus*. The distribution of the new species and of *G. ibericus* are discussed.

INTRODUCTION

Until quite recently the taxonomy of Gammaridae was almost uniquely based on morphological criteria. Stock, 1967 and 1968 discussed the various characters and concluded that distinction should be made between "stable" and "instable" morphological characters. Pinkster (1972) and Goedmakers (1972), in a series of cross breeding experiments with members of the *Gammarus pulex*-group, tested the validity of morphological characters. They concluded that a number of "stable" characters showed considerable variation. Karaman & Pinkster (1977) produced a key for the identification of the *Gammarus pulex*-group, based on a set of "stable" morphological criteria. As stable characters to be used in discriminating the various taxa they mentioned: 1) structure and setation of the mandibular palp; 2) setosity and shape of A1; 3) shape and setosity of peduncle and/or flagellum of A2; 4) presence of medial palmar spines in P1 and P2; 5) length of setae on the posterior margins of P3 and P4; 6) presence or absence of setae on the inner surface of the basis in P5 to P7; shape and setosity of P5 to P7; 8) ratio length exopod/endopod in uropod 3; 9) presence of setae on dorsal surface of metasome.

When using this set of characters, it is quite well possible, to identify at least male gammarids belonging the the *Gammarus pulex*-group in the larger part of Europe, but some problems still remain.

In the winter and early spring of 1980 and 1981 Stock and Pinkster did systematic sampling in the French départements Var and

Bouches-du-Rhone, a geologically old area with streams and rivers. Special emphasis was given to upper regions, wells and otherwise isolated localities. When trying to identify the material thus collected with the aid of the key by Karaman & Pinkster (1977) no satisfactory results were obtained. Some populations could be identified as *G. fossarum* Koch, 1835, but most populations could not be attributed to one of the known taxa, when applying the morphological characters mentioned above. When using the set of so-called stable characters with the material at hand, at least 3 species should be distinguished, but even then uncertainty remained because of the enormous variability. Not one population is identical to any other, although three major "forms" (called A, B, C in the sequel) can be recognized. These populations all have certain characters in common with *G. fossarum*, but differ from it (and often from each other) in two or more characters which should be discriminative according to Karaman & Pinkster (1977). Two of these "forms" resemble *G. ibericus* Margalef, 1951 in more than one respect.

In order to test the importance of the observed morphological differences two series of cross-breeding experiments were executed (in consecutive years), in which we used three different morphological "forms" from the studied area and, in order to bring at least one stable factor in it, two standard populations of "true" *G. fossarum* used in many previous hybridization experiments (Goedmakers, 1972). Since part of the discriminating characters of populations B and C resemble those found in *G. ibericus*, especially in the setosity of the pereiopods, a final series of cross-breeding experiments has been run in which so called *G. ibericus* (after Goedmakers, 1974) from the Massif Central (France) was tested against the same three populations of the earlier experiments.

DESCRIPTION OF THE POPULATIONS USED

All populations used in our cross-breeding experiments resemble each other (and thus *G. fossarum* and *G. ibericus*) in many respects, viz. the shape of eyes and head and the armature of meta- and urosome, epimeral plates, first antenna, first and second gnathopods, pereiopods 3 and 4, uropods 1 to 3 and telson. For a description of these characters see Goedmakers (1972) and figs. 1 A to G. However, in some other details, especially in the shape and setosity of the second antenna and of the pereiopods 5 to 7, they can show important differences, both in male and female as is summarized below.

Population A, from a "source vauclusienne" near the old abbey in the Parc de St. Pons, E. of Aubagne, dépt. Bouches-du-Rhone, France, collected 4-III-1980, and 2-IV-1981. It is characterized by a relatively setose second antenna, the setae being longer than the segments on which they are implanted, in males (fig. 2A) as well as in females (fig. 1H), without calceoli. In males P5 to P7 are moderately slender, armed with only few setae in between the spines (figs. 2E, 3A, and 4A). In females these setae are more numerous and longer (figs. 3E and 4E).

Population B, from the Fontaine de Nans, in the Massif de la Sainte Baume, dépt. Var, France, collected 5-III-1980 and 2-IV-1981. It is characterized by a relatively poorly setose second antenna without calceoli. In males the setae are about as long as or shorter than the diameter of the segments on which they are implanted (fig. 2B), in females these setae are longer (fig. 1I). In addition to the usual spines, setae are implanted along the anterior margins of pereiopods 5 to 7; these setae are usually longer than the spines, both in males and females (figs. 2F, 3B, 4B, and 3F and 4F). The basal segment of P7 is more or less characteristic because of its constricted distal portion, both in males and females.

Population C, from a spring in the Vallat de Fontanieu, dépt. Var, France, collected 5-III-1980, 2-IV-1981, and 2-VII-1981. The animals of this population are relatively small, the extremities are less slender than in populations A and B. The second antenna of the male is moderately slender (fig. 2C) the setae being

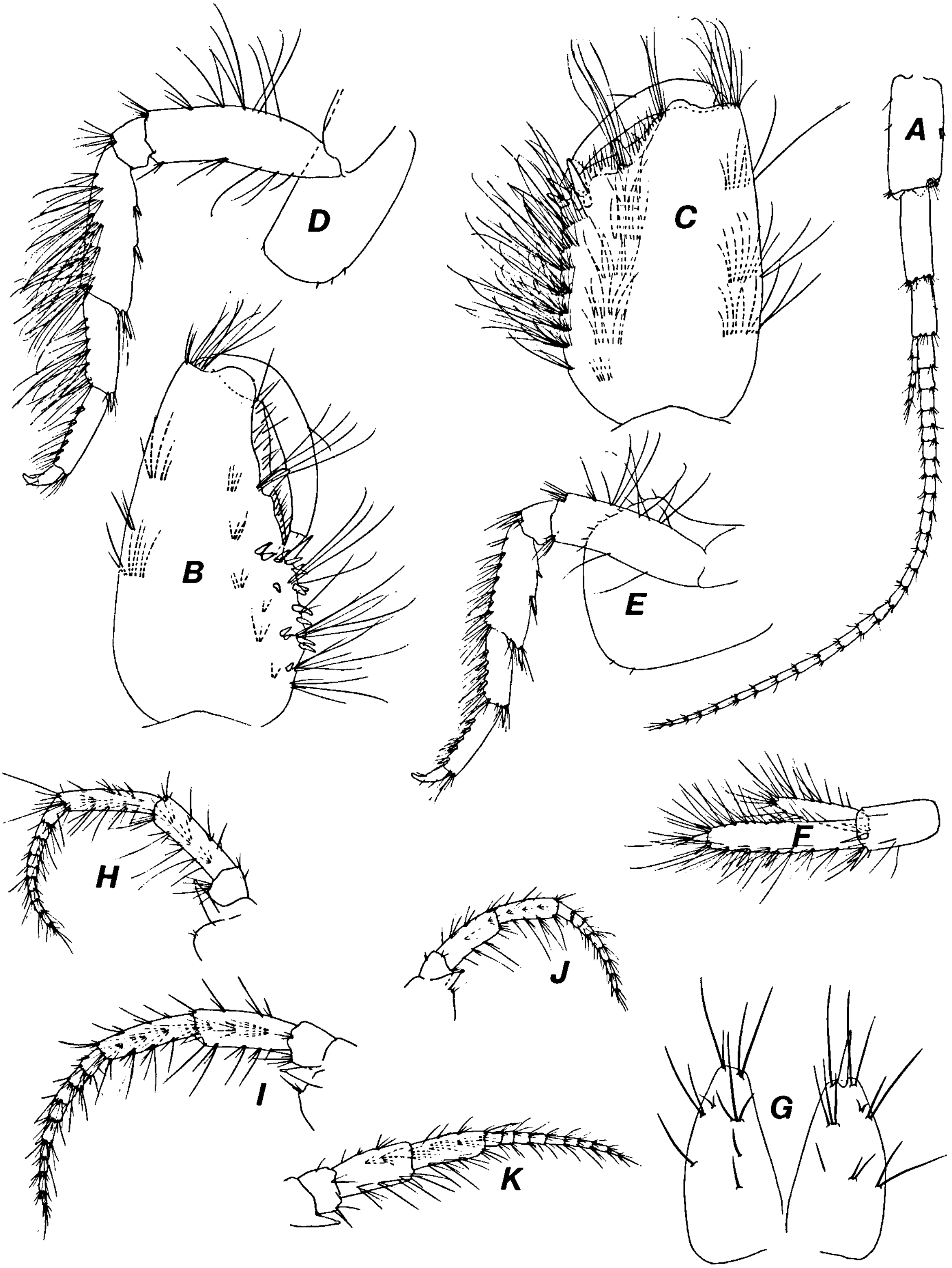

Fig. 1. *Gammarus stupendus* n.sp. A-G, male from type locality (= population B). A, first antenna (scale A); B, propodus of first gnathopod (B); C, propodus of second gnathopod (B); D, third pereiopod (A); E, fourth pereiopod (A); F, third uropod (A); G, telson (B). H to K, second antenna of female: H, population A; I, population B; J, population C; K, population D (all scale A).

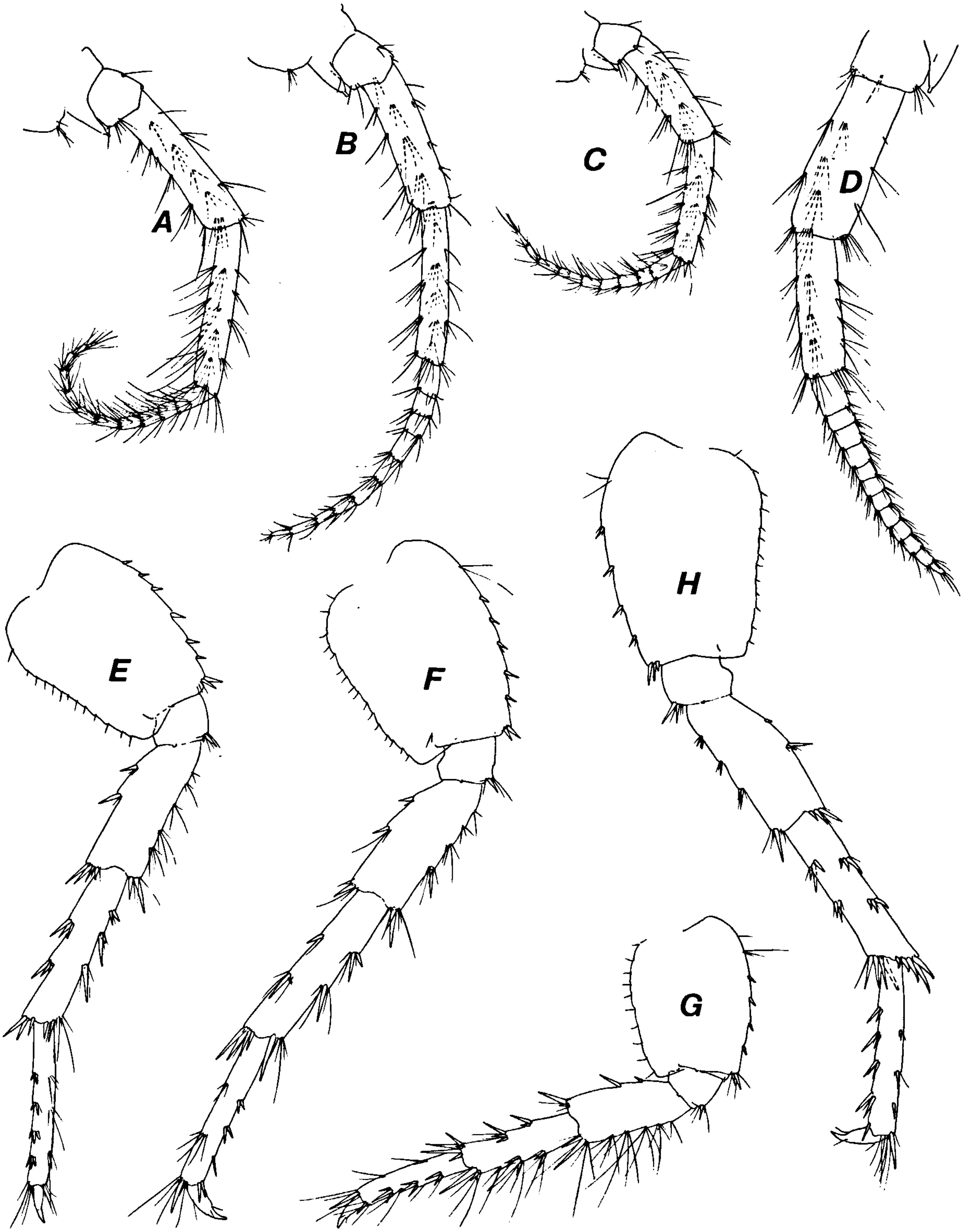

Fig. 2. A-C and E-G, *Gammarus stupendus* n.sp., D and H, *Gammarus fossarum* Koch, 1835 from the river Slack, Pas-de-Calais, France. A, second antenna population A; B, second antenna population B; C, second antenna population C; D, second antenna; E, P5 of population A; F, P5 of population B; G, P5 of population C; H, P5 (all scale A).

about as long as the diameter of the segments on which they are implanted; calceoli are absent. In females this setation is longer (fig. 1J). Pereipods 5 to 7 are densely setose in both sexes (figs. 2H, 3C, 4G and 3G and 4G).

Population D, from a small source in the river Slack, near a farm called Héronval, dépt. Pas-de-Calais, France, collected 22-III-1980. In many previous cross-breeding experiments this population was used as "typical" *Gammarus fossarum* Koch, 1836. The setae on the peduncle of A2 are as long as or shorter than the diameter of the segments; the flagellum is poorly setose (fig. 2D); calceoli are always present in males. In females the setation of A2 is more developed. In both sexes pereiopods 5 to 7 are armed with spines only.

Population E, from a mountain stream near Sarcenas, 12 km N. of Grenoble, dépt. Isère, France, collected 3-IV-1981. In previous hybridization experiments (Goedmakers, 1972) this population was proved to be interfertile with population D, and thus to be conspecific with *G. fossarum*. It differs from population D in a denser setation of A2 and the absence of calceoli (see figs. 4B and 4C in Goedmakers, 1972).

Population F, from the sources of the river Gapeau, dépt. Var, France, collected 3-IV-1981 and 20-VII-1981. This population is morphologically identical to population C.

Population G, from a small stream, N. of Aubenas, dépt. Dordogne, France, collected 20-VII-1981 and 5-VIII-1981. This population was identified as *Gammarus ibericus* by Goedmakers (1974). It is characterized by a rather long setation of both peduncle and flagellum of A2, many of the setae being longer than the diameter of the segments on which they are implanted. Calceoli are always present in males. In both sexes, pereiopods 5 to 7 are armed with numerous long setae in between the spines. Apart from the presence of calceoli, this population looks very much the same as populations C and F (see figs, 5A-H).

METHODS

After being collected and transported (in plastic bags with habitat water, under oxygen) to the laboratory and an adaptation period of some days, the animals were kept in the laboratory at 15°C in a mixture of habitat-water and Amsterdam tap-water in storage basins of 25 x 25 x 10 cm, and a water depth of 7 cm. Rough stones were provided for shelter and air was constantly blown through the water both for aeration and circulation. All experiments were done at 15°C (± 0.5°C) and a light/dark period of 16/8 hours. The animals were fed with *Stellaria media* Vill., a common terrestrial weed, with the addition of freeze-dried Tubicifid worms.

For the experiments sexually mature animals were selected from the various populations by separating animals in precopulation. Females were carefully checked for the possession of eggs in the brood-pouch. If eggs were absent they could be used immediately in our experiments, if (developed) eggs were present, they were kept apart until the eggs hatched or were lost. In the experiments a given number of females (dependant on how many mature females without eggs were at hand at a given moment) were put together with (if possible) the same number of mature males from another population (or the same population in the control experiments).

If females from these experiments produced eggs, they were separated and kept apart until the eggs eventually hatched or otherwise disappeared. Every two days the ovigerous females were checked. Juveniles resulting from these crossings were isolated as soon as possible, in order to prevent them from being eaten by their mother, and together with juveniles from identical crossings, raised till maturity.

It nevertheless appeared to be very difficult to raise the young. The major part of them died before reaching sexual maturity, but nevertheless we were able to raise at least some from almost all our experiments, that could be used to study the morphology and fertility of the F1.

In the first series of experiments, which were run from March 1980 till December 1980, populations A, B, C, and D were used. We also

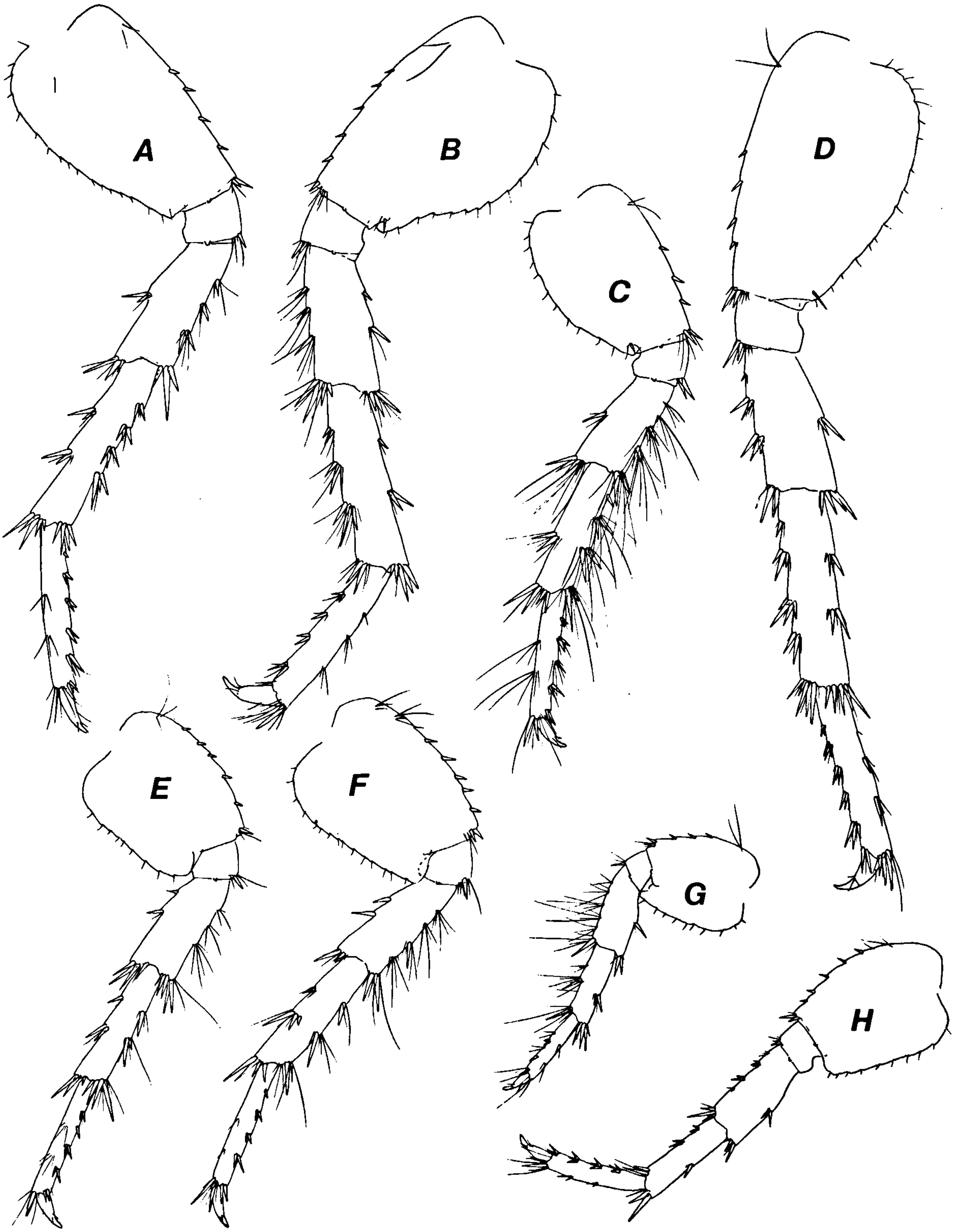

Fig. 3. A-C and E-G, *Gammarus stupendus* n.sp., D and H, *Gammarus fossarum* Koch, 1835 from the river Slack, Pas-de-Calais, France. A, P7 of population A; B, P7 of population B; C, P7 of population C; D, P7 of population D; E, P5 of female of population A; F, P5 of female of population B; G, P5 of female of population C; H, P5 of female. (all scale A).

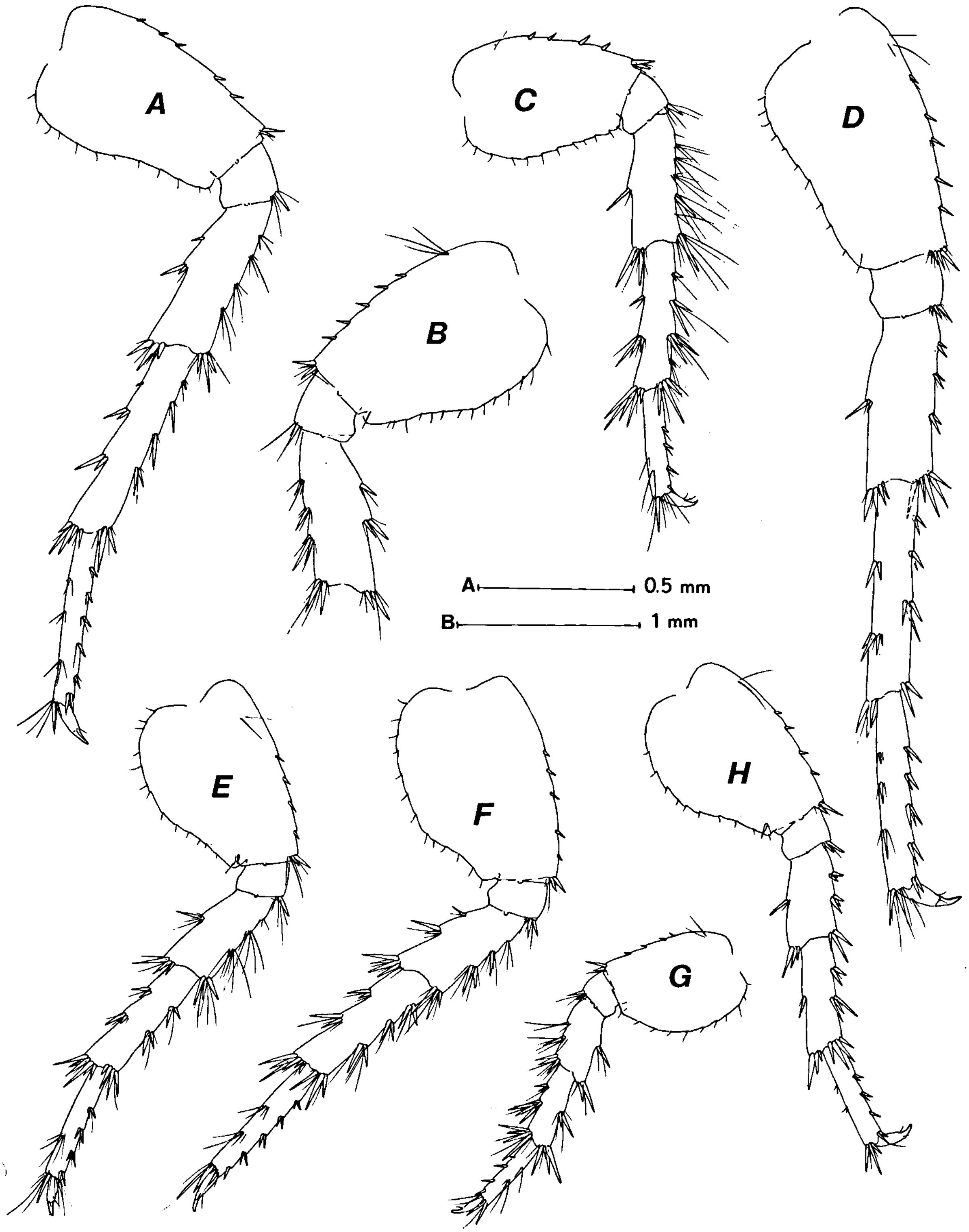

Fig. 4. A-C and E-G, *Gammarus stupendus* n.sp., D and H, *Gammarus fossarum* Koch, 1835 from the river Slack, Pas-de-Calais, France. A, P6 of population A; B, P6 of population B; C, P6 of population C; D, P6 of population D; E, P7 of female of population A; F, P7 of female of population B; G, P7 of female of population C; H, P7 female of population D. (all scale A).

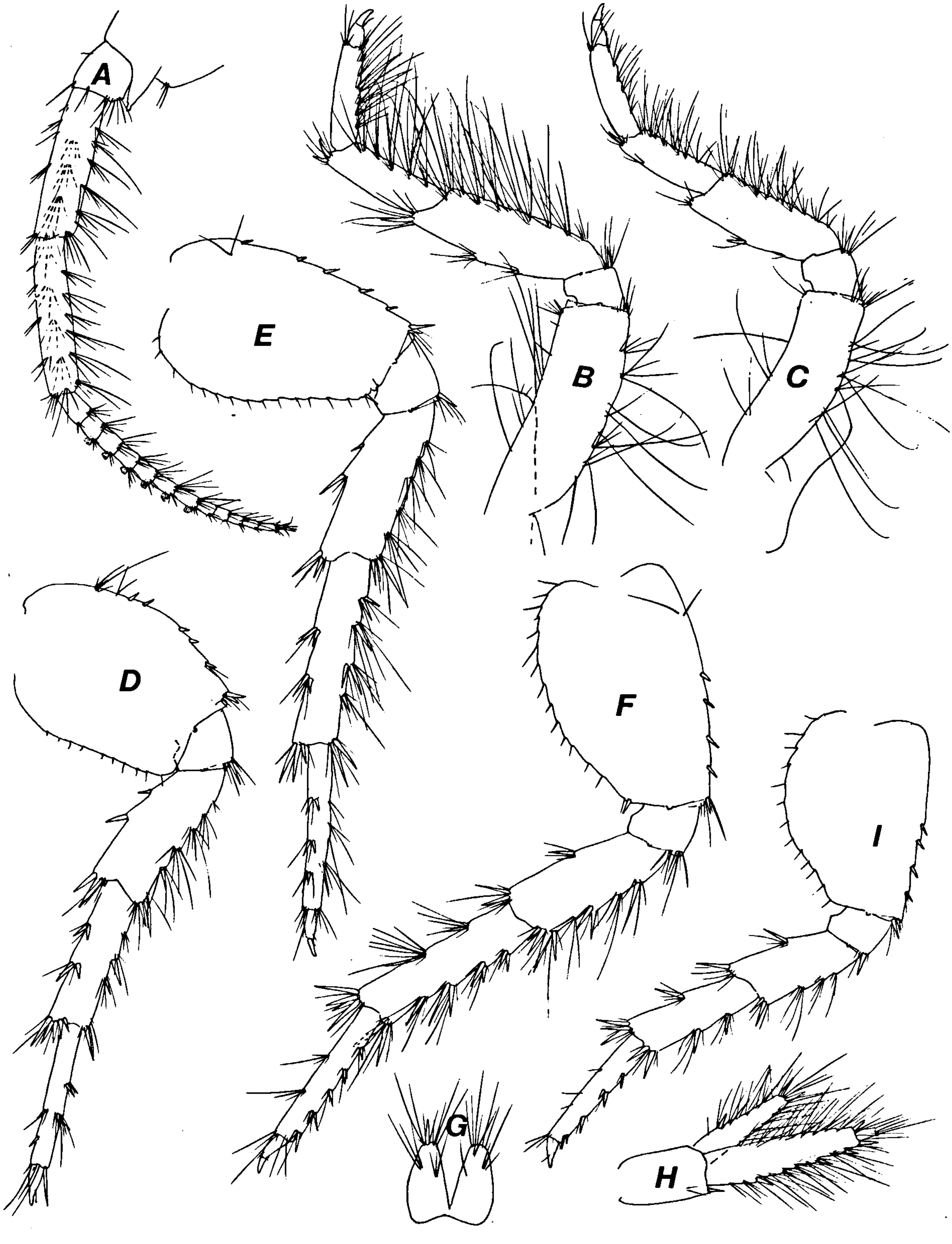

Fig. 5. *Gammarus ibericus* Margalef, 1951. A, second antenna; B, third pereiopod; C, fourth pereiopod; D, fifth pereiopod; E, sixth pereiopod; F, seventh pereiopod; G, telson; H, third uropod; seventh pereiopod of female.

tried to cross-breed populations C and F which turned out to be completely interfertile. So in further experiments we used population F instead of C, simply beause population C is very small and hard to collect in suitable numbers, while F was availabe in unlimited numbers.

In the second series, started in April 1981 and lasting for more than a year, we used populations A, B, F (= C) and population E (instead of D). In the third series, started in August 1981, we tried to cross-breed populations A, B, and F with population G. In this series those crosses that had already proved to be interfertile, have not been repeated.

RESULTS

The results of series I to III have been summarized in Tables I to III. Probably the most striking result from these experiments is that populations A, B, and C (= F) are interfertile in reciprocal crosses. The offspring of all combinations could be raised to sexual maturity and proved to be fertile. All crosses between any of these populations and a population of *Gammarus fossarum* (population D) failed to give any offspring (Table I). Population F which looks morphologically identical to population C also yielded crosses in both ways with populations A and B.

When trying to hybridize populations A, B, and F, with a *Gammarus fossarum* population from the French Alps (population E) no offspring could be obtained when using males from populations A, B, or F with females of E (*fossarum*). However, when trying to cross-breed male *fossarum* with females of the other three populations a low percentage of offspring was obtained in two crosses (E x A and E x B), see Table II. We failed to raise these juveniles to maturity but this may be an artefact due to the limited number of juveniles (and the "normal" mortality-rate when raising juvenile gammarids).

Experiments to hybridize populations A, B, and F with *G. ibericus* from the Massif Central (population G) all failed (Table III). In all three series of experiments, the intra-populational (homogeneous) crosses proved to be successful.

Table I. Results of interpopulational (heterogenous) and intra-populational crosses. First series of experiments, March-December, 1980. For explanation of the code see text.

cross	no. of ♂♂	no. of ♀♀	no. of ovig. ♀♀	no. with offspring
AA	50 A	50 A	42	37
AB	44 A	49 B	32	15
AC	65 A	53 C	35	14
AD	41 A	46 D	22	--
BA	31 B	27 A	21	4
BB	50 B	50 B	46	41
BC	35 B	37 C	22	8
BD	51 B	51 D	11	--
CA	34 C	43 A	21	6
CB	24 C	39 B	27	8
CC	50 C	50 C	36	27
CD	62 C	60 D	17	--
DA	42 D	38 A	18	--
DB	58 D	55 B	21	--
DC	44 D	47 C	6	--
DD	50 D	50 D	41	38

Table II. Results of interpopulational (heterogenous) and intra-populational crosses. Second series of experiments, April 1981-April, 1982. For explanation of the code see text.

cross	no. of ♂♂	no. of ♀♀	no. of ovig. ♀♀	no. with offspring
AA	50 A	50 A	40	33
AB	57 A	56 B	51	8
AE	49 A	43 E	24	--
AF	42 A	41 F	26	9
BA	30 B	39 A	28	1
BB	40 B	40 B	27	7
BE	30 B	61 E	49	--
BF	25 B	40 F	86	4
EA	31 E	41 A	35	2
EB	50 E	49 B	40	1
EE	20 E	17 E	15	13
EF	34 E	30 F	9	--
FA	29 F	43 A	34	9
FB	19 F	39 B	27	8
FE	35 F	56 E	25	--
FF	15 F	15 F	14	12

Table III. Results of interpopulational and intra-populational (homogenous) crosses. Third series of experiments, August 1981-June, 1982. For explanation of the code see text.

cross	no. of ♂♂	no. of ♀♀	no. of ovig. ♀♀	no. with offspring
GA	31 G	31 A	16	--
GB	50 G	50 B	43	--
GF	77 G	77 F	68	--
GG	50 G	50 G	42	28
AG	30 A	30 G	16	--
BG	35 B	35 G	12	--
FG	64 F	94 G	91	--

Morphology of F1.-

Juveniles from successful crosses have been raised to sexual maturity in order to study the morphology of the F1. Although much more work needs to be done, on a larger scale than so far, it seems that specimens originating from crosses A x B and B x A resemble animals from population B in morphology. Crossings between populations A and C resemble population C (although not entirely). Hybrids of populations B and C are somewhat intermediate between B and C, although in most respects they resemble population B.

However, it must be taken into account that variability is rather high in all populations and that no individual is completely like another. More work should be done on the heredity of the morphological characters of the various populations.

CONCLUSIONS OF THE HYBRIDIZATION EXPERIMENTS

Regarding the successful crosses between populations A, B, C (and F), all from a rather limited area in southern France, it must be concluded that these populations belong to one and the same species. Likewise, it is clear that this species cannot be *Gammarus ibericus* from the Massif Central although this species has many morphological characters in common with at least populations of "form" C.

The results from the hybridization experiments with *G. fossarum* are less clear. Indeed, none of the populations of "form" A, B, or C hybridized with *G. fossarum* from northern France. In crosses with *fossarum* populations from the French Alps (population E), females from population E never produced offspring with males from populations A, B, or C. However, males of population E, in combination with females from A, B, or F, produced juveniles in a few cases (E x A and E x B). This means that at least a partial intersterility exists.

It therefore must be decided that populations of "forms" A, B, and C belong to a hitherto undescribed species. However, because of its enormous variability of morphological characters, it is very hard to give a diagnosis of the new species and to name characters in which it can be distinguished from *G. fossarum* at the one hand and *G. ibericus* at the other hand.

Gammarus stupendus n.sp.

Diagnosis.—Small to medium-sized species with smooth slender body. Poorly to moderately setose second antenna. Pereiopods 5 to 7 are always armed with spines with at least some setae in between them. The dorsal surface of the urosome is flat.

Description.—Male: maximum length observed 13 mm. The urosome segments have no dorsal excavation or elevations. The dorsal armature generally consists of a mid-dorsal group of elements and a lateral group on each side. The eyes are relatively small, of the usual *G. pulex*-type.

The first antenna (fig. 1 A) shows no special features and is poorly setose. The gland cone of the second antenna (fig. 2 A-C) is rather long, attaining the distal end of the third peduncle segment. Usually peduncle segments 4 and 5 make a slender impression. The setation of these segments and the flagellum is very variable. Calceoli are always absent.

The mandible palp is of the usual *G. pulex*-type.

The first and second gnathopods (figs. 1 B, C) are always armed with a medial palmar spine.

Pereiopods 3 and 4 are not very characteristic (figs. 1 D, E). The setation is rather short in P4.

Pereiopods 5 to 7 are very variable, both in shape and setation. In P7 the basal segment is often constricted near the distal end. Spines form the dominant armature along the anterior margins of the segments, but nevertheless always some setae can be found. The length and number of these setae is very variable both within and between populations (figs. 2 E-G, figs. 3 A-C and figs. 4 A-C).

The endopod of uropod 3 is about as long as the exopod (fig. 1 F); the setae along the outer margin of the exopod are medium long, usually naked. The telson lobes are twice as long as wide, set with a varying number of setae on the dorsal surface (fig. 1 G).

Epimeral plates 2 and 3 are almost rectangular to slightly pointed. The armature is scanty.

Female: The characters observed in the males are more conspicuous in the females. So, the setation of A 2 and pereipods 5 to 7 is denser and longer than in the other sex. Likewise, the constriction of the basal segement of P7 is more pronounced (figs. 1 H-J, 3 E-G, and 4 E-G).

REMARKS AND AFFINITIES

Gammarus stupendus seems morphologically related to both *G. fossarum* and *G. ibericus*. Hybridization experiments however showed

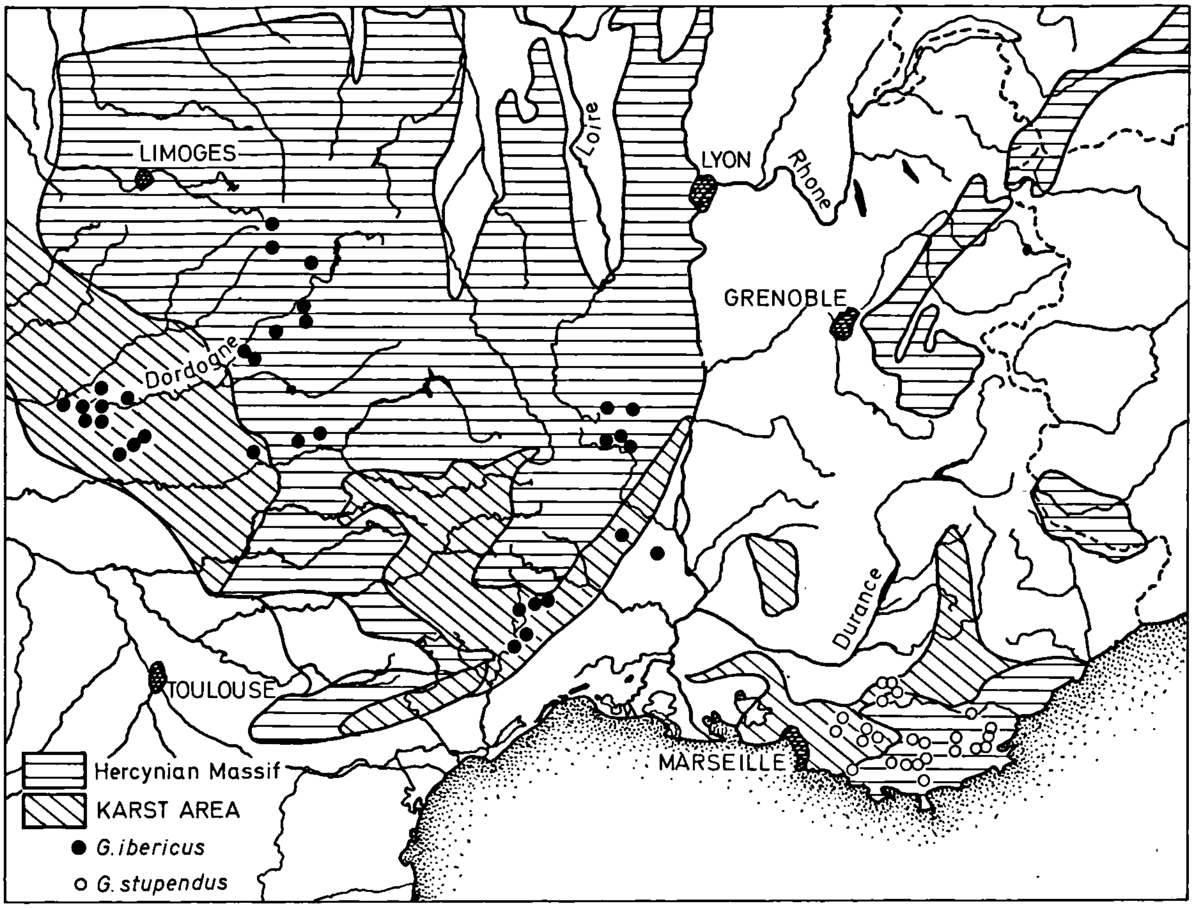

Fig. 6. Distribution of *G. stupendus* and *G. ibericus*.

that it must be regarded a valid species. Nevertheless it is often difficult to separate it from these two species. The only character in which it always differs from *G. fossarum* is the presence of setae, however few, between the spines along the anterior margin of P5 to P7. The only character in which it always differs from *G. ibericus* is the absence of calceoli on the flagellum of A 2. All other characters, even those which have so far been considered as stable in other species are too variable and cannot be used as discriminating factors.

DISTRIBUTION

Systematic sampling in southern France revealed that the new species is limited to a small area where it can be found, partly overlapping with *G. fossarum* and *G. p. gallicus*. When plotting the localities on the geological map of France (Vincent, 1956; Bonnet, 1956), it strikes that they fall within the limits of the Hercynian Massif and the adjacent Karst area. The same holds true for *G. ibericus* in the Massif Central (with one exception, see fig. 6). This may suggest that in both cases we deal with old species that have been isolated since the end of the carboniferous.

This probably also explains the intra-specific variation observed. When the distribution area of the new species (fig. 7) is studied in detail (fig. 7) no pattern can be discovered in the distribution of the various "formae" A, B, or C. Often, more than one "form" is found within the same stream system (but not at the same station). Likewise, so far no correlation could be found between the type of habitat

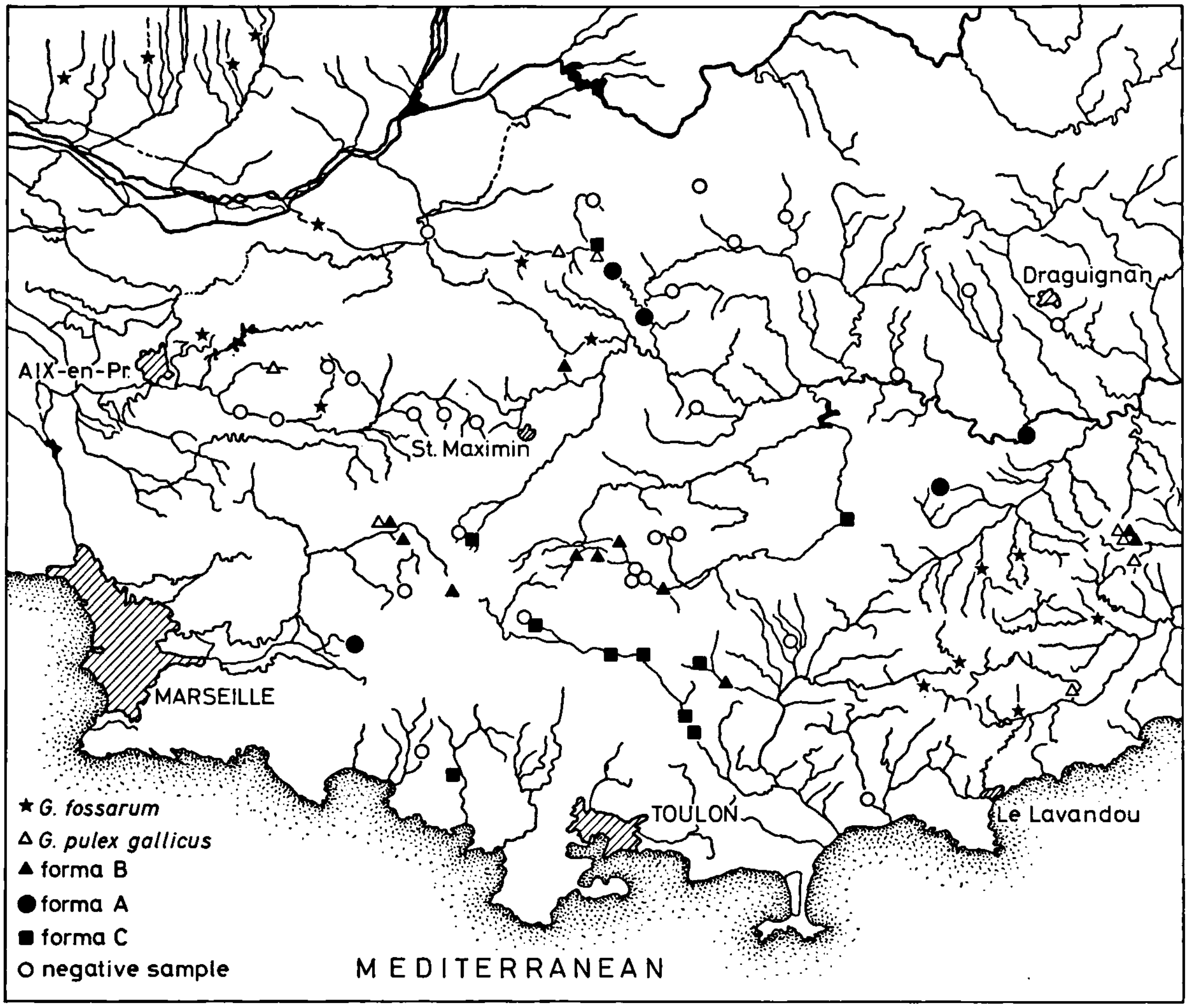

Fig. 7. Distribution of the various "types" of *Gammarus stupendus* and other *Gammarus* species.

(wells, brooklets, rivers) and any of the "formae".

Loc. typ.—Spring in the Vallat de Fontanieu, dépt. Var, France. The ♂ holotype, ♀ allotype and many paratypes, collected 12-I-1980, have been deposited in the collections of the Zoologisch Museum Amsterdam, cat. no. ZMA Amph. 107-570 a, b, and c.

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