# Description of *Gammarus balutchi* spec. nov. (Amphipoda: Gammaridae) from Iran, based on light and electron microscopy

**V. Khalaji-Pirbalouty & A. Sari**

Khalaji-Pirbalouty, V. & A. Sari. Description of *Gammarus balutchi* spec. nov. (Amphipoda: Gammaridae) from Iran, based on light and electron microscopy.
Zool. Med. Leiden 80-1 (6), 10-iii-2006: 91-100, figs 1-4.— ISSN 0024-0672.
V. Khalaji-Pirbalouty & A. Sari. Department of Biology, Faculty of Science, University of Tehran, Tehran, Iran (e-mails: khalajiv@yahoo.com; sari@khayam.ut.ac.ir). Correspondence to second author.

Key words: Iran; new species; endemic; SEM; *Gammarus balutchi.*
In the present study, a new endemic gammarid amphipod, *Gammarus balutchi* spec. nov. is described from Charmahal-Va-Bakhteyari province, Iran. A description is given using light and electron microscopes. Comparison of SEM micrographs shows that pore density and patterns are reliable diagnostic characters at the species level based on ultrastructural study of the head.

## Introduction

Comprehensive studies of Iranian limnic and brackish water amphipods is rather new (Karaman, 1998; Stock et al., 1998; Yavari, 2000; Banakar, 2001; Amraii, 2001; Khalaji-Pirbalouty, 2002; Pourmohammadi-Sarbanani, 2002; Naghib 2002) but limited to some previous works (Karaman, 1934; Birstein, 1945; Ruffo, 1979; Pesce et al., 1982). The recent study by Stock et al. (1998) examined 29 species, including six new species: *Gammarus crinicaudatus, G. paricrenatus, G. parthicus, G. proiectus, G. anodon,* and *G. lobifer,* from various parts of Iran. Most of the Iranian species belong to the artificial *Gammarus pulex*-group, and the rest are grouped in the *Gammarus locusta*-, *G. roeseli*- and *G. duebeni*-species groups (see Stock et al., 1998). Following upon the excellent contributions to Iranian amphipod fauna by Stock et al. (1998), several studies were started from 1999 in the Department of Zoology, University of Tehran, one of which was a thorough survey of amphipods of Chaharmahal-Va-Bakhteyari (Khalaji-Pirbalouty, 2002). This is a unique area in regards to habitat diversity and water sources in the Central Zagros Mountains, Iran. The present study resulted in recognizing a new species of *Gammarus* with close morphological affinities to *G. lobifer* from adjacent provinces.

Due to the variability of useful characters at species and population levels (Karaman & Pinkster, 1977), morphological characters were found to be less suitable for detection of phenotypically similar species (Pinkster, 1983). Some examples of studies on phenotypically similar amphipod species such as Wellborn (1993), Meyran, et al. (1997 & 1998), Witt & Hebert (2000), Müller (2000), Müller et al. (2000), Witt et al. (2003) and Wellborn & Cothran (2004) demonstrated the significance of studies on ecology and evolution of life history, population dynamics, behaviour and genetic diversity in taxonomy and phylogeny rather than morphological characters alone. In the present study, despite some morphological similarities at lower magnification, the description of *Gammarus balutchi* spec. nov. is based on use of light and scanning electron microscopes at higher magnification. For comparative purposes, some materials of *G. lobifer* from

adjacent areas (Yasooj and Ilam) and *G. duebeni* from England are partially described. SEM study was in the line with previous surveys on crustacean cuticular microsculpturings and their significance for phylogeny and taxonomy at either interspecific, or family levels (see Platvoet, 1985; Al-Yahya, 1991; Elfimov, 1995; Grygier, 1995; Sari, 1997). The main objectives of the SEM study were morphological comparison of the members of the *G. duebeni*-group (*G. lobifer* from Iran and *G. duebeni* from England) with *Gammarus balutchi* spec. nov. to find useful discriminating characters at ultrastructural level to help with identification of morphologically similar species. In this view, some characters on the head cuticle, including pores density and patterns, were considered.

## Materials and methods

Sampling was carried out in the Atashgah fall (31°14′N 5°00′E), southeast of Lordegan, Iran. Most of the specimens were collected from aquatic plants or beneath small stones. Captured specimens were washed in the clean freshwater, then narcotized with ether, and finally fixed in 70% alcohol. Large male and female specimens were selected for drawing. Partial dissection was facilitated using a pair of fine needles under a low power microscope. Drawings, mostly based on male specimen, were made using a Camera Lucida on a light microscope (Reichert biovar).

Scanning electron microscopy.— All specimens were twice washed with agitation in chilled 1% sodium acetate solution, first for 5 minutes, then for 15 minutes. Specimens were washed several times to remove the adhered sediment and debris from cuticle according to Haley (1997). Then specimens were dehydrated in a graded acetone series (30, 50, 70, 85, 95 and 100%). Dried specimens were mounted on stubs with two-sided adhesive or silver glue. Then stubs were coated with gold under a spatter coater (Blazers/SCD 004). Gold coated specimens were studied under a digital scanning electron microscope (Zeiss/DSM 960A). Some materials were provided by Zoological Museum University of Amsterdam. Material examined were *G. lobifer* from Yasooj (ZMA AMPH 201 936, 30°40′N 51°30′E) and *G. duebeni* (Cornwall, England).

Symbols.— A1 and A2: antenna 1and 2; BP6 and BP7: basal part of pereopod 6 and 7; DCU: dorsal contour of urosomes; EP1 to EP3: epimeral plates 1 to 3; GN1and GN2: gnathopod 1 and 2; H: head; MNDP: mandible palp; O3: oostegite of pereopod 3; P3 to P7: pereopod 3 to 7; PGN1 and PGN2: propodus of gnathopod 1 and 2; PLMX: palp of left maxilla 1; PRMX: palp of right maxilla 1; T: telson; U1 to U3: uropod 1 to 3.

## Systematic description

***Gammarus balutchi*** spec. nov.
(figs 1-4)

Material.— one ♂ holotype, one ♀ allotype, many paratypes, Zoological Museum University of Tehran, ZUTC-Amph. 2070, ZMA AMPH 204 658, Atashgah-e-Lordegan fall (31°14′N 51°00′E), altitude 2100 m, 8 August 2001.

Description.— Male, body length up to 12.5 mm.

Lateral cephalic lobe (fig. 1, H) rounded; eye, ovoid large and longer than diameter of the first peduncular segment of antenna 1.

Antenna 1 (fig. 1, A1) both peduncular and flagellar segments poorly setose. The main and accessory flagella have 23-28, and 3-4 segments, respectively.

Antenna 2 (fig. 1, A2) shorter than antenna 1; gland cone slightly curved upwards, its tip not reaching the end of third peduncular segment; peduncular segments 4 and 5 almost equal in length, both armed with 3-4 transverse rows of long straight setae (2 to 3 times as long as the diameter of the segments); flagellum, 10-12 segmented, without calceoli, armed with setae (2 times as long as diameter of the segments on which they are implanted).

Mandible palp (fig. 1, MNDP) first segment unarmed; segment 2 with 8-10 long ventral setae; segment 3 with one group of A-setae, one to two groups of B-setae, without C-setae, a row of 20-25 D-setae and four to five E-setae.

Maxilla 1: Left palp (fig. 1, PLMX) slender and narrow, distal segment with six slender spines+1 seta; right palp (fig. 1, PRMX) wider than left, armed with four heavy and short spines+1 slender spine+1 seta.

Gnathopod 1 (fig. 2, GN1) poorly setose, first gnathopod propodus palmar margin (Fig. 2, PGN1) not very oblique, sinuous, palmar angle with four spines, mid-palmar spine present.

Gnathopod 2 (fig. 2, GN2) same size as gnathopod 1; palm (fig. 2, PGN2) less oblique, rectangular shape, palmar angle with three spines; mid-palmar spine present.

Pereopod 3 (fig. 3, P3) with long straight setae (up to seven groups on merus but fewer on carpus and propodus).

Pereopod 4 (fig. 3, P4) coxal plate broad and emarginate; distal segments with long setae as in pereopod 3.

Pereopod 5 (fig. 3, P5) with subrectangular basal segment; posterior margin with few setae; postero-distal corner freely produced; postero-distal segments predominantly spinous, with few setae intermixed with spines.

Pereopod 6, similar to pereopod 5 but with an elongate basis (fig. 3, BP6).

Pereopod 7 (fig. 3, P7) basis with some setae along posterior margin; postero-ventral corner freely produced; article 2 without submarginal setae; distal segments mainly spiniferous, with a few setae among the spines.

Epimeral plate 1 (fig. 2 EP1) with rectangular postero-ventral corner, antero-ventral margin with some long setae.

Epimeral plates 2 and 3 (fig. 2, EP2 and EP3) with two to three spines on ventral margin and some setules on posterior margin; postero-ventral corner weakly pointed in EP2 and more strongly pointed EP3.

Urosome segments (fig. 1, DCU) without obvious dorsal elevation; dorsal armature consisting of one dorso-medial and two dorso-lateral groups of short spine and long setae (urosomite 1-2); urosomite 3 with some mid-dorsal setae and spines.

Uropod 1 (fig. 1, U1) mid-dorsal margin of peduncles with two spines; endopode and exopode with one mid-dorsal and some terminal spines.

Uropod 2 (fig. 1, U2) exopode slightly shorter than endopode; both rami with one mid-dorsal and some terminal spines.

Uropod 3 (fig. 1, U3) exopode more than twice as long as endopode; distal segment minute; setae on endopode and exopode mostly plumose, some glabrous.

Telson lobes (fig. 1, T) up to twice as long as wide; laterally armed with one lateral spine and one to two setae, two subdistal medial setae (or spine+setae); distal armature includes three spines and some setae; two sensory setules located near base of second lateral setae.

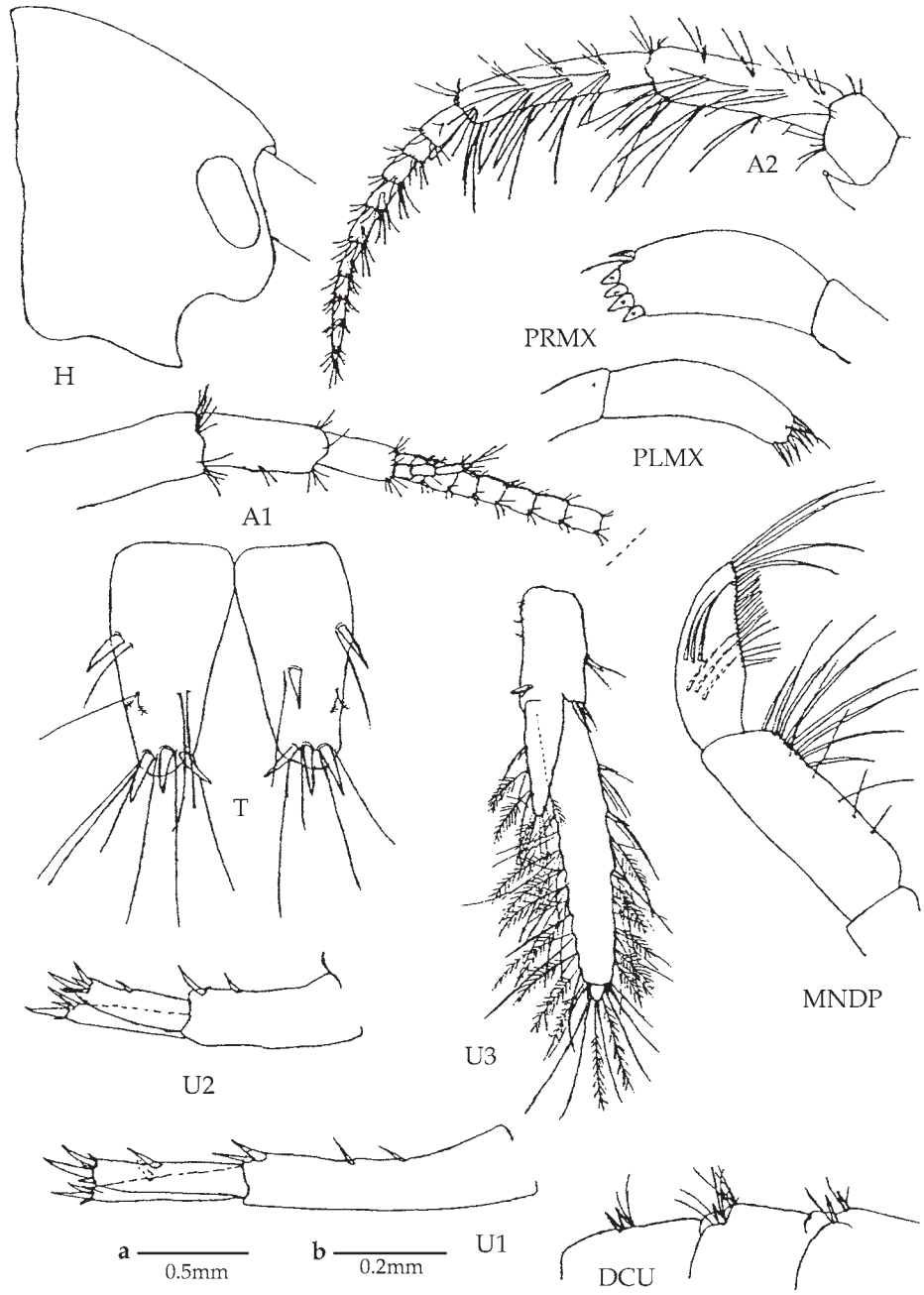


Fig. 1, *Gammarus balutchi* spec. nov., ♂ paratype, 12.5mm; H, A1, A2, U1-U3, and DCU (scale a) PRMX, PLMX, MND and T(scale b).

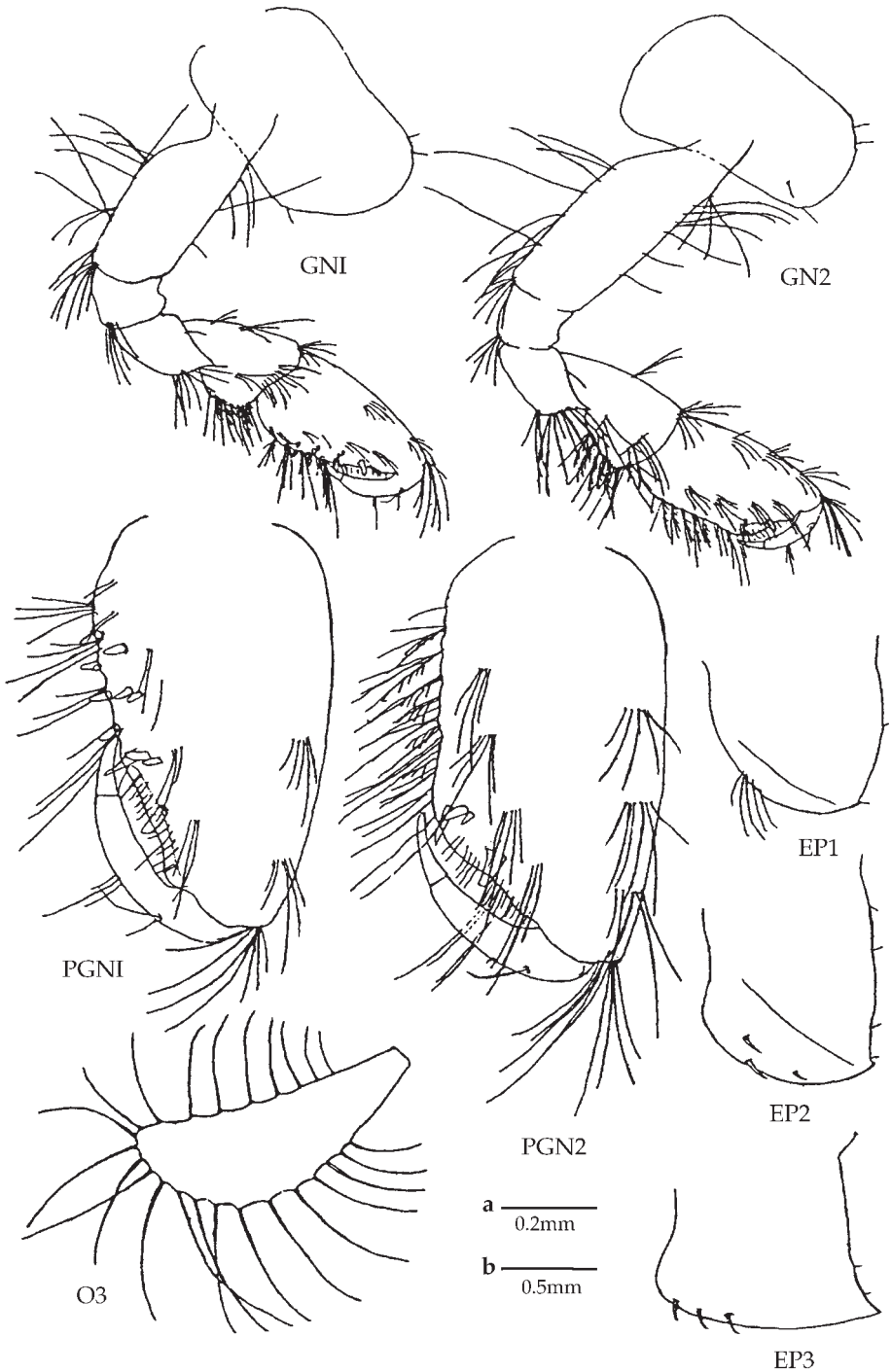


Fig. 2, *Gammarus balutchi* spec. nov., ♂ paratype,12.5mm; GN1, GN2, and EP1-EP3 (scale a); PGN1, PGN2 and O3 (scale b).

Female.— Smaller than male; gnathopod 1 without mid-palmar spine; both gnathopods 1 and 2 smaller than in male; compared to male, setae on antenna 2 and also pereopod 3 and 4 are shorter; uropod 3 in female is not densely setose; basis of perepods 6 and 7 (fig. 3, BP7) more convex than in male. Oostegites 3 as illustrated (fig. 2, O3).

Etymology.— The species is named *Gammarus balutchi* to acknowledge many years of contribution to the zoology of Iran by Professor Mohammad Balutch at the University of Tehran.

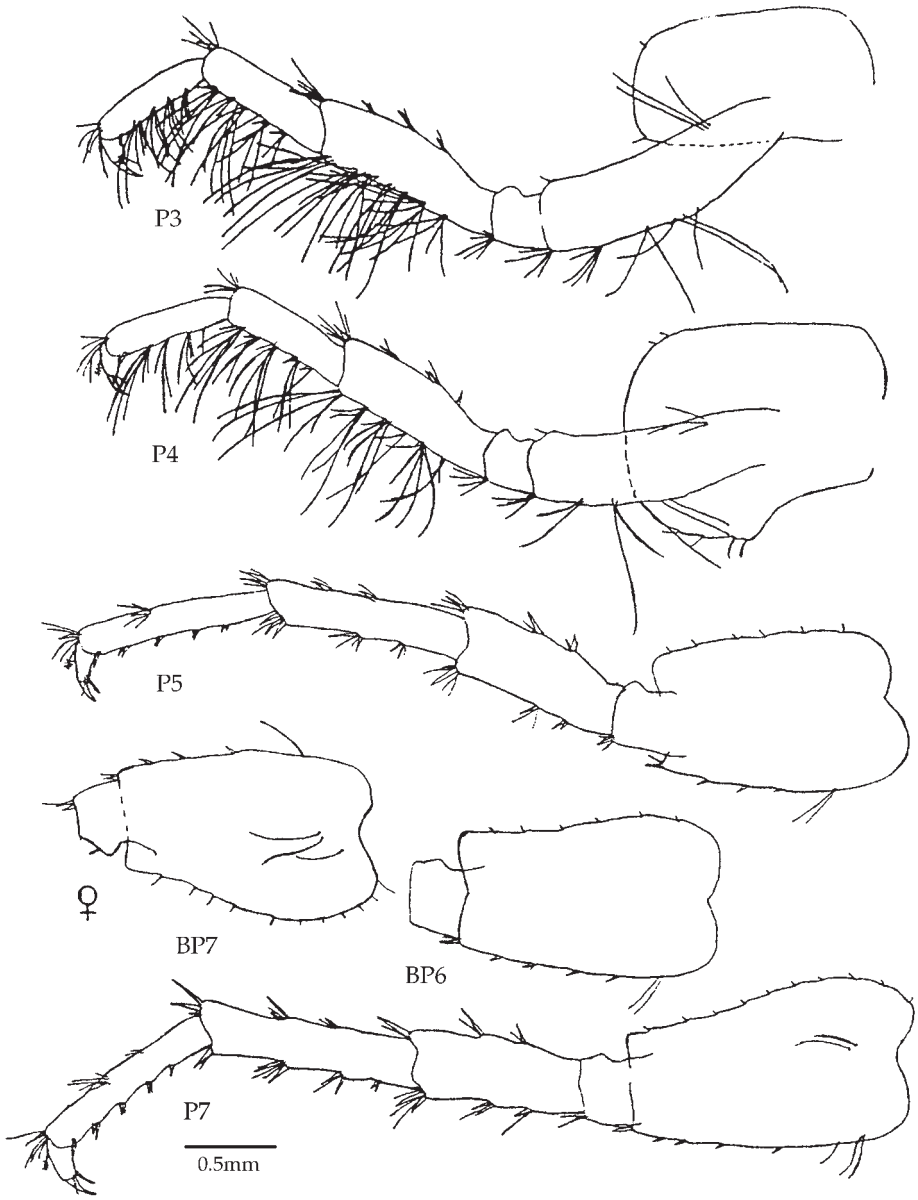


Fig. 3, *Gammarus balutchi* spec. nov., ♂ paratype,12.5mm; P3-P7, BP6 and ♀BP7 to same scale.

Remarks.— This species was compared with all European and Iranian gammarids deposited in the zoological museum, University of Tehran (ZUTC), and found to be similar to *G. duebeni* Liljeborg, 1852 and *G. lobifer* Stock et al, 1998 (see the original description) in regards to the presence of large, elongate eyes (compared to small, rounded to reniform eyes in members of *G. pulex*-group**)**. *G. duebeni*, however, has longer setae on pereopods 5 to 7 and on the posterior margin of the epimeral plates. The endopode of uropod 3 is half or nearly more than half the length of the exopode outer margin. The outer margin of the exopode is armed with simple setae. Moreover, antenna 2 bears calceoli. In contrast, *G. lobifer* has a truncate lateral head lobe and one subangular seta on

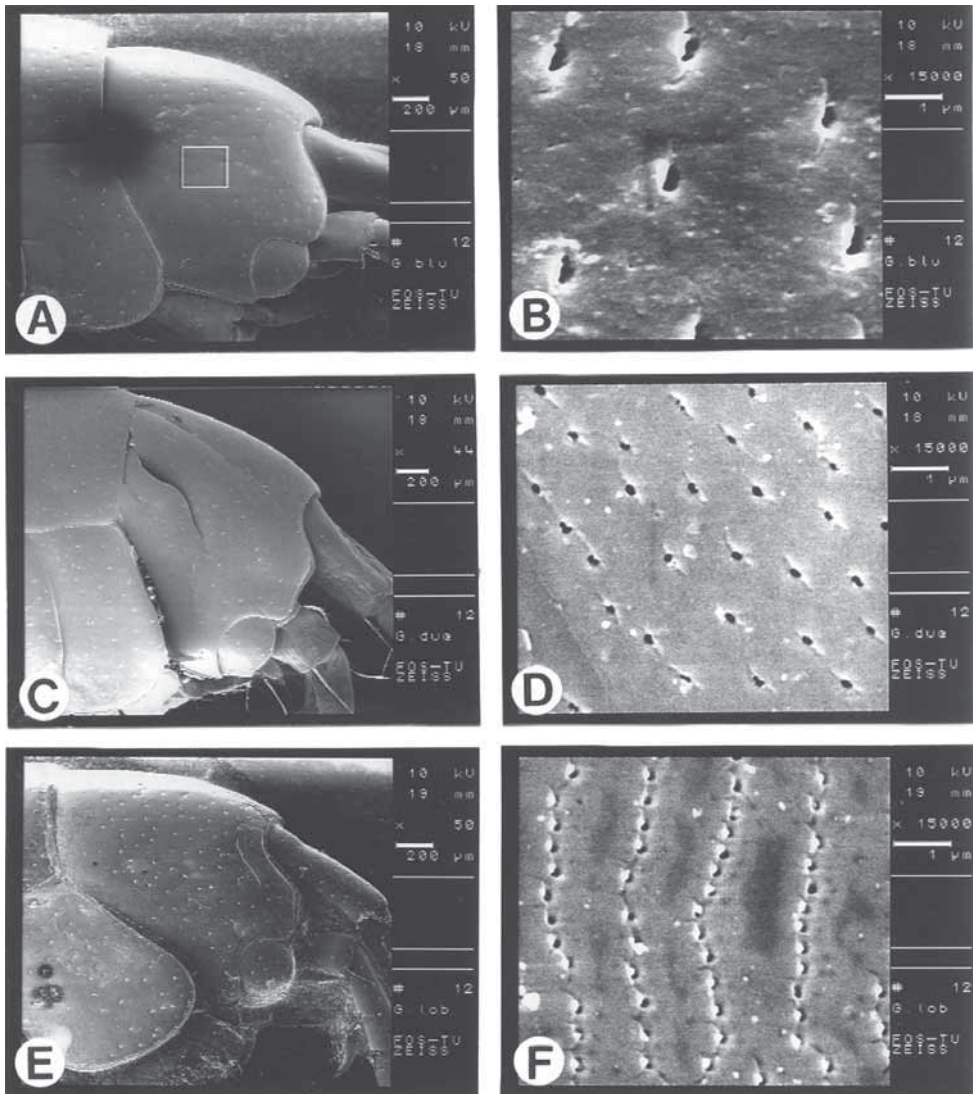


Fig. 4, Scanning electron micrographs of head and details of head microsculpturing in *Gammarus balutchi* spec. nov. (A & B); *G. duebeni* (C & D); and *G. lobifer* (E & F).

the postero-ventral corner of pereopod 7, but *G. balutchi* spec. nov. has a round lateral head lobe and lacks subangular seta. The gland cone in *G. lobifer* is longer than the third peduncular segment, whereas in *G. balutchi* spec. nov. the gland cone tip does not reach the third peduncular segment. In *G. balutchi* spec. nov., the setae of antennular peduncles 4 and 5 are longer than those in *G. lobifer*.

Some characters found in *G. balutchi* spec. nov. were observed in *G. birsteini* (see Karaman & Pinkster, 1977). These characters are: rounded lateral head lobes, antenna 2 with short gland cone, peduncle segments 4 and 5 bearing long and straight setae, and the endopode of uropod 3 less than half of the exopode. However *G. birsteini* has eyes as long as or shorter than the diameter of first pedunclar segment of antenna 1, fourth and fifth peduncular segments bear 8-10 transverse rows of long straight setae on peduncular segments, and the exopode of uropod 3 is armed with long simple setae on the inner and outer margins.

Description using Scanning Electron Microscope.— SEM studies also revealed that the lateral head lobes are truncate in both *G. lobifer* (fig. 4E) and *G. duebeni* (fig. 4C), but rounded in *G. balutchi* spec. nov. (fig. 4A). Head microsculpturing shows some marked differences in the number and patterns of pore distribution. These are arranged in discrete rows of pores in *G. lobifer* (fig. 4F). In contrast, pores pattern in *G. balutchi* spec. nov. is scattered and pores are taken apart (fig. 4B). *G. duebeni* has cuticular pores similar to *G. balutchi* spec. nov. but with much denser or higher frequency of pores in a given area (fig. 4D).

## Discussion

According to the description of *G. lobifer* in Stock et al. (1998), three main diagnostic characters were found in the *G. duebeni*-group: a pair of large and elongated eyes, protruding postero-ventral basis lobe of pereopod 5-7, and the endopode of uropod 3 half or less than the exopode length. In the present study, it has been found that the endopode length does not show consistency, even after re-examination of some *G. duebeni* from England, and comparison of material with drawing in Pinkster et al. (1970) for *G. duebeni*, wherein all specimens have the endopode larger than half the exopode length. In *G. balutchi* spec. nov., which is restricted to a specific habitat at high altitude of 2100 m of a fall, little variation in characters was found. With use of the SEM to study microsculpturing of the head, it has been revealed that there are species specific pore patterns (figs. 4B, D, F). These are unique patterns, and further unpublished surveys on other species show no marked ultrastructural variations at the population level. These patterns include well marked rows of pores on the head cuticle. To compare these patterns, some species were used including previously described new species from different localities in Iran, *G. lobifer* by Stock et al. (1998) and *G. duebeni* from England. Comparatively, in *G. balutchi,* eyes are elongated similar to those in *G. duebeni* from England. SEM study of the head microsculpturing of both species shows similarity in pore pattern, but their density is species specific (Fig. 4B & D). It seems that the sieve-like pore pattern is characteristic of *G. balutchi, G. duebeni* (Fig. 4B & 4D) and *G. pulex* (unpublished data). However, the former species shows fewer pores in a given area of head cuticle. In contrast, *G. lobifer* shows parallel chains of pores (Fig. 4F). Although recent genetic studies of amphipods (Wellborn, 1993; Meyran, et al., 1997, 1998; Witt & Hebert, 2000; Müller,

2000; Müller et al., 2000; Witt et al., 2003; Wellborn & Cothran, 2004) shed more light on population level taxonomy and revealed identity of some cryptic species, here we place emphasis on morphological data obtained by easy to use tools such as light and electron microscopes. Based on the present data, *G. balutchi* spec. nov. is a new species with a great similarity to *G. lobifer,* but it has marked differences as revealed by light and electron microscopic studies. Moreover, this endemic species has a unique habitat at high latitude of about 2100 m which is some distance from the areas in which *G. lobifer* populations are found. The results of present study shed some light to knowledge of the Iranian species within the genus *Gammarus*. Further study should be carried out with further re-examination of Iranian gammarids using more species.

## Acknowledgements

The authors greatly acknowledge helps of Dr D. Platvoet for loan of materials, his hospitality during our visit to the ZMA collection and also fruitful discussion; Dr D.T. Bilton, from University of Plymouth for providing specimens from Cornwall, England; Dr N. Rastegar-Pouyani for critical review of the manuscript; and Mr S.M. Hashemi for technical SEM support. We also acknowledge the financial help and facilities provided by University of Tehran and the Natural Resources and Animal Science Research Centre of Chaharmahal-Va-Bakhteyari province.

## References

Al-Yahya, H., 1991. Studies on cirripede larvae with special reference to external features of cyprids from five families.— Ph.D. dissertation, University of Wales, Swansea.

Amraii, R., 2001. Biosystematics survey on some amphipods (Crustacea: Amphipoda) in the Lorestan province rivers.— Unpublished M. Sc. thesis, Faculty of Science, University of Tehran.

Banakar, F., 2001. Biosystematics study of Amphipoda in the Tehran Province Rivers and population dynamics of species in Khojir area, Iran.— Unpublished M. Sc. thesis, Islamic Azad University, North Tehran branch.

Birstein, J.A., 1945. Zametka o presnovodnykh visshikh rakoobraznykh Turkmenii i Irana.— Uchen. Zap. mosk. gos. Univ. 83: 151-164.

Elfimov, A. S., 1995. Comparative morphology of the thoracican cyprid larvae: studies of the carapace. In: F.R. Schram & J.T. Høeg (eds), New frontiers in barnacle evolution: 137-152. Crustacean issues 10.— A.A. Balkema, Rotterdam.

Grygier, M.J., 1995. An unusual barnacle nauplius illustrating several hitherto un- appreciated features useful in cirripede systematics. In: F.R. Schram & J.T. Høeg (eds), New frontiers in barnacle evolution. Crustacean issues 10.— A.A. Balkema, Rotterdam.

Haley, C.J., 1997. Comparison of secondary production, life history, and mouthpart functional morphology between two populations of amphipod *Gammarus minus*.— Unpublished Ph.D. thesis, State University, Virginia.

Karaman, G., 1998. First discovery of the family Niphargidae (Gammaridea) in Iran. (Contribution to the knowledge of the Amphipoda 234).— Glasn. odjel. prirod. nauka CANU, Podgorica 12: 9-22.

Karaman, S., 1934. Über asiatische Süsswassergammariden.— Zool. Anz. 106 (5/6): 127-134.

Karaman, G.S. & S. Pinkster, 1977. Freshwater *Gammarus* species from Europe, North Africa and adjacent region of Asia (Crustacea: Amphipoda ), Part 1. *Gammarus Pulex*-group and related species.— Bijdr. Dierk. 47 (1): 1- 97.

Khalaji-Pirbalouty, V., 2002. A study on species and populations of Amphipoda (Crustacea) in Charmahal-Va-Bakhteyari province with emphasis on aquaculture.— Unpublished M.Sc. thesis, Faculty of Science, University of Tehran.

Meyran, J.C., M. Monnerot & P. Taberlet, 1997. Taxonomic status and phylogenetic relationships of some species of the genus *Gammarus* (Crustacea, Amphipoda) deduced from mtDNA sequences.— Mol. Phylogenet. Evol. 8: 1-10.

Meyran, J.C., L. Gielly & P. Taberlet, 1998. Environmental calcium and mitochondrial DNA polymorphism among local populations of *Gammarus fossarum* (Crustacea, Amphipoda).— Mol. Ecol. 7: 1391-1400.

Müller, J., 2000. Mitochondrial DNA variation and the evolutionary history of cryptic *Gammarus fossarum* types. Mol. Phylogenet. Evol.— 15 (2): 260-268.

Müller, J., E. Partsch & A. Link, 2000. Differentiation in morphology and habitat partitioning of genetically characterized *Gammarus fossarum* forms (Amphipoda) across a contact zone.— Biol. J. Linn. Soc. 69: 41-53.

Naghib, M., 2002. A study on distribution, embryology and karyology of amphipods from Qom and Isfahan provinces.— Unpublished M. Sc. thesis, Faculty of Science, University of Tehran.

Pesce, G.L., R. Pace, & D.Maggi, 1982. Ricerche faunistiche in acque sotterranee fratiche dell'Iran nordoccidentale.— Riv. Idrobiologia 21 (1/3): 37-74.

Pinkster, S., 1983. The value of morphological characters in taxonomy of *Gammarus*.— Beaufortia 33: 15-28.

Pinkster, S., A.L. Dennert, B. Stock, & J.H. Stock, 1970. The problem of European freshwater population of *Gammarus duebeni*.— Bijdr. Dierk. 40 (2): 116 – 147.

Platvoet, D., 1985. Side-line organ in gammarids (Crustacea: Amphipoda).— Beaufortia 35 (7): 129-133.

Pourmohammadi-Sarbanani, M., 2002. A study on species and populations of Amphipoda (Crustacea) in Kerman province with emphasis on aquaculture.— Unpublished M. Sc. thesis, Faculty of Science, University of Tehran.

Ruffo, S., 1979. Descrizione di due nuovi Anifipodi anoftalmi dell'Iran e del Madagascar.— Boll. Museo civ. Storia Nat. Verona 6: 419-440.

Sari, A., 1997. Aspects of breeding biology and cypris fine structure in some balanoid barnacles (Crustacea: Cirripedia).— Ph.D. dissertation, University of Wales, Swansea.

Stock, J.H., A.R. Mirzajani., R.Vonk., S. Naderi, & B.H. Kiabi, 1998. Limnic and brackish water Amphipoda (Crustacea) from Iran.— Beaufortia 48 (9): 173 – 234.

Wellborn, G.A., 1993. Ecology and evolution of life history variation among populations of a freshwater amphipod, *Hyalella azteca*.— PhD thesis, Department of biology, University of Michigan, Ann Arbore.

Wellborn, G.A. & R.D. Cothran, 2004. Phenotypic similarity and differentiation among sympatric cryptic species in a freshwater amphipod species complex.— Freshwater Biol. 49: 1-13.

Witt, J.D. S. & P.D.N. Hebert, 2000. Cryptic species diversity and evolution in the amphipod genus *Hyalella* within central glaciated North America: a molecular phylogenetic approach.— Can. J. Fish. Aqua. Sci. 57: 687-698.

Witt, J.D.S., Dean, W.B. & P.D.N. Hebert, 2003. The recent evolutionary origin of the phenotypically novel amphipod *Hyalella montezuma* offers an ecological explanation for morphological stasis in a closely allied species complex.— Mol. Ecol. 12: 405-413.

Yavari, Y., 2000. Biosystematics study of four local populations of Amphipoda from Chareh-Chai Basin, Markazi province, Iran.— Unpublished M.Sc. thesis, Shiraz University.

Received: 26.viii.2003
Accepted: 1.iv.2005
Edited: W.H. de Weerdt, P.J.H. van Bree