Summer 2000

Comparative Systematics of Subterranean Amphipod Crustaceans in the Families Crangonyctidae and Bogidiellidae

Stefan Koenemann
Old Dominion University

Recommended Citation

Koenemann, Stefan. "Comparative Systematics of Subterranean Amphipod Crustaceans in the Families Crangonyctidae and Bogidiellidae" (2000). Doctor of Philosophy (PhD), dissertation, Biological Sciences, Old Dominion University, DOI: 10.25777/bjvj-vj04
https://digitalcommons.odu.edu/biology_etds/69

COMPARATIVE SYSTEMATICS OF SUBTERRANEAN AMPHIPOD CRUSTACEANS IN THE FAMILIES CRANGONYCTIDAE AND BOGIDIELLIDAE

by

Stefan Koenemann
B.S. January 1994, Carl-von-Ossietzky Universität (Oldenburg, Germany)
M.S. January 1997, University of Amsterdam

A Dissertation Submitted to the Faculty of Old Dominion University
in Partial Fulfillment of the Requirement for the Degree of

DOCTOR OF PHILOSOPHY

ECOLOGICAL SCIENCES

OLD DOMINION UNIVERSITY
August 2000

Approved by:

John R. Holsinger (Director)

Kent E. Carpenter (Member)

Michael F. Gable (Member)

ABSTRACT

COMPARATIVE SYSTEMATICS OF SUBTERRANEAN AMPHIPOD CRUSTACEANS IN THE FAMILIES CRANGONYCTIDAE AND BOGIDIELLIDAE

Stefan Koenemann
Old Dominion University, 2000
Director: Dr. John R. Holsinger

The research project presented in this doctoral dissertation deals with the systematics of two different taxonomic groups of subterranean amphipods (Crustacea). Therefore, rather than being a single project, this study is divided into several sub-projects. Altogether, the chapters for the sub-projects are composed of five publications. An introductory chapter and a summarizing discussion are added to provide a structural unit for the collection of papers and to compare the results of the individual projects.

The thesis investigates the systematics of the amphipod families Bogidiellidae Hertzog, 1936, and Crangonyctidae Bousfield, 1973. Based on descriptive taxonomy according to modern standards, revisions are given for the crangonyctid genus *Bactrurus* Hay, 1902, the bogidiellid genus *Spelaeogammarus* da Silva Brum, 1973, and the family Bogidiellidae. The bogidiellid sub-project also includes the description of the new genus and species *Megagidiella azul*.

This study makes an attempt to compile and evaluate molecular and morphological data for two families of gammaridean amphipods. To research the phylogenetic relationships of the Bogidiellidae and Crangonyctidae, cladistic analyses, using external morphological characters, are conducted for each family. In addition, sequence analyses of the 18S (small subunit) rDNA gene are carried out for three species of *Bactrurus* and several selected amphipod taxa.

The results of morphological and molecular analyses are compared and the phylogenetic relationships of the taxa under investigation are discussed, especially emphasizing their biogeographic distribution patterns and current taxonomic classification.

In a summarizing chapter, the biogeography and evolutionary history of both families are compared. Moreover, the application of different methods of phylogenetic reconstructions is discussed for the Bogidiellidae and Crangonyctidae, as well as for stygobiont amphipods in general.

This thesis is dedicated to my wife Cora who never seemed to get tired listening to the story of the Amphipoda, to my father who is very proud of me because I made a profession of my admiration for life and nature, and to my mother who certainly is equally proud of me but still not quite sure what exactly I am doing.

"In science one tries to tell people, in such a way as to be understood by everyone, something that no one ever knew before. But in poetry, it is the exact opposite."
Paul Dirac (1902-1984)

ACKNOWLEDGMENTS

I would like to thank my major advisor Dr. John R. Holsinger, for his assistance and support throughout the whole research project and being the second author of the papers presented in Chapters 2-5. I am also very grateful to my graduate committee members, Drs. Kent E. Carpenter and Michael F. Gable, for their constructive contributions to this dissertation.

I would like to acknowledge numerous people who assisted with the individual sub-projects:

- I thank Eleonora Trajano (University of São Paulo) for providing me with some of the specimens examined in this study and for helpful comments on Brazilian caves, and Paolo S. Young, curator of the Museum Nacional, Rio de Janeiro, for making the paratypes of *Spelaeogammarus bahiensis* available to me.
- I am grateful to the National Museum of Natural History (Smithsonian Institution) for lending us the topotypes of *Paracrangonyx compactus*, and L. B. Holthuis, who read manuscript drafts of the revision of the Bogidiellidae and offered helpful suggestions for improvement.
- I thank Ulrike Englisch for joining me on a 4-week field trip in May 1999 and sharing her experience during the DNA cooperation project at Ruhr-Universität Bochum (Germany), Don Web and Larry Page (Illinois Dept. of Natural Resources) for assistance in locating *Bactrurus* habitats in Illinois, and Lynn M. Ferguson for making the initial collections of the new species *Bactrurus wilsoni*. The following persons spent several days with me in the field, often hiking over rough terrain in search of springs, caves or wells: Johnny R. Wilson in Alabama; Chris Hobson in Virginia; Julian J. Lewis (Conservation Consultant) in Indiana; Earl Biffle and Jane Walker (Tyson Research Center) in Missouri (special thanks to Earl. Biffle for showing us his artificial cave called "Biff's bunker"); William R. Elliott (Missouri Dept. of Conservation) and Neil R. Babik (Watershed Program Manager, Mark Twain National Forest) in Missouri. I am particularly grateful to Jane Culver (Hayden, Alabama) for her persistent "hunting" efforts that resulted in two additional, badly needed specimens of the new species *B. wilsoni*. I also thank Dave Larson and Brandon Curry of the Illinois State Geological Survey for sending me numerous relevant publications on the geology/hydrology of Illinois.

Finally, I would like to acknowledge the Graphic Office at Old Dominion University for generating numerous distribution maps, slides, posters, etc., during the entire project.

This study was supported by a PEET (Partnership for Enhancing Expertise in Taxonomy) grant from the National Science Foundation to JRH (DEB-9521752). I extend sincere thanks to the Cave Research Foundation and the Cave Conservancy of the Virginias for providing additional funds for fieldwork and travel to Ruhr-Universität Bochum in 1999.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The crustacean order Amphipoda is represented by approximately 7,000 described species (Holsinger, 1994a), commonly occurring in aquatic and occasionally even in terrestrial habitats. Their worldwide distribution and taxonomic diversity is probably unparalleled among all crustacean groups. Amphipod crustaceans inhabit freshwater, brackish, and marine environments. They are recorded from the benthos of arctic seas to shallow tropical shores, from thermal desert springs to cold mountain trickles up to 2,500 m elevation and higher.

The Amphipoda are divided into the suborders Gammaridea, Caprellidea, and Hyperiidea, and sometimes the Ingolfiellidea as a fourth suborder. One remarkable characteristic of the Gammaridea is their abundant presence in hypogean (subterranean) environments. The vast majority of gammaridean stygobionts (obligatory hypogean, aquatic organisms) is distributed among 12 families, some of which are composed exclusively of hypogean species, whereas others comprise both epigean and hypogean taxa (Holsinger, 1993, 1994a).

This research project compares the systematics of two of the larger hypogean groups within the suborder Gammaridea, the families Bogidiellidae and Crangonyctidae.

The family Bogidiellidae

All species of the Bogidiellidae occur in subterranean environments. To date, the family comprises 23 genera, 11 subgenera, and a total of 110 species. The bogidiellid sub-project of the thesis presented yielded three publications:

- The description of the new species and genus *Megagidiella azul* (Chapter II).
- A revision of the genus *Spelaeogammarus*, including the description of four new species (Chapter III).
- A revision and phylogenetic analysis of the family Bogidiellidae (Chapter IV). To obtain additional support for a revised classification, a cladistic analysis, using 27 morphological characters, was performed on 41 taxa of the unrevised family. The revision excluded five genera from the family, all remaining subgenera were elevated to generic level. Four taxa were split, resulting in five new genera. The family Bogidiellidae now consists of 33 genera.

The genus *Bactrurus* (Crangonyctidae)

The Crangonyctidae are composed of only six extant genera and a total of approximately 150 species, including both hypogean and epigean taxa. The exclusively subterranean genus *Bactrurus* is a relatively small group, endemic to the United States. Descriptions of four new species are given, resulting

---

The model journal used to format this thesis was *Crustaceana*.

in a total of seven species for *Bactrurus*. The genus is redescribed and its phylogenetic relationships to its potential sister-genus *Stygobromus* Cope, 1872, and other crangonyctid taxa are investigated. New geological data are given to clarify the rather unusual geographic distribution of *Bactrurus*.

As part of an international cooperative project, 18S (small subunit) rDNA sequencing of *Bactrurus* and several additional amphipod taxa was accomplished in the laboratory of Dr. J. Waegele at Ruhr-University Bochum (Germany). For the first time, analyses of DNA data are applied to subterranean crustaceans.

The revision of *Bactrurus* resulted in the preparation of a monograph for the genus (Chapter V). The DNA-sequencing project yielded a separate publication (Chapter VI).

A summary in Chapter VII compares the evolutionary history, biogeography and phylogeny of the Bogidiellidae and Crangonyctidae.

This thesis aims to answer several key questions about the evolution of stygobiont amphipods:

1. The family Bogidiellidae has an enigmatic geographic distribution. Some taxa inhabit isolated, continental freshwater habitats whereas more abundant groups occur in coastal regions, including marine shores. How can this pattern be interpreted with regard to the evolutionary history of the family?

2. The crangonyctid genus *Bactrurus* is endemic to parts of central and eastern US. The distribution of two of its species extends into regions that were covered by ice sheets during Pleistocene glaciation periods. Other species occur in karst regions that may have been isolated freshwater habitats since the late Paleozoic. How can the geographic distribution of *Bactrurus* be explained? Which assumptions does the biogeography of *Bactrurus* allow us to make about their evolutionary history?

3. How old are lineages of the subterranean freshwater amphipods investigated and which estimates about their evolutionary age can be derived from the results of this study?

4. Techniques and even methods of phylogenetic reconstruction usually differ, depending on the group or taxonomic level under investigation. In which way do these differences affect phylogenetic analyses of stygobiont amphipods? Which methods/techniques should be applied to perform analyses at the family level and above?

CHAPTER II

MEGAGIDIELLA AZUL, NEW GENUS AND NEW SPECIES OF A CAVERNICOLOUS AMPHIPOD CRUSTACEAN OF THE FAMILY BOGIDIELLIDAE FROM BRAZIL, WITH REMARKS ON ITS BIOGEOGRAPHIC AND PHYLOGENETIC RELATIONSHIPS

Introduction

Recent biological exploration of caves by speleologists in several karst areas in Brazil has revealed many new localities for gammaridean amphipod crustaceans and other subterranean organisms (Pinto-da-Rocha, 1995). One such investigation in the Serra da Bodoquena Karst of central-western Brazil resulted in the discovery of a new stygobiont amphipod genus of the family Bogidiellidae, described below. The specimens were collected from a deep, subterranean lake in Gruta do Lago Azul (Blue Lake Cave).

Taxonomic part

Megagidiella, new genus

Diagnosis: Eyes absent. Body smooth, unpigmented. Uronites not fused. Coxal plates longer than wide, not overlapping. Coxal gills occurring on pereopods 4-6; sternal gills absent. Oostegites on pereopods 2-5, sublinear. No sexual dimorphism in any characters. Interantennal (lateral) lobe of head narrowly rounded anteriorly. Mandibular palp absent. Maxilla 1: palp 2-segmented; outer plate with 7 serrate spines; inner plate with 3 apical plumose setae. Gnathopod 1 propodus much larger than gnathopod 2 propodus. Pereopods 5-7 with narrow bases. Pleopods and uropods unmodified. Pleopods biramous; outer ramus 3-segmented; inner ramus reduced, 1-segmented. Uropods biramous; peduncle of uropod 1 with several ventrolateral (basofacial) spines; uropod 3 relatively long. Telson about as long as broad, with shallow excavation.

Type species: *Megagidiella azul*, new species by monotypy; gender feminine.

Etymology: The generic name, referring to the relatively large size of the type species, is a combination of the Greek prefix "mega" (= large) and part of the family name.

Remarks and relationships: Bogidiellids are relatively small amphipods, their body lengths generally range between 1-3 mm, occasionally exceeding 5 mm. With adult specimens reaching a body length of 16.2 mm, *Megagidiella* is an extraordinary exception. The more significant diagnostic character, however, is the absence of a mandibular palp, a morphological reduction to date unparalleled in the family Bogidiellidae (sensu Stock, 1981). Apart from its size and absence of a mandibular palp, *Megagidiella* closely matches the typical morphology of *Bogidiella*, s. str., e. g., gnathopod 1 larger than gnathopod 2; pereopods 3-7 with narrow bases; coxal plates not overlapping, wider than long; 3-segmented pleopodal outer ramus; reduced, 1-segmented pleopodal inner rami. Minor exceptions from the general bogidiellid model are a 1-segmented accessory flagellum and the armature of the telson. Of all described bogidiellid species, a 1-segmented accessory flagellum is known only in 4 genera: *Artesia* Holsinger (*in:* Holsinger & Longley, 1980), *Kergueleniola* Ruffo, 1970, *Marigidiella* Stock, 1981, and *Parabogidiella* Holsinger (*in:* Holsinger & Longley, 1980).

Interestingly, the armature of the telson shows a remarkable resemblance to that of *Spelaeogammarus* da Silva Brum, 1975, from caves in eastern Brazil: *Megagidiella* has 2-3 apical and 3-5 subapical (lateral) spines per telsonic lobe in comparison with 2 apical and 3-4 subapical spines in *Spelaeogammarus*. The combination of 2 apical spines with more than 2 subapical spines is exceptional for bogidiellids. Moreover, the armature and shape of uropods 1-3 show noteworthy similarities in both genera, for example, a row of long setae on the medial margin of the outer ramus of uropod 3. Along with *Artesia*, from an Artesian Well in Texas, these are the only bogidiellids known to us with setae on the rami of uropod 3.

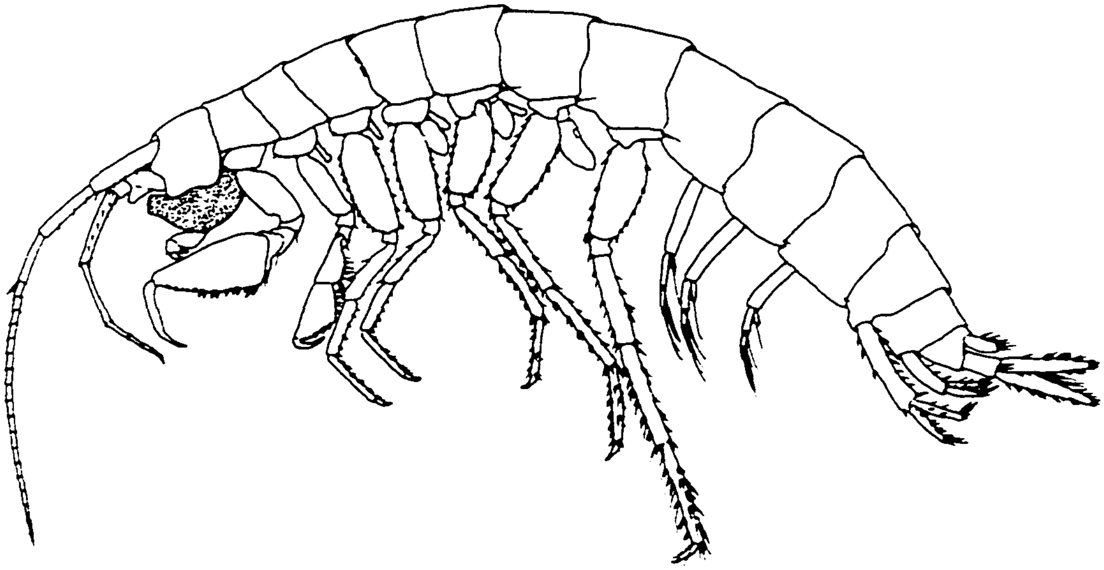


Fig. 2.1. *Megagidiella azul* n. sp., holotype female (16.2 mm) from Lago Azul Cave, Bonito, Estado Mato Grosso do Sul, Brazil. Note: buccal mass is shaded.

***Megagidiella azul*, new species**

Figs. 2.1-4

Material examined: Holotype female (16.2 mm), allotype male (15 mm), and 3 paratypes (1 male, 1 female, 1 juvenile), collected by Adrian Boller, 1 July, 1991.

Type locality: Gruta do Lago Azul, northwest of Bonito, Estado Mato Grosso do Sul, Brazil.

The holotype is dissected and mounted on microscope slides in Faure's medium. It will be deposited in the Museu Nacional (UFRJ) in Rio de Janeiro, Brazil. The allotype and paratypes are preserved in alcohol and will be retained in the research collection of JRH under the catalog no. H-3487.

Diagnosis: With the characters of the genus. Largest male 15 mm, largest female 16.2 mm (Fig. 2.1).

Description: Antenna 1 (Fig. 2.2a) about 50% length of body length. Peduncular segments 1-3 gradually decreasing in length distally. Primary flagellum longer than peduncle, with up to 19 articles in adult specimens, without aesthetascs. Accessory flagellum 1-segmented.

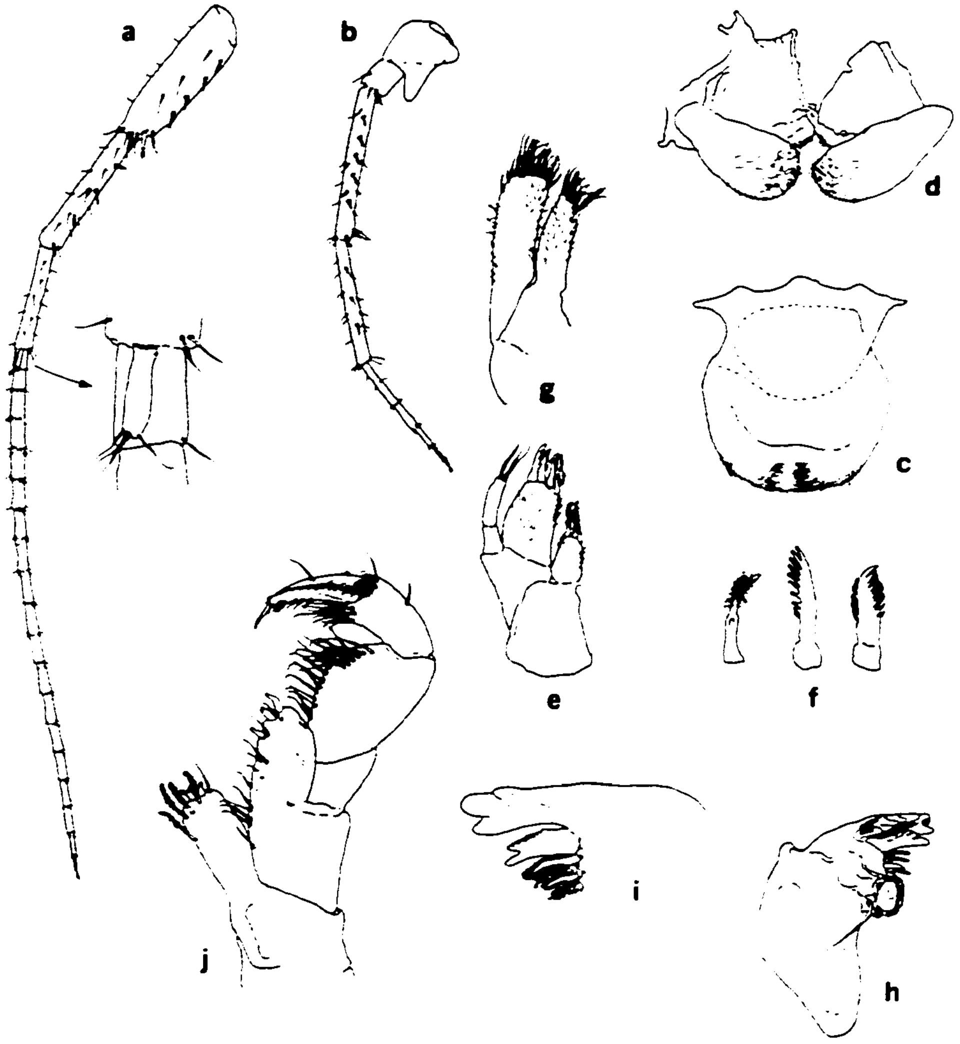


Fig. 2.2. *Megagidiella azul* n. sp., holotype female (16.2 mm): a) antenna 1 (accessory flagellum enlarged), b) antenna 2, c) upper lip, d) lower lip, e) maxilla 1, f) enlarged spine and seta types of maxilla 1, g) maxilla 2, h) left mandible, i) incisor, lacinia mobilis, and spine row of right mandible, j) maxilliped.

Antenna 2 (Fig. 2.2b) about half as long as antenna 1. Peduncular segment 4 longer than segment 5. Flagellum as long as peduncular segment 5, with 5 articles.

Upper lip (Fig. 2.2c) rounded apically, with setules along distal margin.

Mandible (Figs. 2.2h, i): palp absent; molar prominent, rounded, weakly triturative, bearing 1 long, finely serrate seta; left lacinia mobilis 5-dentate, right lacinia 2-dentate, with serrate upper margin; left and right mandible with 4-6, variably plumose accessory spines.

Lower lip (Fig. 2.2d) bearing setules on outer lobes and on distal margins of inner lobes; inner lobes small but distinct; lateral processes short with bluntly rounded corners.

Maxilla 1 (Fig. 2.2e): Palp 2-segmented, with 3 apical setae. Outer plate with 7 comb-like spines (Fig. 2.f), bearing loosely inserted setules on surface and in row along medial margin. Inner plate with marginal setules and 3 apical plumose setae.

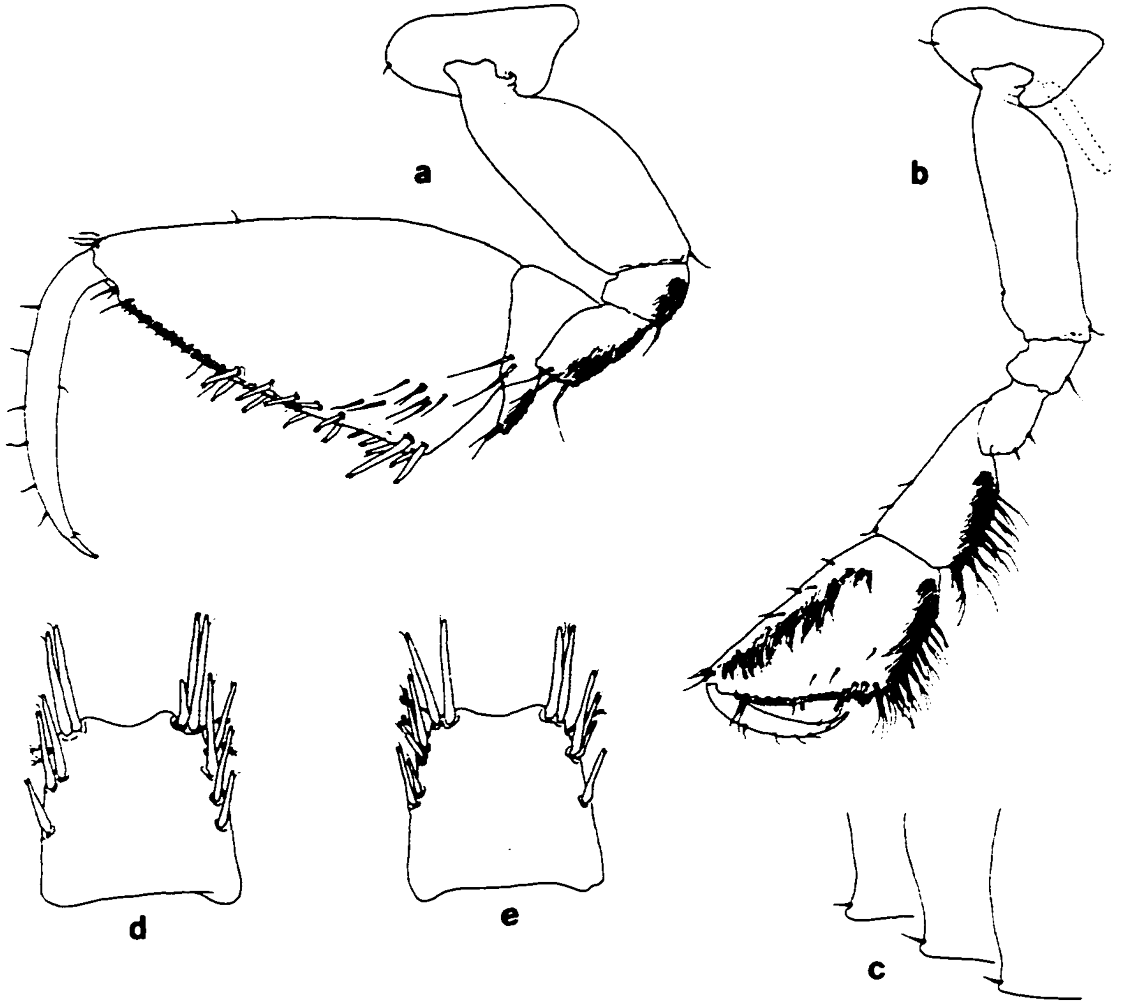


Fig. 2.3. *Megagidiella azul* n. sp., holotype female (16.2 mm): a) gnathopod 1, b) gnathopod 2, c) epimeral plates, d) telson, e) telson, allotype male (15 mm).

Maxilla 2 (Fig. 2.2g): Outer plate with approximately 24 naked apical setae; apical margin of inner plate bearing about 17 naked setae and 3 plumose setae; both plates with fine setules.

Maxilliped (Fig. 2.2j): Palp 4-segmented; 3 blunt spines along apical margin of outer plate; apical margin of inner plate with 2 bifid (y-shaped) spines, 4 plumose setae, and 1 naked seta.

Gnathopod 1 (Fig. 2.3a): Basis naked, bearing only 1 short seta at distoposterior corner. Carpus short, triangular shaped, with 2 setae on pointed posterior lobe. Propodus almost twice as long as broad, approximately twice the size of gnathopod 2 propodus. Palmar margin oblique and even, finely serrate along whole margin, with 5 medial and 5-6 lateral spines; medial margin with about 27 short setae and 4 angular spines of unequal length. Dactyl about 80% length of propodus.

Gnathopod 2 (Fig. 2.3b): Basis naked, bearing only 1 short seta at distoposterior corner. Propodus bearing 18-20 short setae (12-13 laterally and 6-7 medially), 5 spines near corner, and a single lateral spine at mid-palmar margin. Palm with distinctly oblique, finely serrate margin. Dactyl about 60% length of propodus.

Pereopods 3 and 4 subequal (Figs. 2.4a, b). Bases narrow, anterior margins little expanded. Dactyls 24-27% length of propods.

Pereopods 5-7 (Figs. 2.4c-e) increasing in length posteriorly. Bases narrow, posterior margins very weakly expanded. Dactyls about 22, 26, and 28% length of propods, respectively.

All pereopod bases apparently without lenticular organs.

Coxal plates small, wider than long; plates 1-4 rectangular, plates 5-7 at least 2 times wider than long.

Coxal gills (Figs. 2.4a, d) present in 3 pairs, ovate on pereopods 4 and 5 and sack-shaped on pereopod 6.

Oostegites (Figs. 2.3b; 2.4a, c) small, sublinear, occurring on pereopods 2-5 (not setose in material examined).

Epimeral plates (Fig. 2.3c) with small, but distinct distoposterior corners, bearing 1 setule each in groove immediately above corner.

Pleopods 1-3 (Fig. 2.4f) alike. Inner ramus reduced, 1-segmented, with terminal plumose seta . Outer ramus 3-segmented, with 2 terminal plumose setae per segment.

Uropod 1 (Fig. 2.4g) biramous, outer ramus slightly shorter than inner ramus; rami about 64% length of peduncle. Peduncle bearing 14-15 spines, 3 of which inserted along ventrolateral (basofacial) margin. Outer ramus with 12 lateral spines and 4 apical spines. Inner ramus with 4-5 apical and 5 dorsomedial spines.

Uropod 2 (Fig. 2.4h): Inner and outer rami subequal, slightly longer than peduncle. Peduncle with 6 spines. Outer ramus bearing 8 lateral spines and 4 apical spines (2 long ones and 2 short ones). Inner ramus bearing 5 spines along medial and lateral margins and 5 apical spines (3 long ones and 2 short ones).

Uropod 3 (Fig. 2.4i) long, with subequal, 1-segmented rami. Peduncle about 48% length of rami, with 2-4 spines. Outer ramus with 6 apical spines and 6 sets of spines along lateral margin (with 1-5 spines per set); medial margin bearing 4-5 long plumose setae. Inner ramus with 6-7 apical spines and about 19 medial and lateral spines (some doubly inserted).

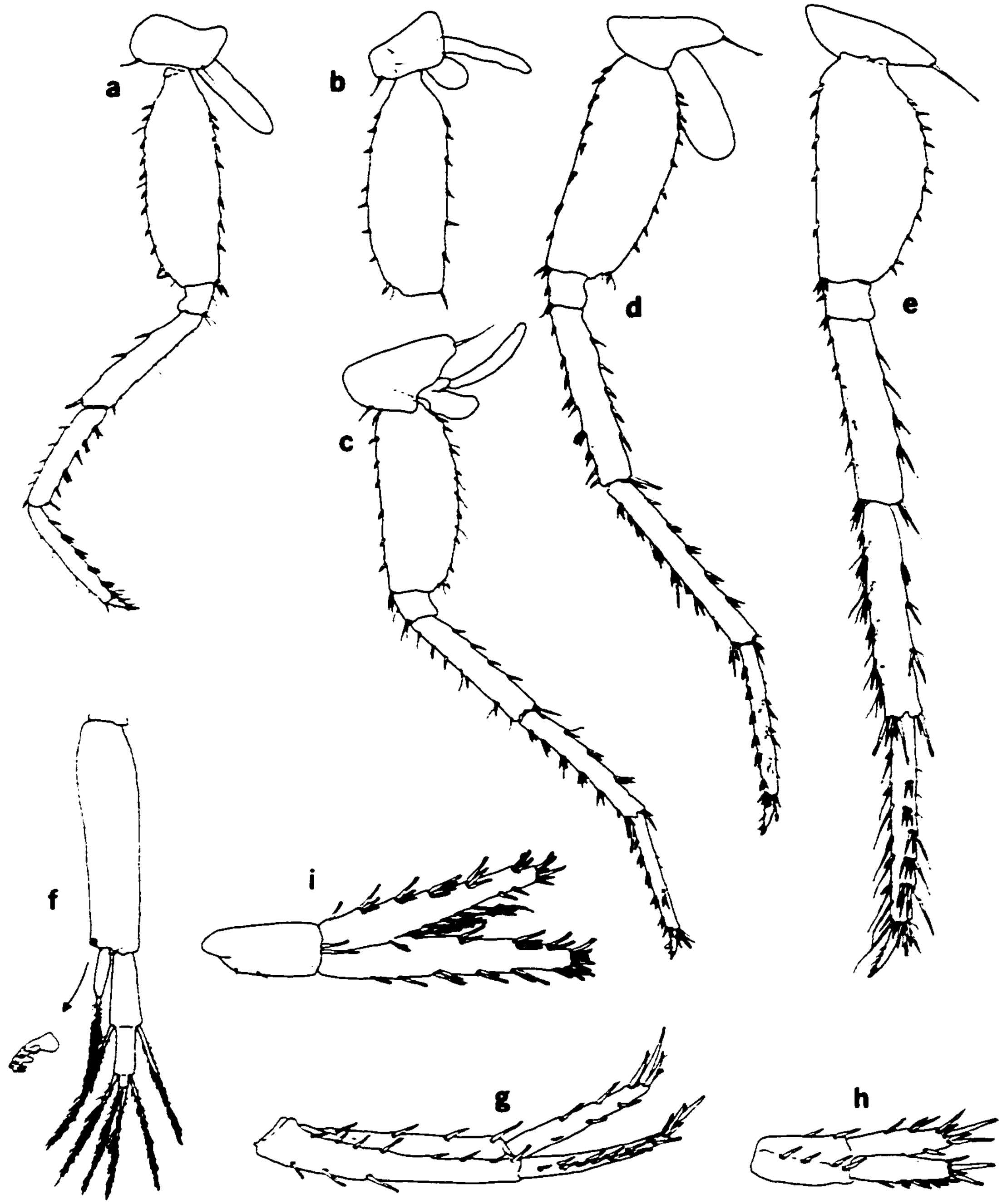


Fig. 2.4. *Megagidiella azul* n. sp., holotype female (16.2 mm): a) pereopod 3, b) pereopod 4, c) pereopod 5, d) pereopod 6, e) pereopod 7, f) pleopod 2, g) left uropod 1, h) left uropod 2, i) left uropod 3.

Telson (Fig. 2.3d, 2.3e) about as broad as long; apex with shallow excavation (8% length of telson); each half bearing 2 plumose setae, 2 (sometimes 3) apical and 3-5 subapical spines.

Variation: Morphological variation, apart from usual differences between juveniles and adults (e. g., number of spines on appendages, flagellum articles, etc.), was observed most obviously in the armature of the telson. The number of subapical spines in the adult females (16 mm in length) varied from 3 to 5 per side, whereas both adult males (15 and 11 mm in length) had a constant number of 3 subapical spines. In the holotype female, a short third apical spine was inserted on the left telsonic apex (Fig. 3d).

Etymology: The epithet *azul* is based on the name of the type locality and is used as a noun in apposition.

Discussion

The type material was collected at a depth between 6 and 12 m from a deep, turquoise-blue lake inside Blue Lake Cave. The cave is located at the southern edge of the world's largest wetland area along the Serra da Bodoquena in central-western Brazil (Pinto-da-Rocha, 1995). Because of the large cave entrance, the lake, about 50 m inside the cave, receives light during some hours of the day (Pires, 1987). The water in the lake presumably marks the upper portion of a subterranean aquifer.

Blue Lake Cave was already biogeographically significant prior to the discovery of *Megagidiella azul*, inasmuch as it is the only known locality in the western hemisphere for the extremely rare crustacean order Spelaeogriphacea. Prior to the discovery of *Potiicoara brasiliensis* Pires, 1987 in Blue Lake Cave, the only other spelaeogriphacean known to science was *Spelaeogriphus lepidopus* Gordon 1957 from caves on Table Mountain in South Africa. One explanation for the occurrence of freshwater stygobiont spelaeogriphaceans in caves on opposite sides of the Atlantic Ocean is that these species are derived from a common ancestor which inhabited Gondwana prior to the separation of Africa and South America in the Early Cretaceous. Although it is tempting to speculate that bogidiellids and spelaeogriphaceans share a similar evolutionary history affected by continental drift, there is to date no evidence that the ranges of these groups form a generalized distribution track. Bogidiellids are recorded only from a few localities near coastal regions in northeastern and northern Africa, whereas the freshwater amphipod fauna in central and southern Africa is composed primarily of epigean paramelitids, and stygobiont ingolfiellids and sternophysingids.

From an ecological perspective, it is important to note that *M. azul* dwells in a large lake of phreatic water. The extraordinary size of this species might imply a correlation of body size and available habitat space. An interesting parallel example of this phenomenon can be observed in the amphipod family Ingolfiellidae. Most ingolfiellids, like many bogidiellid taxa, are less than 3 mm long and live in interstitial habitats. In contrast to the norm, however, species of the ingolfiellid genus *Trogloleleupia* live in large "open" cave lakes in central and southern Africa and may reach 23 mm in length (Griffiths, 1989).

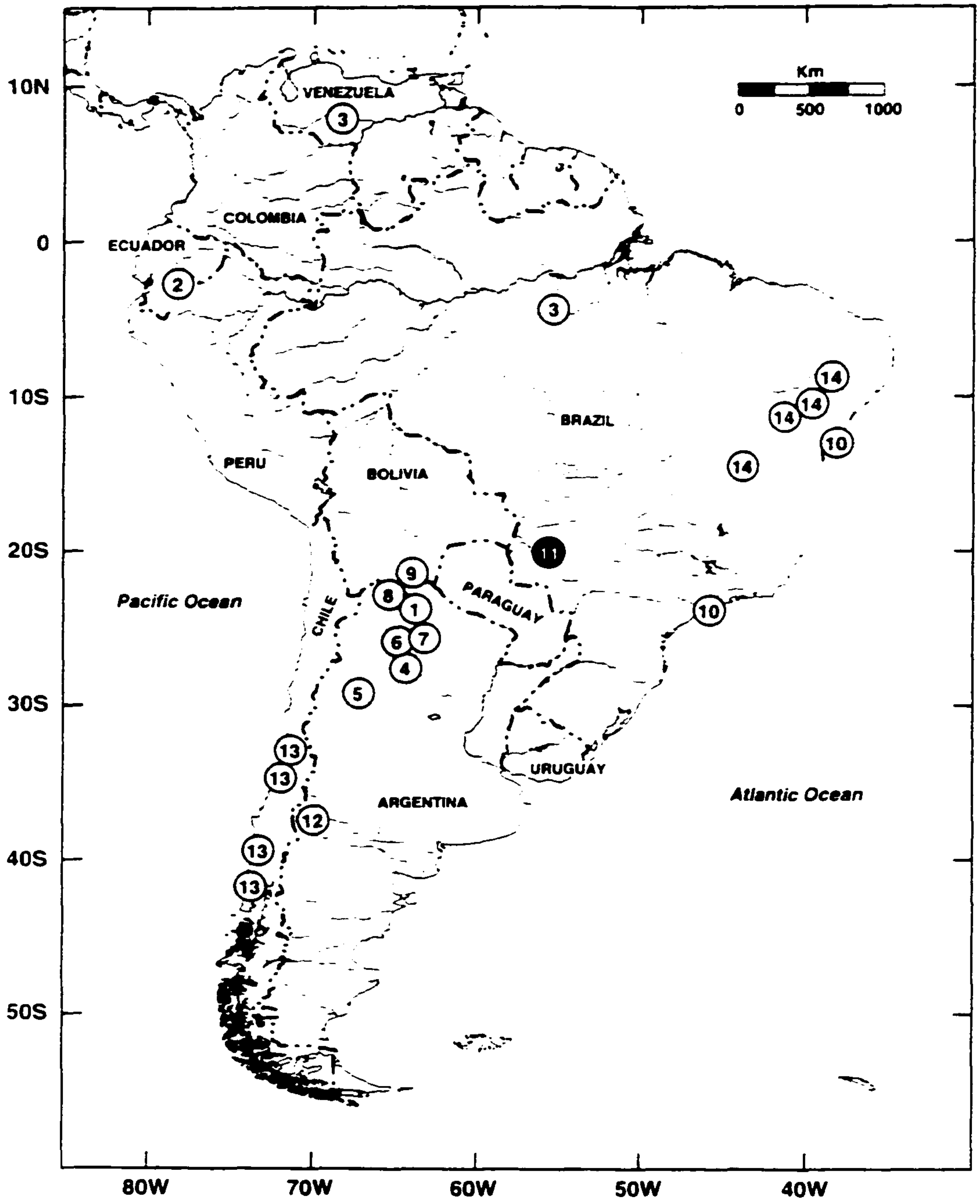


Fig. 2.5. Geographic distribution of bogidiellid amphipods in continental South America: (1) *Bogidiella cooki* Grosso & Ringuelet (1979); (2) *B. gammariformis* Sket (1985); (3) *B. neotropica* Ruffo (1952); (4) *B. (Dycticogidiella) ringueleti* Grosso & Fernández (1988); (5) *B. (Dyct.) talampayensis* Grosso & Claps (1985); (6) *B. (Mesochthongidiella) tucumanensis* Grosso & Fernández (1985); (7) *B. (Stygogidiella) hormocollensis* Grosso & Fernández (1988); (8) *B. (Styg.) lavillai* Grosso & Claps (1984); (9) *Eobogidiella purmamarcensis* Karaman (1982); (10) *Marigidiella brasiliensis* Stock (1981; see also Siewing, 1953); (11) *Megagidiella azul* n. gen., n. sp. (background darkened for emphasis); (12) *Patagongidiella danieli* Grosso & Fernandez (1993) and *P. mauryi* Grosso & Fernández (1993) (both in same locality); (13) *Pseudingolfiella chilensis* Noodt (1965); (14) *Spelaeogammarus bahiensis* da Silva Brum (1975) and 3 n. ssp., Koenemann & Holsinger (2000).

Bogidiellid amphipods have a near worldwide distribution pattern, occurring exclusively in subterranean habitats. Their distribution pattern is characterized by several regions with relatively dense concentrations of species. For example, the South American continent shows the highest generic diversity as opposed to the Mediterranean region where species richness is higher but generic diversity is lower. To date, 18 species, distributed among 10 genera and subgenera, are known from South America (Fig. 2.5).

The discovery of *Megagidiella azul* in the interior of South America, approximately 1,000 km from the nearest coast, is biogeographically significant because the vast majority of bogidiellids occupy ranges between 100-200 km from marine coastal regions. South America shows a remarkable pattern of isolated aquatic habitats, and has promise for the future study of stygobiont organisms and their environments.

Summary

Megagidiella azul, a new genus and species, is described from Gruta do Lago Azul, a cave in central-western Brazil. With a body length of more than 16 mm, this species is the largest bogidiellid recorded to date. In addition to its large size, the absence of a mandibular palp is a unique diagnostic character for the family Bogidiellidae and alone merits recognition of a new genus. The occurrence of *Megagidiella azul* in an isolated, inland cave habitat marks another exceptional biogeographic record of a bogidiellid amphipod from South America.

CHAPTER III

REVISION OF THE SUBTERRANEAN GENUS *SPELAEOGAMMARUS* (BOGIDIELLIDAE) FROM BRAZIL, INCLUDING DESCRIPTIONS OF THREE NEW SPECIES AND CONSIDERATIONS OF THEIR PHYLOGENY AND BIOGEOGRAPHY

Introduction

Exploration of caves in eastern Brazil in the early 1970s resulted in the discovery of the new bogidiellid genus and species *Spelaeogammarus bahiensis* (da Silva Brum, 1975). The specimens were collected from a cave near Curaçá, capital of the district Matamuté, in the state of Bahia. Subsequently, between 1989 and 1993, many additional specimens from various caves in Bahia were collected by Brazilian speleologists and sent to us for identification. Because these specimens differed morphologically from the description of *Spelaeogammarus bahiensis*, we borrowed paratypes of this species from the Museu Nacional in Rio de Janeiro, for a comparison with the new material. The paratypes enabled us to identify and describe three new species and also to partially redescribe the genus *Spelaeogammarus*. In addition, a key to the four species of the genus is provided as well as a table detailing morphological differences.

The holotypes of the new species are deposited in the Museu Nacional in Rio de Janeiro (MNRJ), Brazil, as indicated.

Taxonomic part

Genus *Spelaeogammarus* da Silva Brum, 1975

Spelaeogammarus da Silva Brum, 1975: 125-128.

Type species (by monotypy): *Spelaeogammarus bahiensis* da Silva Brum, 1975.

Diagnosis: Eyes absent. Body smooth, unpigmented. Coxal plates 1-2 small, wider than long; plates 3-6 longer than wide, overlapping. Antenna 1 about 45-50% of body length, primary flagellum longer than peduncle, with 16-20 segments. Accessory flagellum with 4-5 segments. Antenna 2 flagellum bearing 7-10 segments. Mandibular palp 3-segmented. Maxilla 1 with symmetrical, 2-segmented palp; inner plate with 3 plumose setae; outer plate bearing 6-7 serrate spines. Inner plate of maxilliped bearing apically 2 bifid (y-shaped) spines; outer plate with 3 or 4 blade-like spines apically and subapically. Propodus of gnathopod 1 larger than that of gnathopod 2. Dactyls of both gnathopods distinctly serrate along inner margins. Pereopods without any trace of lenticular organs; pereopods 5-7 bases broad, propodus and/or carpus with long, bifurcate setae. Pleopods and uropods unmodified. Pleopods biramous, with 3-segmented outer ramus and 1-segmented inner ramus, rami subequal in length. Uropods biramous: peduncle of uropod 1 with 3 or 4 large basiofacial (ventrolateral) spines; uropod 3 with subequal, 1-segmented rami, outer ramus bearing a row of long, bifurcate setae along medial margin. Telson typically longer than wide, apex with shallow excavation, bearing apical and subapical spines. Coxal gills present on pereopods 4-6. Oostegites linear and elongate, on pereopods 2-5. No sexual dimorphism in any characters.

Key to the species of *Spelaeogammarus* (based on males and females)

1. Accessory flagellum of antenna 1 with 4 segments; flagellum of antenna 2 with 7 segments; propodus of gnathopod 1 slightly larger than basis 2

   Accessory flagellum of antenna 1 with 5 segments; flagellum of antenna 2 with 8-10 segments; propodus of gnathopod 1 much larger than basis .... *S. santanensis*

2. Coxal plate 6 bearing 1 spine, setae absent; outer plate of maxilla 1 with 6 serrate spines and 1 plumose seta; inner plate of maxilliped with 4 plumose setae 3

   Coxal plate 6 bearing 1 spine and about 20 setae; outer plate of maxilla 1 with 7 serrate spines; inner plate of maxilliped without plumose setae .... *S. bahiensis*

3. Anterior margin of basis of gnathopod 1 with 5-9 short setae; coxal plate 5 bearing 1 spine and 17-18 setae .... *S. trajanoae*

   Anterior margin of basis of gnathopod 1 with 2-4 spines (or longer setae) and 1 short seta; coxal plate 5 bearing 1 spine and about 9 setae .... *S. spinilacertus*

Spelaeogammarus spinilacertus, new species

Figs. 3.1-5; 3.6c

Material examined: Estado do Bahia, Brazil: holotype male (6.1 mm) and 1 paratype male (7.5 mm) from Baixa do Salitre Cave, Iraquara, collected by J. A. Cardoso, Sep 1993; 4 paratypes (3 females, 1 male) from Baixa do Salitre Cave, collected by L. Mendes and E. Rubbioli, 24 May, 1997; allotype female (8.1 mm) and 2 paratypes (1 male, 1 female), from Jaburu Cave, Iraquara, collected by J. A. Cardoso, Sep 1993.

The holotype and allotype are dissected and mounted on microscope slides in Faure's medium. Holotype (MNRJ 13340) and allotype are deposited in the Museu Nacional. The remaining 7 paratypes are retained in the research collection of the second author.

Diagnosis: *Spelaeogammarus spinilacertus* is easily distinguished from other species in the genus by 2-4 spines or setae of corresponding length on the distoanterior margin of basis of gnathopod 1. Largest male 10.5 mm, largest female 10 mm.

Description: Antenna 1 (Fig. 3.2a) about 45-50% of body length. Peduncular segments 1-3 gradually decreasing in length; peduncular segment 1 with 3-4 spines; peduncular segment 2 with 2-3 short spines; peduncular segment 3 bearing 0-2 spines. Primary flagellum longer than peduncle, with 17-20 segments; aesthetascs on most segments; accessory flagellum with 4 segments, terminal segment vestigial.

Antenna 2 (Fig. 3.2b) about 85% length of antenna 1. Peduncular segment 4 longer than peduncular segment 5; peduncular segment 5 with 3-4 ventral and 2-4 medial spines. Flagellum with 7 segments, 1st segment twice as long as average length of segments 2-6.

TABLE III.1

Major morphological differences of the four species of the genus *Spelaeogammarus*.

Character	***S. spinilacertus***	***S. trajanoae***	***S. santanensis***	***Sp. bahiensis***
Accessory flagellum	4 segments	4 segments	5 segments	4 segments
Antenna 2 flagellum	7 segments	7 segments	8-10 segments	7 segments
Maxilla 1: outer lobe	6 serrate spines + 1 plumose seta	6 serrate spines + 1 plumose seta	7 serrate spines	7 serrate spines
Maxilliped: apical margin of inner lobe	4 plumose setae	4 plumose setae	2 plumose setae	no plumose setae
Gnathopod 1	propod a little larger than basis	propod a little larger than basis	propod much larger than basis	propod a little larger than basis
Gnathopod 1: post. margin of basis	6-8 setae (some double)	9-10 setae (all single)	~ 20 setae (all single)	7-9 setae (all single)
Gnathopod 1: ant. margin of basis	2-4 spines + 1 short seta	5-9 short setae	4 short setae	3-5 short setae
Gnathopod 2: post. margin of basis	9-10 setae	8-9 setae	21-23 setae	~ 9 setae
Coxal plate 5	1 spine + 9 setae	1 spine + 17-18 setae	1 spine + 12 setae	1 spine + 20-21 setae
Coxal plate 6	1 spine - no setae	1 spine - no setae	1 spine + 1 seta	1 spine + 20-21 setae
Pleopods: inner ramus	4-5 setae	5-7 setae	7-8 setae	~ 7 setae
Uropod 3: outer ramus	~ 20 bifurcate setae	~ 20 bifurcate setae	~ 8 bifurcate setae	missing
Telson: spines per lobe	2 apical + 3-4 subapical	3 apical + 2-3 subapical	1 apical + 3 subapical	2 apical + 3-4 subapical

Upper lip (Fig. 3.2c) as long as broad, trapezoidal, with few apical setules.

Mandible (Fig. 3.2h, 3.2i): Palp 3-segmented, with 3-4 terminal setae; 2nd segment bearing 2 apical and 2-3 subapical setae. Molar rounded and well developed, with 1 long lateral seta. Both incisor and lacinia mobilis on left mandible with 5 irregular, rounded cusps (Fig. 3.2h); 3 long and 3 short plumose spines between lacinia and molar. Right mandible (Fig. 3.2i): lacinia apically serrated, consisting of irregularly pointed denticles; 2 long and 2 short plumose spines between lacinia and molar.

Lower lip (Fig. 3.2d) bearing setules on outer lobes, outer and mandibular lobes with rounded corners.

Maxilla 1 (Fig. 3.2e): Palp 2-segmented, with 5-6 apical setae and few lateral setules. Outer plate with 6 serrate spines and 1 plumose spine; inner plate with 3 plumose setae.

Maxilla 2 (Fig. 3.2f): Outer plate apically with 1-2 comb-like setae, ± 15 medium-sized plumose setae and 2 large plumose spines (slightly subapical); medial margin with few fine setules; apical margin of inner plate bearing ± 18 long comb-like setae and ± 5 short naked setae (seta/spine types in Fig. 3.2g).

Maxilliped (Fig. 3.2j, 3.6c): Segment 1 of palp with 1-2 medial setae; segment 2 bearing 12-13 medial setae; dactyl long and slender, bearing a row of marginal setules. Inner plates apparently fused along medial margins.

Gnathopod 1 (Fig. 3.3a): Posterior margin of basis with 6-8 long setae (some doubly inserted); anterior margin bearing 2-4 spines plus 1 short seta (sometimes 2-3 setae plus 1 short seta). Carpus with 8 setae on posterior lobe (4-6 comb-like and 2-3 naked). Propodus ovate, almost twice as long as broad, larger than gnathopod 2 propodus; palm uneven, serrate with minute setules at corner (Fig. 3.3b); palmar margin bearing 7-8 normal spines and 15-19 short bifid spines on lateral margin; medial margin with 6 short setae, 1 normal angular spine, and 4-6 oblique subangular spines (1-2 relatively long). Dactyl about 70% length of propodus; inner margin with distinct row of denticles (Fig. 3.3c).

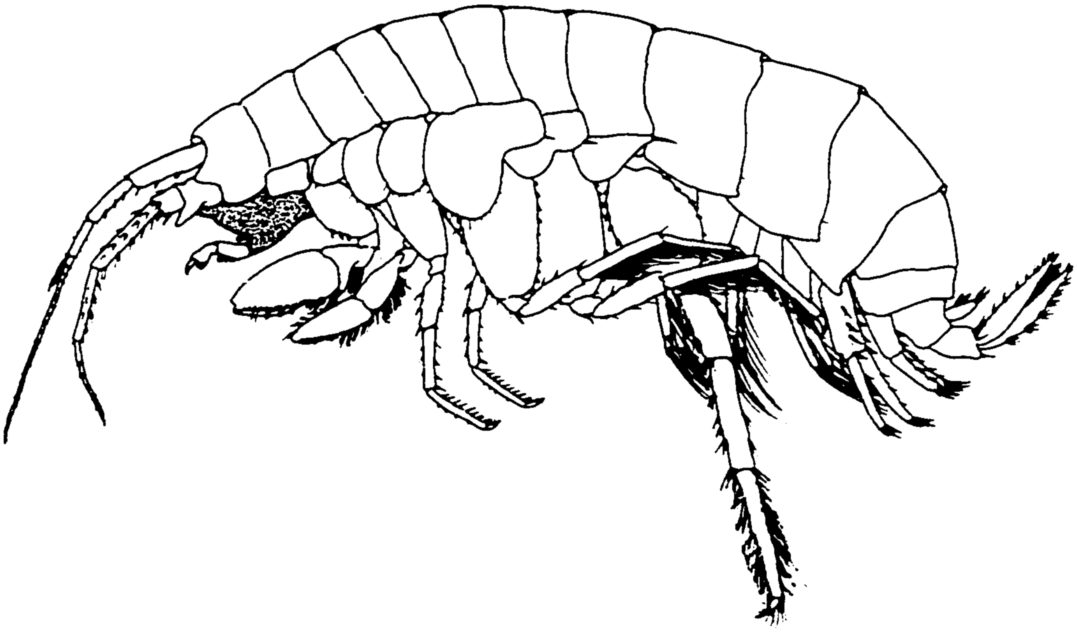


Fig. 3.1. *Spelaeogammarus spinilacertus* n. sp., allotype female (8.1 mm) from Jaburu Cave, Estado do Bahia, Brazil.

Gnathopod 2 (Fig. 3.3d): Posterior margin of basis bearing 9-10 long setae. Carpus posteriorly with fine setules and 7 sets of setae (1-5 setae per set). Propodus ovate, almost twice as long as broad; palm oblique, with 5-6 corner spines (Fig. 3.3e), 11-14 short lateral bifid spines, and 7-8 short medial setae; palmar margin finely serrate at whole margin, with minute setules at corner. Dactyl about 50% length of propodus; inner margin with distinct row of denticles.

Pereopods 3 and 4 subequal (Fig. 3.4a). Basis without spines, anterodistal margin even (pereopod 4 basis with 0-1 spine plus 1 seta at anterodistal margin). Posterior margin of carpus bearing 4-5 spines. Propodus with 8-9 spines along posterior margin and 2 apical spines. Dactyl about 24% length of propodus (Fig. 3.4b).

Pereopod 5 (Fig. 3.4c): Basis with 10-11 spines at posterior margin (distal and proximal group of spines separated by a gap); anterior margin bearing 13-15 spines; anterior lateral surface with 6 short setae; 10-11 short setae at posterior margin and posterior lateral surface. Ischium with 1 spine and 3 setae. Anterior margin of carpus with a row of long, bifurcate setae (Fig. 3.4f) and 6-12 spines (some doubly inserted). Propodus anteriorly with a row of long, bifurcate setae, occurring progressively shorter distally, with slightly thicker bases; lateral margin with 17-19 spines (some doubly inserted); proximal part with 3-4 spines. Dactyl 14-20% length of propodus.

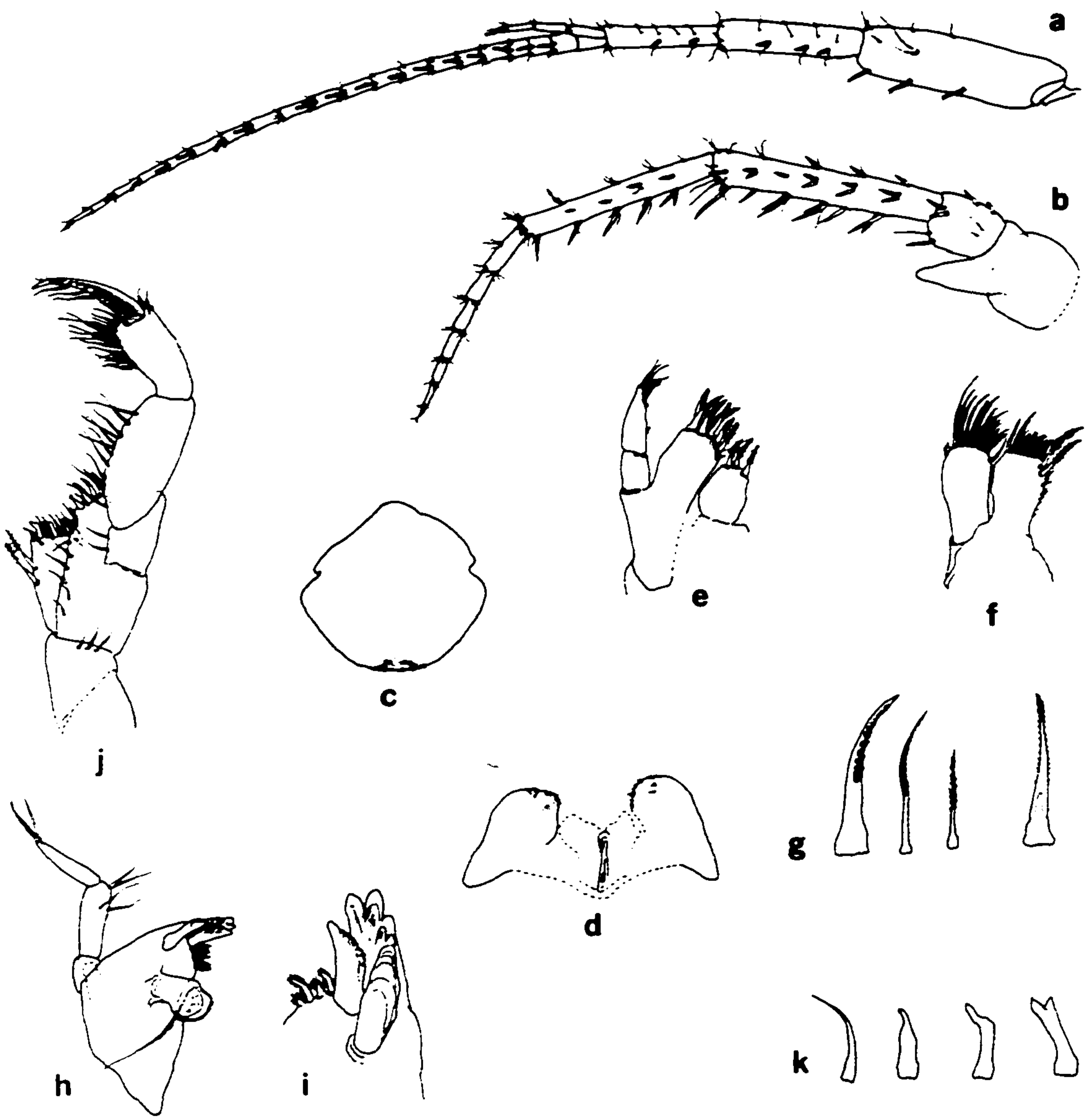


Fig. 3.2. *Spelaeogammarus spinilacertus* n. sp., allotype female: a) antenna 1, b) antenna 2, c) upper lip, d) lower lip, e) maxilla 1, f) maxilla 2, g) spine and seta types (on maxilla 1 and 2, maxilliped, and gnathopod 1 and 2), from left: serrate spine, comb-like seta, plumose seta, and plumose spine, h) left mandible, i) right incisor and lacinia mobilis, j) maxilliped, k) spine types of maxilliped outer lobe (far left 2) and inner lobe (far right 2).

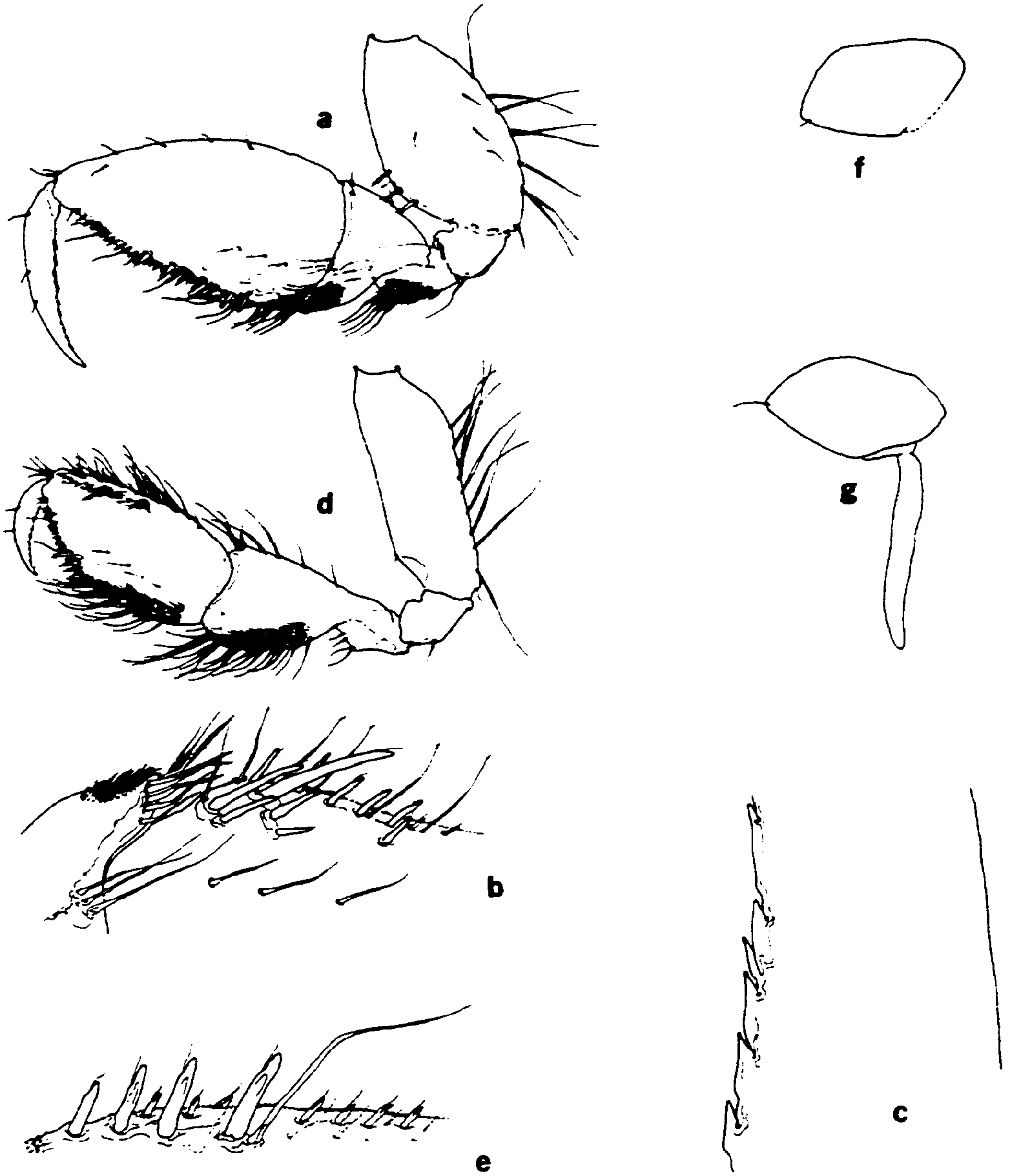


Fig. 3.3. *Spelaeogammarus spinilacertus* n. sp., holotype male: a) left gnathopod 1, b) detail of right gnathopod 1, c) detail of right gnathopod 1 dactyl, d) left gnathopod 2, e) detail of left gnathopod 2; allotype female: f) coxal plate of gnathopod 1, g) coxal plate of gnathopod 2.

Pereopod 6 subequal to pereopod 5 but slightly longer.

Pereopod 7 (Fig. 3.4d): Basis ovate, bearing 8 spines on anterior margin and 7-9 spines on posterior margin. Ischium with 2 spines. Merus with 3 spines on posterior margin and 5 spines at anterior margin (1 singly and 2 doubly inserted). Carpus with 13 spines, occurring in 6-7 sets (with 1-3 spines per set) on anterior margin, 4 spines (2 doubly inserted) plus 4 setae on posterior margin, and 10-11 terminal spines. Propodus bearing 12 slender spines on anterior margin and rows of long, bifurcate setae along posterior and anterior margins (Fig. 3.4f). Dactyl about 27 % length of propodus.

Pereopods 1-7 without any trace of lenticular organs; pereopods 5-7 with broad bases.

Coxal gills ovate, present on pereopods 4-6.

Coxal plates 1 and 2 (Fig. 3.3f, 3.3g) about equal in size, subrectangular to ovate in shape, wider than long; plates 3-6 longer than wide, overlapping; plates 3 and 4 about the same size, plate 5 largest; plate 5 and 6 with distinct anterodistal lobes (Fig. 3.4c, 3.4g).; plate 7 (Fig. 3.4e) subtriangular to irregular in shape, with single long spine at tapered posterior corner.

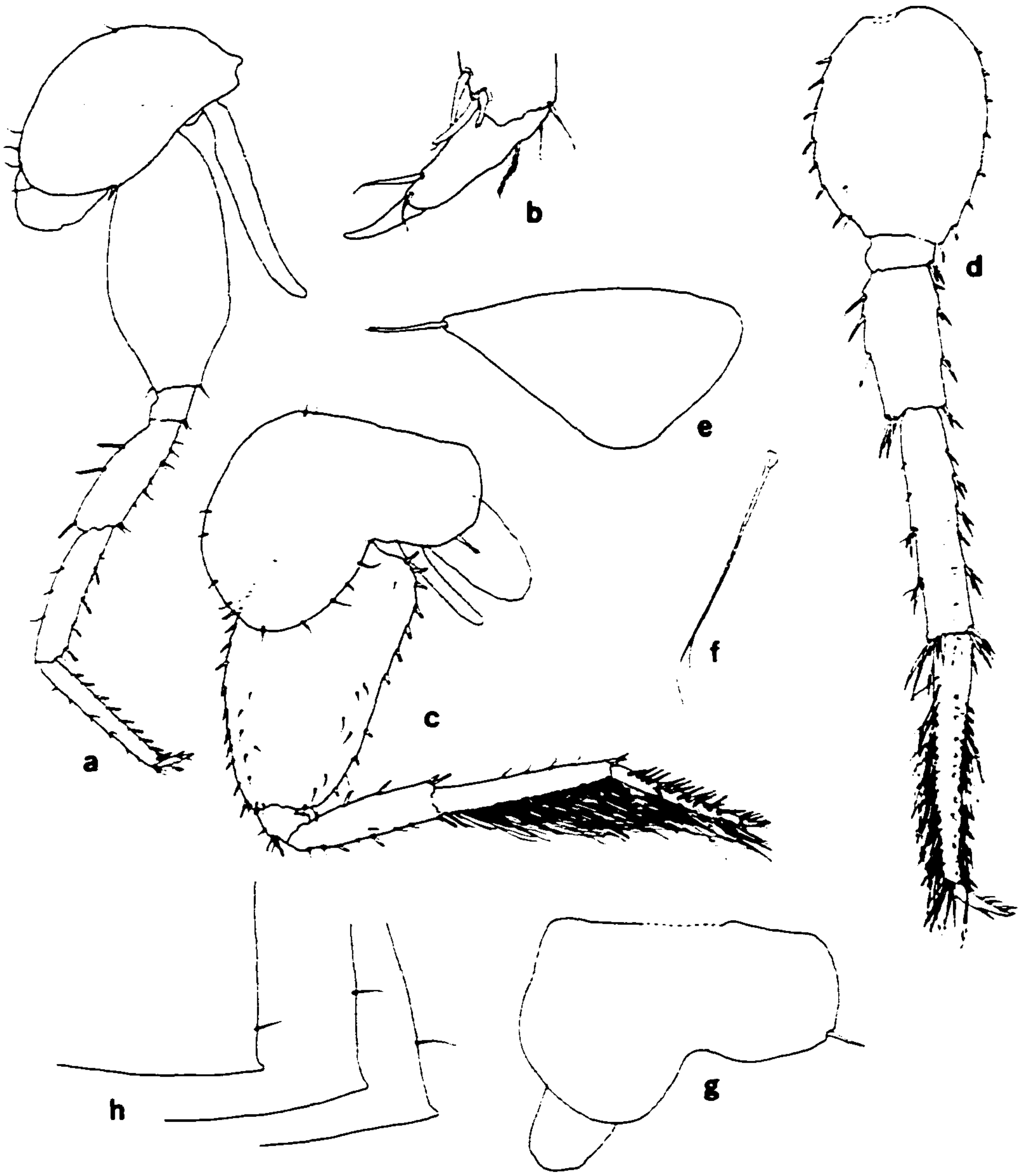


Fig. 3.4. *Spelaeogammarus spinilacertus* n. sp., allotype female: a) left pereopod 4, b) right pereopod 4 dactyl, c) left pereopod 5, d) right pereopod 7, e) coxal plate of right pereopod 7, f) bifurcate seta (on carpus and propodus of pereopod 5-7), g) coxal plate of left pereopod 6, h) epimeral plates.

Oostegites linear and elongate, on pereopods 2-5.

Pleopods 1-3 morphologically alike (Fig. 3.5a), decreasing slightly in size posteriorly, with subequal outer and inner rami. Outer rami 3-segmented, with 2 terminal plumose setae per segment; segment 1 bearing 6-10 lateral plumose setae and 5-8 medial plumose setae. Inner rami 1-segmented, with 4-5 medial plumose setae.

Epimeral (pleonal) plates subquadrate, with small, subacute posterior margins, bearing 1 setule each (Fig. 3.4h).

Uropod 1 (Fig. 3.5b): Rami subequal in length, slightly shorter than peduncle. Peduncle with 3 spines on dorsolateral, dorsomedial, ventrolateral (basofacial), and apical margin, respectively. Outer ramus bearing 3-4 dorsolateral and 4 apical spines. Inner ramus with 5 apical and 4-5 dorsolateral spines, the latter occurring as 3-4 singly and 1-2 doubly inserted.

Uropod 2 (Fig. 3.5c): Peduncle bearing 1 dorsomedial spine, 2 dorsolateral spines, and 2 apical spines. Outer ramus slightly shorter than inner ramus, as long as peduncle, bearing 2-3 spines dorsolaterally and 4 spines apically. Inner ramus with 5 dorsolateral and 4-5 apical spines.

Uropod 3 (Fig. 3.5d) with subequal, lanceolate rami, both 1-segmented. Peduncle about 46% length of rami, with 2 apical spines, 1 subapical spine, and 1 small dorsoproximal spine. Outer ramus bearing 3 apical spines, 6 sets of spines (with 2-3 spines per set) along lateral margin, and about 20 bifurcate long setae along medial margin (Fig. 3.5e). Inner ramus with 3 apical spines; lateral margin with 5 spines; medial margin bearing 9 spines (some doubly inserted).

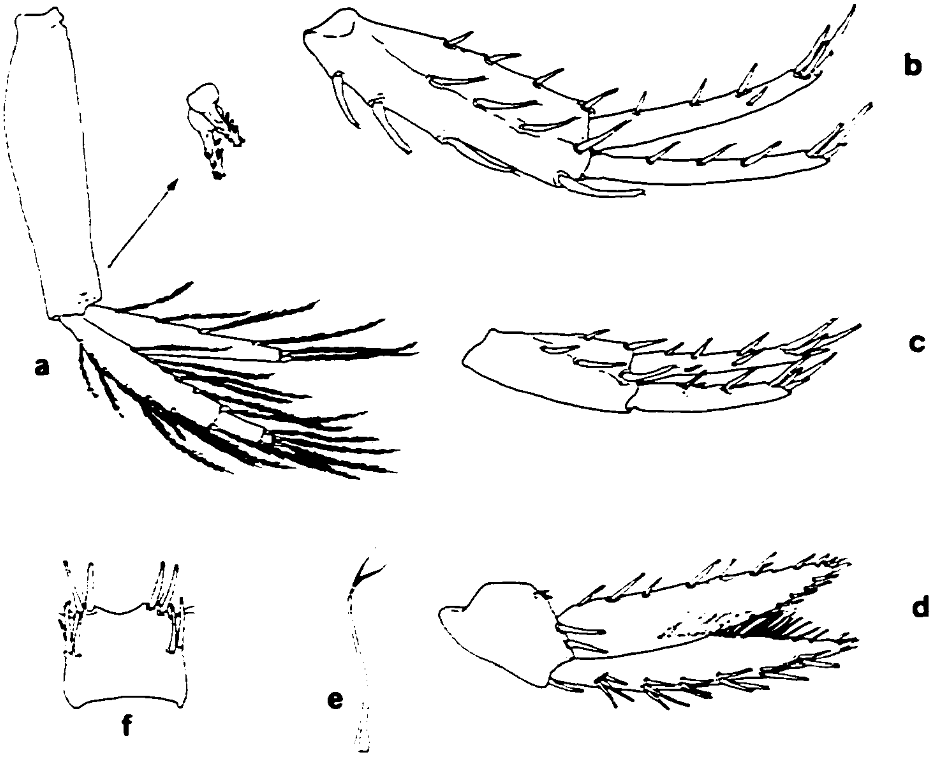


Fig. 3.5. *Spelaeogammarus spinilacertus* n. sp., holotype male: a) left pleopod 3, b) left uropod 1, c) left uropod 2, d) left uropod 3, e) bifurcate seta of outer ramus of uropod 3, f) telson.

Telson (Fig. 5f) width about 84% of length, with shallow excavation (10% of length); each side bearing 3 setae, 2 apical and 2 (sometimes 3) subapical spines.

Etymology: The epithet *spinilacertus* is a noun in apposition, alluding to the presence of spines on the anterior margin of the basis of gnathopod 1. It is formed by combining *spini*, from Latin meaning 'thorn' or 'spine', with *lacertus*, from Latin meaning 'upper arm' (~ basis).

Remarks: Two of the three specimens from Jaburu Cave showed variation in some characters, one of which is diagnostic for the species: instead of 2-4 spines, the basis of the female gnathopod 1 had 2-3 setae along the anterodistal margin. These setae had the same length as the spines they replaced and could be clearly distinguished from corresponding setae in *S. santanensis* and *S. trajanoae* (described below). Furthermore, both specimens (10 mm male and 7 mm female) had 3 subapical spines on each lobe of the telson. The male from the Jaburu sample also showed morphological variability in its appendages, e.g., the bases of both gnathopods and pereopods 3-6 were relatively narrow and elongate; similarly, the propods of both gnathopods appeared relatively longer and larger, with a conspicuously sinusoid palmar margin.

Spelaeogammarus trajanoae, new species

Figs. 3.6b, i; 3.7c, d; 3.8a, b

Material examined: Campo Formoso, Estado do Bahia, Brazil: holotype female (10.4 mm) and 5 paratypes (2 males, 3 females) from Toca do Pitu Cave (= Gruta do Pitu?), collected by A. Auler and M. Martins, Sep 1989; 1 male and 1 female paratype from Toca do Pitu Cave, collected by E. Rubbioli, Jan 1992; 3 paratypes (1 female, 2 juveniles) from Buraco do Teodoro Cave, collected by J. A. Cardoso (no date given); 1 female paratype from Toca do Gonçalo Cave, collected by P. Gernhard, 4 Jul, 1997; 1 fragmented specimen from Convento Cave, collected by S. Larizotti, 1986 (?).

The holotype is dissected and mounted on microscope slides in Faure's medium. Holotype (MNRJ 13341) and 2 paratypes from the type locality are deposited in the Museu Nacional. The allotype and the remaining paratypes are retained in the collection of the second author.

Diagnosis: *Spelaeogammarus trajanoae* is morphologically closely allied with *S. spinilacertus* but can be distinguished from that species by 5-9 short setae instead of spines or spine-length setae on the distoanterior margin of the basis of gnathopod 1 and coxal plate 5 with approximately 18 setae plus 1 spine on margins instead of 9 setae plus 1 spine. Largest males 10.0 mm, largest female 10.5 mm. *S. trajanoae* is moreover distinguished from *S. spinilacertus* as indicated in the following description.

Description: Antenna 1: Peduncular segment 1 with 5-7 spines; peduncular segment 2 with 3-4 short spines; peduncular segment 3 bearing 2 spines.

Lower lip (Fig. 3.6i) bearing setules on medial margin of outer lobes.

Maxilliped: Segment 2 with ± 17 setae along medial margin. Inner and outer plate shown in Fig. 3.6b.

Gnathopod 1 (Fig. 3.7c): Posterior margin of basis bearing 9-10 singly inserted, long setae; anterior margin with 5-9 short setae. No setules on posterior margin of ischium. Carpus bearing 6-9 setae on pointed posterior lobe (4-6 comb-like and 2-3 naked). Palm of propodus even; lateral margin with 5-7 normal spines and 16-17 short bifid spines; medial margin with 6-8 short and 6 long setae.

Gnathopod 2: Posterior margin of basis with 8-9 long setae. Carpus posteriorly with 5-6 rows of setae (1-5 setae per row). Propodus with 6-8 rows of setae (1-3 setae per row) at proximoposterior margin; palmar margin with 3-4 lateral corner spines, 16-17 short lateral bifid spines, and 8-9 short medial setae.

Pereopods 3 and 4 (Fig. 3.8a): Basis bearing 1 spine at posterodistal margin; anterodistal margin sinusoid, with 1 spine and 1 seta (pereopod 3) or 2 spines and 1 seta (pereopod 4). Carpus bearing 6-7 spines posteriorly. Propodus with 13-15 spines along posterior margin.

Pereopod 5: Coxal plate with 17-18 marginal setae on anterior lobe. Anterior margin of basis bearing 10-12 spines; 15 short setae on both posterior and anterior lateral surfaces. Dactyl about 22% length of propodus.

Pereopod 6: Ischium with 2 spines and 1-2 setae. Dactyl about 26% of propodus length.

Pereopod 7: Basis with 11 spines on anterior margin and 10 spines on posterior margin. Merus with 3 sets of doubly inserted spines at posterior margin and 3 sets of spines at anterior margin (with 3-4 spines per set).

Pleopods: Outer ramus with 8-10 lateral plumose setae and 8-9 medial plumose setae on first segment. Inner ramus bearing 5-7 medial plumose setae.

Epimeral plates (Fig. 3.7d) with produced, bluntly rounded distoposterior corners.

Uropod 1: Peduncle with 3-4 spines on dorsolateral margin, 4-5 spines on dorsomedial margin. Inner ramus with 4-5 marginal spines, occurring as 4 dorsomedial and 0-1 dorsolateral spines.

Uropod 2: Peduncle with 1-2 dorsomedial spines, 2-3 dorsolateral spines.

Uropod 3: Outer ramus bearing 3-5 apical spines, 4-6 rows of spines (with 1-3 spines per row) along lateral margin. Inner ramus bearing 10-11 rows of spines (with 5 singly and 5 doubly inserted) at medial margin.

Telson (Fig. 3.8b) width about 81% of length, with u-shaped apical excavation (19% of length); each side with 3 apical setae, 2 apical spines, and 3-4 subapical spines.

Etymology: The species is named in honor of Professor Dr. Eleonora Trajano of the University of São Paulo, who has made important contributions to Brazilian biospeleology.

Remarks: Out of a total of 12 specimens examined, 2 individuals were found with 2 and 3 subapical spines on each telsonic lobe, respectively. The majority (10 specimens) had 4 subapical spines on one lobe and 3 subapical spines on the other lobe.

***Spelaeogammarus santanensis*, new species**

Figs. 3.6a, e-g; 3.7a, b; 3.8c-e

Material examined: Padre Cave, Santana, Estado do Bahia, Brazil: holotype male (13.6 mm), 3 male and 3 female paratypes (11.5-13.6 mm), and 1 juvenile paratype (10.6 mm), collected by F. Chaimowicz, July 1987.

The holotype is dissected and mounted on microscope slides in Faure's medium. Holotype (MNRJ 13342) and 2 paratypes are deposited in the Museu Nacional. The remaining paratypes are retained in the collection of the second author.

Diagnosis: A comparatively large cavernicolous species, easily distinguished from other species in the genus by having: 5-segmented accessory flagellum; 20-23 setae on posterior margins of the bases of gnathopods 1 and 2; propodus of gnathopod 1 proportionally larger. Largest male 13.6 mm, largest female 10.5 mm. *S. santanensis* is furthermore distinguished from *S. spinilacertus* according to the following description.

Description: Antenna 1 about 40-45% length of body. Primary flagellum bearing 20-21 segments, some of which with multiple inserted aesthetascs (Fig. 3.6e). Peduncular segment 1 with 5-6 spines; peduncular segment 3 with 2 ventromedial spines. Accessory flagellum 5-segmented.

Antenna 2 slightly shorter than antenna 1. Peduncular segment 5 with 5 ventral spines. Flagellum as long as peduncular segment 5, with 8-10 segments.

Mandible (Fig. 3.6f, 3.6g): Palp with 3 terminal setae. Palp segment 2 of left mandible with 3 setae and 1 spine; 3 short and 1(-2?) long plumose spines between lacinia and molar. Palp segment 2 of right mandible with 4 setae and 2 spines.

Lower lip with few thin setules on medial margin of outer and inner lobes; corner of outer lobes with slightly pointed corners.

Maxilla 1: Palp with 8-9 apical and subapical setae and a few lateral setules. Outer plate with 7 serrate spines apically.

Maxilla 2: Outer plate apically with 0-1 comb-like setae, ± 21 plumose setae, and 1-2 plumose spines (slightly subapical). Inner plate bearing ± 22 long comb-like setae and several short naked setae (two of which on medial margin).

Maxilliped: Palp segment 1 with 2 medial and 2 proximomedial setae; segment 2 with ± 25 medial setae; segment 3 bearing ± 17 medial and 3 dorsomedial setae. Dactyl with 8 setae on outer margin. Inner and outer plate shown in Fig. 6a.

Gnathopod 1 (Fig. 3.7a): Posterior margin of basis bearing 20 long, singly inserted setae; anterior margin with 4 setae. Carpus with distinctly pointed posterior lobe, which bears 13-14 plumose setae. Propodus bearing 7-8 setae on medial surface; palmar margin sinusoid, with 10 short medial setae, 4 long lateral setae, 8-9 normal lateral spines, 19-20 short bifid lateral spines, 1 normal angular spine medially, 2-3 oblique subangular spines (1-2 relatively long), ± 13 subangular lateral setae, and 6-7 subangular medial setae. Inner margin of dactyl with row of blunt denticles.

Gnathopod 2 (Fig. 3.7b): Posterior margin of basis bearing 21-23 long setae. Carpus posteriorly with 8-9 rows of setae and 5 short setae distolaterally. Propodus twice as long as broad; palm with 6 lateral spines and 1 medial spine, 18 short lateral bifid spines, 1 long lateral seta, and 12 short medial setae; palmar margin with blunt serration of whole margin, distinct serration and minute cilia at corner. Dactyl about 53% length of propodus, inner margin with row of blunt denticles.

Fig. 3.6. *Spelaeogammarus santanensis* n. sp., holotype male: a) inner and outer plate of maxilliped, e) detail of antenna 1, f) left mandible, g) detail of right mandible; *S. trajanoae* n. sp., paratype female (10.36 mm): b) inner and outer plate of maxilliped, i) lower lip; *S. spinilacertus* n. sp., holotype male: c) inner and outer plate of maxilliped; *S. bahiensis*, paratype (8 mm juvenile): d) inner and outer plate of maxilliped, h) left incisor and lacinia mobilis.

Pereopod 3: Basis (Fig. 3.8c) with 2 spines at posteriodistal margin. Carpus bearing 5-6 spines posteriorly and 2-3 spines plus 1-2 setae anteriorly. Propodus with 11-12 spines along posterior margin (some doubly inserted) and 2 spines plus 4 setae apically. Dactyl about 25% length of propodus.

Pereopod 4 subequal to pereopod 3, except for the following differences: coxal plate with 1 spine and 8 setae along distal margin, 2 setae at proximal margin, and 3 setae on lateral surface. Basis with 2-4 spines at posterodistal margin.

Pereopod 5: Coxal plate with 12 setae on anterior lobe and 2 setae on lateral surface. Basis bearing 12 spines on anterior margin; anterior and posterior lateral surface with 4 short setae respectively. Ischium with 4 setae. Anterior margin of carpus with 5-6 sets of spines (2-3 singly and 2-3 doubly inserted). Lateral margin of propodus with 13 spines (5-6 doubly inserted). Dactyl about 18% length of propodus, bearing 1 plumose seta posteriorly.

Fig. 3.7. *Spelaeogammarus santanensis* n. sp., holotype male: a) left gnathopod 1, b) right gnathopod 2; *S. trajanoae* n. sp., paratype female (10.36 mm): c) left gnathopod 1, d) epimeral plates.

Pereopod 6 subequal to pereopod 5 except for the following characters: coxal plate with 1 spine and 1 seta on anterior lobe. Basis without setae on posterolateral surface; anterolateral surface with 2-6 setae; posterior margin bearing 14 spines (pereopod 5: 11 spines). Merus posteriorly with 3 spines and anteriorly with 3-4 spines plus 3-4 setae (see *S. spinilacertus* for pereopod 5). Anterior margin of carpus with 8 spines (3 doubly inserted). Dactyl about 20% length of propodus, bearing 1 plumose seta and 1 spine posteriorly.

Pereopod 7: Coxal plate (Fig. 3.8d) irregular in shape. Basis with 10-11 spines on anterior margin. Ischium bearing 1 spine and 1-2 setae. Merus with 6 spines (3 doubly inserted) on anterior margin. Carpus with 3 sets of spines (with 3-4 spines per set) on anterior margin and 4 sets of spines (with 1-3 spines per set) on posterior margin. Anterior margin of propodus additionally with 10 sets of 1-3 slender spines. Dactyl about 21% length of propodus, with 1 plumose seta at posterior margin.

Pleopods: Outer rami bearing 12-13 lateral plumose setae and 7-8 medial plumose setae. Inner rami with 7-8 medial plumose setae.

Epimeral plates subquadrate, with small, subacute posterior margins, bearing 1 setule each.

Uropod 1: Peduncle with 4 spines on dorsolateral, 4 on dorsomedial, 3 on ventrolateral, and 3 on apical margin, respectively. Inner ramus with 7 dorsolateral spines (3-4 dorsomedial and 1-2 dorsolateral spines).

Uropod 2: Outer ramus bearing 4 dorsolateral spines (doubly inserted).

Uropod 3: Outer ramus bearing 2 spines and 1 seta apically, 5 sets of spines (with 1-3 spines per set) along lateral margin; medial margin with ± 8 bifurcate setae proximally and ± 6 slender setae distally. Medial margin of inner ramus with 8 sets of singly and doubly inserted spines.

Telson (Fig. 8.e) width about 76% of length; apex with shallow excavation (5% of length); each lobe bearing 1 spine plus 1 seta apically and 3 spines plus 1 seta subapically.

Etymology: The proposed epithet *santanensis* is a toponym, referring to the capital city Santana, which is situated near the type-locality.

Remarks: In marked contrast to the 3 other species of the genus, the preserved specimens of *S. santanensis* appeared whitish and almost transparent. Specimens of *S. spinilacertus* and *S. trajanoae* were yellowish-grey, whereas *S. bahiensis* showed a dark, brownish tone. Since these variations appeared to be interspecific (i.e., consistent for species from different localities) it is possible that they were caused by structural differences of the exoskeletons.

***Spelaeogammarus bahiensis* da Silva Brum, 1975**

Figs. 3.6d, h; 3.7f, g

Material examined: Patamute Cave (type locality), Curaça, Distrito de Matamuté, Estado do Bahia, Brazil: 1 male paratype, 11.1 mm, and 1 juvenile paratype, 8 mm (Museu Nacional catalogue no.

MN 5725), collected by P. Magalhães, 1972/1973 (?).

Diagnosis: *Spelaeogammarus bahiensis* is morphologically more similar to *S. spinilacertus* and *S. trajanoae* than to *S. santanensis* but differs from the former two species in having 7 serrate spines on the outer plate of maxilla 1, 20-21 setae on coxal plate 5, and the presence of dorsal setules on pereonite 7 (1 setule), pleonites 1-3 (2-10 setules), and uronites 1-2 (1-7 setules). In addition, it is distinguished from *S. spinilacertus* by short setae instead of spines on the anterior margin of the basis of gnathopod 1. Male specimen 11.1 mm in length. Corresponding to the original description by da Silva Brum (1975) with the additions and modifications given below.

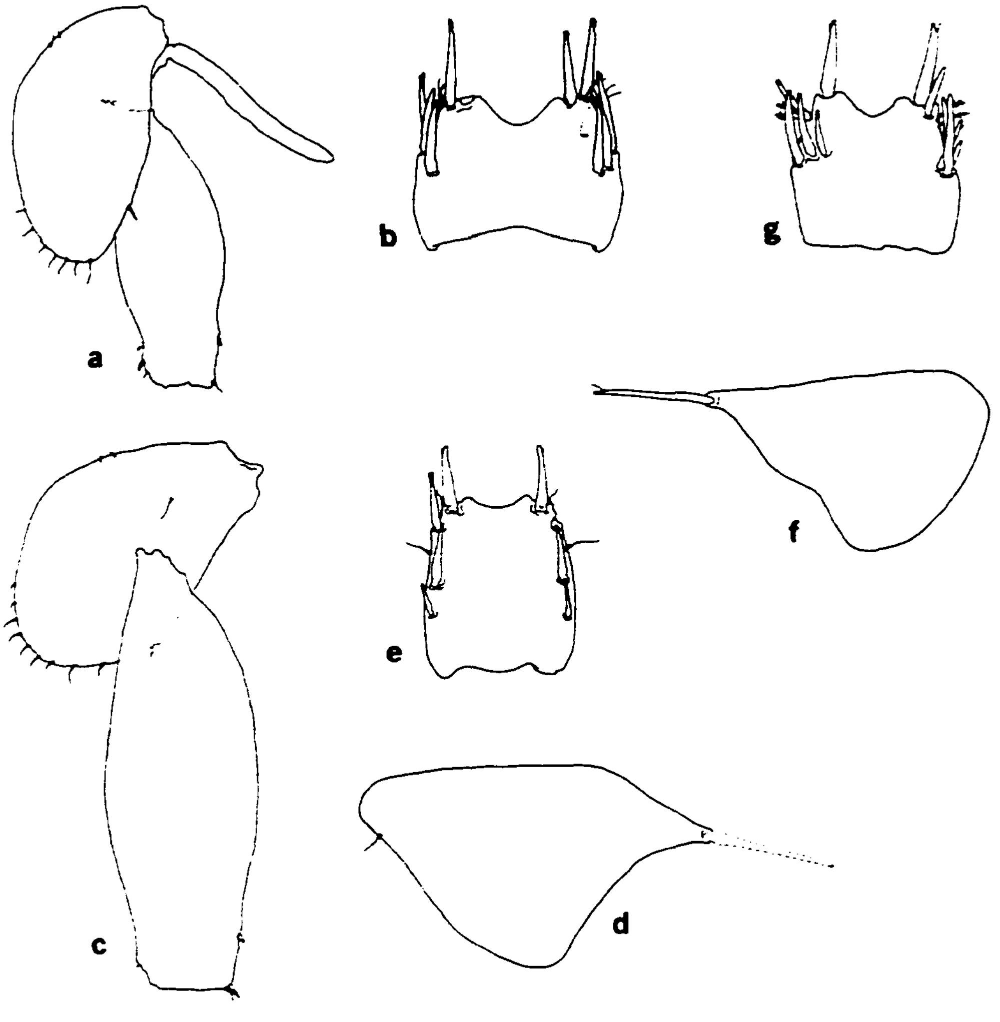


Fig. 3.8. *Spelaeogammarus trajanoae* n. sp., paratype female (10.36 mm): a) left pereopod 3. b) holotype female: telson; *S. santanensis* n. sp., holotype male: c) right pereopod 3, d) coxal plate of pereopod 7, e) telson; *S. bahiensis*, paratype (8 mm juvenile): f) coxal plate of pereopod 7, g) telson.

Redescription: Antenna 1: Peduncular segment 1-3 gradually decreasing in length; peduncular segment 1 with 6 ventral spines. Aesthetascs on most segments of primary flagellum (as long as segments); accessory flagellum with 4 segments, terminal segment vestigial.

Antenna 2: Peduncular segment 3 with 3-4 spines. Flagellum with 7 segments.

Mandible: Left lacinia mobilis distinctly toothed. Long and short plumose spines between lacinia and molar more dissimilar than in other species (Fig. 3.6h).

Maxilla 1: Palp without lateral setules. Outer lobe with 7 apical spines (3 different types) and distinct row of setules at medial margin, subapically.

Maxilliped: Dactyl bearing 3-4 relatively long setae along inner margin. Inner plate bearing apically 2 bifid (y-shaped) spines; outer plate with 3 blade-like spines subapically and 2 strong setae apically (Fig. 3.6d).

Gnathopod 1: Posterior margin of basis with 7-9 long setae (all singly inserted); anterior margin bearing 3-5 short setae. Carpus with rectangular posterior lobe, bearing 3-5 long, sickle-shaped, naked setae, 3-6 comb-like setae, and 3-4 long, naked setae. Palmar margin of propodus bearing 5-9 normal spines and 13-18 short bifid spines on the lateral margin; medial margin with 8-10 short setae, 1 normal angular spine, and 3-5 corner spines (1-2 relatively long). Dactyl with 9-10 denticles, each with 1 short seta.

Gnathopod 2 basically like that of *S. spinilacertus*. Palm with 2 normal spines and 4 spines at corner. Dactyl with 6-7 denticles.

Pereopods 3 and 4 subequal. Pereopod 3 basis with 1 distoanterior spine, and 1 distoposterior seta. Pereopod 4 basis with 1-2 distoanterior spines and 1 seta plus 1 spine at distoposterior margin; dactyl with 1 spine similar to *S. santanensis*.

Pereopods 5-7 missing in specimens examined.

Pleopods basically like those of *S. spinilacertus*. Segment 1 bearing 7-8 lateral plumose setae and 5-6 medial plumose setae. Inner ramus with 7 plumose setae on medial margin.

Coxal plates 3-4 with 9-10 setae and 1 spine; plates 5-6 identical, bearing 20-21 setae and 1 spine, respectively; plate 7 (Fig. 3.8f) irregular, slightly lobed.

Uropod 1: Peduncle with 4 spines on dorsolateral, dorsomedial, and ventrolateral margins, respectively, and 2 on apical margin. Outer ramus with up to 5 apical spines. All spines on inner ramus singly inserted.

Uropod 2: Peduncle bearing 1-2 dorsomedial, 1-2 dorsolateral, and 2 apical spines.

Uropod 3 missing in specimens examined.

Telson (Fig. 3.8g) width about 80% of length; each lobe bearing 2 setae, 2 apical and 3-4 subapical spines.

Discussion

The four species described above are recorded from eight caves in a series of discontinuous karst areas that extend over a linear distance of ca. 1200 km from north to south in eastern Brazil (Fig. 9). The records for each species per karst area are as follows: *S. santanensis* - 1 cave, *S. bahiensis* - 1 cave, *S. spinilacertus* - 2 caves, and *S. trajanoae* - 4 caves. Each species is obviously restricted to one or more caves in a separate karst area. These areas are disjunct and apparently physically isolated from each other.

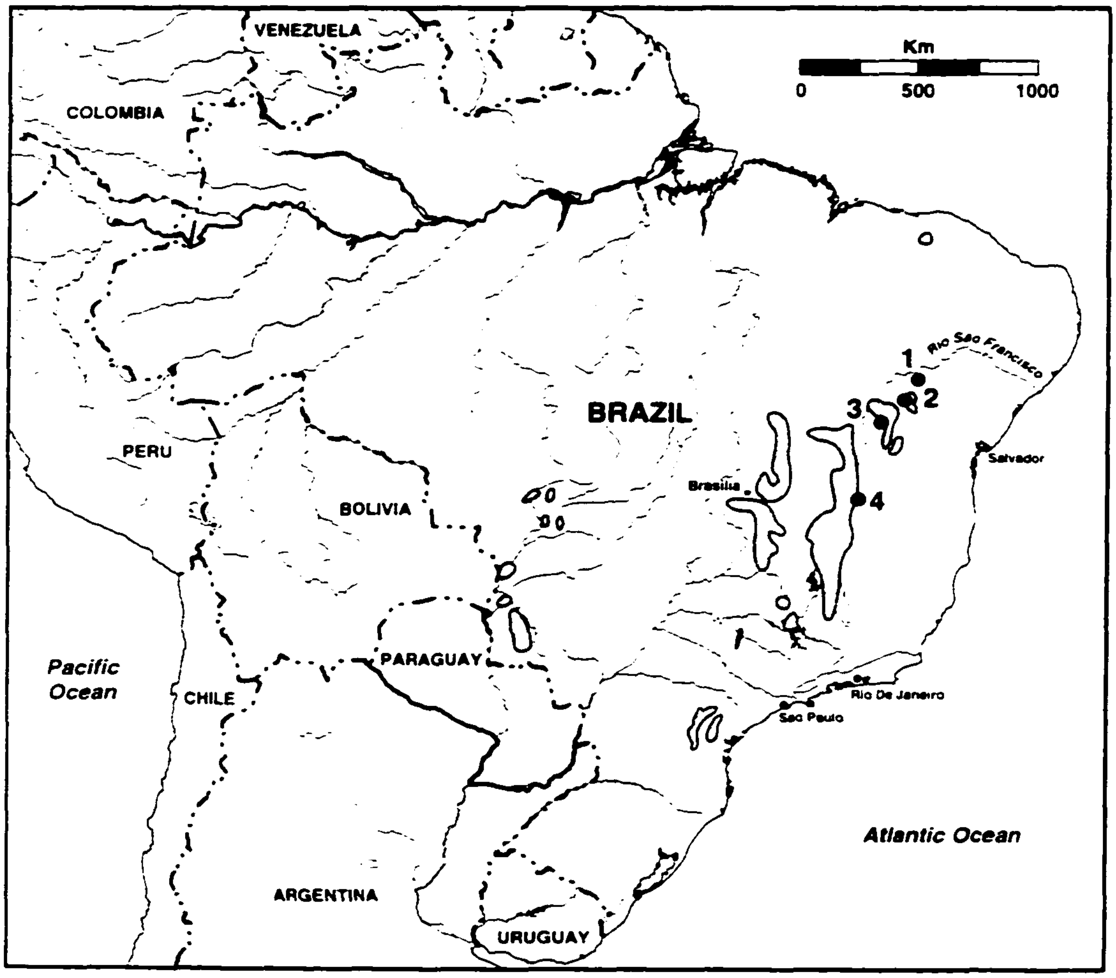


Fig. 3.9. Distribution of species of *Spelaeogammarus* in eastern Brazil: 1) *S. bahiensis* (1 cave); 2) *S. trajanoae* (4 caves); 3) *S. spinilacertus* (2 caves); 4) *S. santanensis* (1 cave). Shading indicates principal cave and/or karst areas. Map adapted from Trajano & Sanchez (1994).

The greatest distance between caves with two species is roughly 650 km (*S. bahiensis* and *S. santanensis*), whereas the shortest distance is only about 135 km (*S. bahiensis* and *S. spinilacertus*). Caves situated within a single, continuous karst area that are inhabited by the same species are never more than ca. 100 km apart.

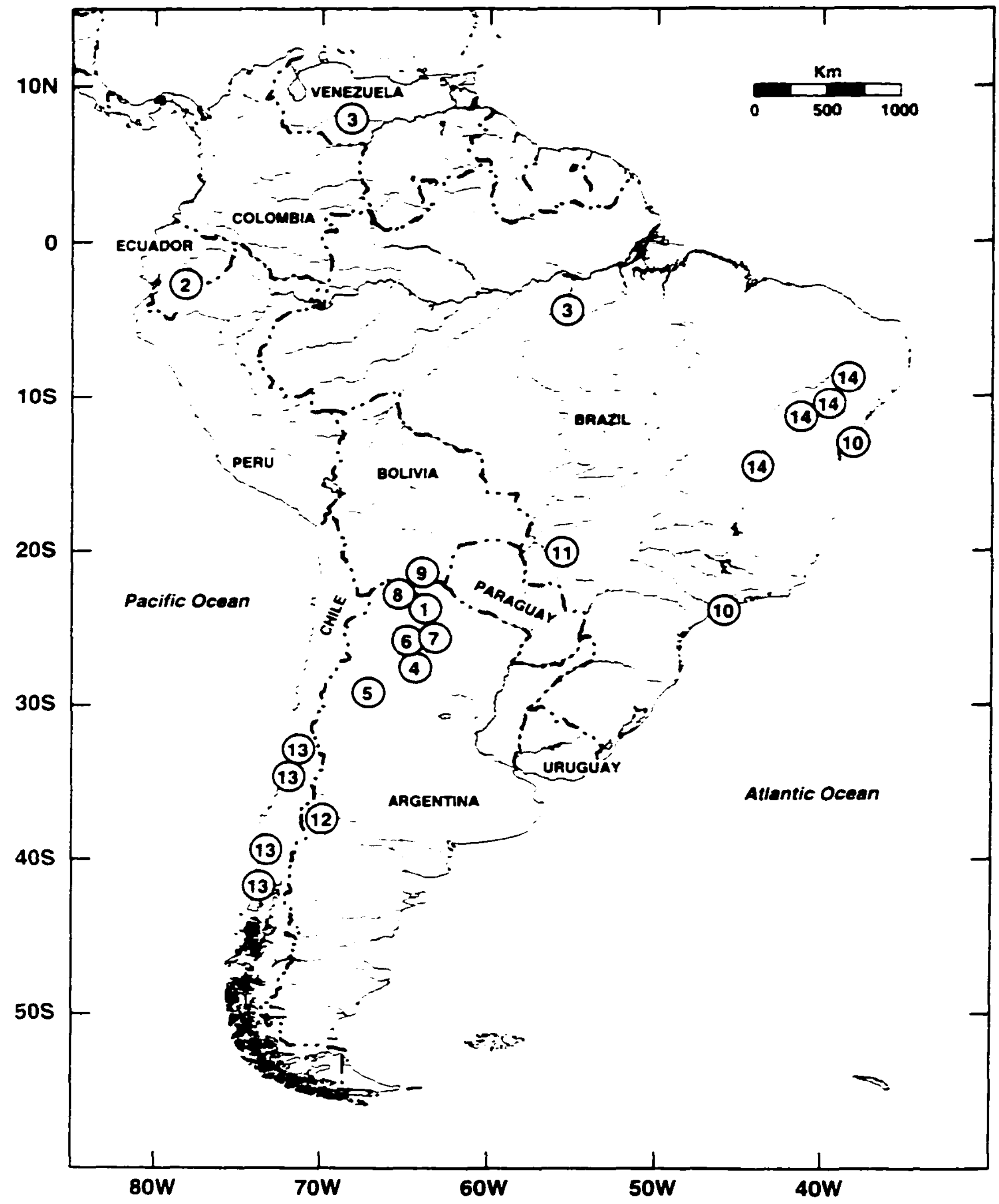


Fig. 3.10. Distribution of bogidiellid genera in continental South America: 1) *Bogidiella cooki* Grosso & Ringuelet, 1979; 2) *B. gammariformis* Sket, 1985; 3) *B. neotropica* Ruffo, 1952; 4) *B.* (*Dycticogidiella*) *ringueleti* Grosso & Fernandez, 1988; 5) *B.* (*Dyct.*) *talampayensis* Grosso & Claps, 1985; 6) *B.* (*Mesochthongidiella*) *tucumanensis* Grosso & Fernandez, 1985; 7) *B.* (*Stygogidiella*) *hormocollensis* Grosso & Fernandez, 1988; 8) *B.* (*Styg.*) *lavillai* Grosso & Claps, 1984; 9) *Eobogidiella purmamarcensis* Karaman, 1982; 10) *Marigidiella brasiliensis* Stock, 1981; 11) *Megagidiella azul* (Koenemann & Holsinger, 1999); 12) *Patagongidiella danieli* Grosso & Fernandez, 1993 and *P. mauryi* Grosso & Fernandez, 1993 (sympatric species); 13) *Pseudingolfiella chilensis* Noodt, 1965; 14) *Spelaeogammarus bahiensis* da Silva Brum, 1975, *S. santanensis* n. sp., *S. spinilacertus* n. sp., and *S. trajanoae* n. sp.

Both the elongate coxal plates and aequiramus pleopods found in the genus *Spelaeogammarus* are characters usually considered plesiomorphic for the family Bogidiellidae (Stock, 1981; Barnard & Barnard, 1983). Apart from *Spelaeogammarus*, coxal plates that are longer than wide are known only for the genus *Artesia* Holsinger, 1980 (*in* Holsinger & Longley, 1980), and this genus, like *Spelaeogammarus*, also has pleopods with aequiramus inner rami. However, they are 5-segmented in *Artesia* and only 1-segmented in *Spelaeogammarus*. *Artesia* can also be distinguished from *Spelaeogammarus* by 1-segmented accessory flagellum, fewer flagellar segments in both antennae, 1-segmented palp of maxilla 2, 6-segmented pleopodal exopodite, unlobed coxal plates 5 and 6, and the telson, which is deeply cleft and bears 4-6 apical spines on each lobe.

Equally long rami are also found in *Aequigidiella* Botosaneanu & Stock, 1989, *Kerguelenicola* Ruffo, 1974, and *Parabogidiella* Holsinger, 1980 (*in:* Holsinger & Longley, 1980). However, in these three genera the coxal plates are typically wider than long. Moreover, *Aequigidiella* differs from *Spelaeogammarus* by sexually dimorphic inner rami and spines of the second uropods of the male and a telson that is much longer than wide. Although to date only one specimen of the genus *Kerguelenicola* is known, there are several characters that distinguish it from *Spelaeogammarus*: 1-segmented accessory flagellum, distinct shape of and lack of armature on the telson, large mandibular molar, and reduced number of spines and setae on the outer and inner lobes of maxilla 1. *Parabogidiella* differs from *Spelaeogammarus* by 1-segmented accessory flagellum, 5-segmented flagellum of antenna 2, 1-segmented palp of maxilla 2, characteristically elongated pereopod 7, 5 pairs of coxal gills, and armature of the telson.

The most closely related bogidiellid taxon to *Spelaeogammarus* described to date may be *Bogidiella gammariformis* Sket (1985) from a cave in Ecuador. This species features some interesting characters that might be interpreted as intermediate states between the relatively primitive *Spelaeogammarus* and the more derived *Bogidiella* s. str. For example, *B. gammariformis* has enlarged, bilobed coxal plates 5 and 6, which are longer than wide, therefore showing a strong resemblance to the coxae of *Spelaeogammarus*. The inner rami of the pleopods of *B. gammariformis* are also 1-segmented, but show the same reduction as in most other species of *Bogidiella* s. str. (i.e., shorter than segment 1 of the outer ramus).

The concentration of the four morphologically closely similar species of *Spelaeogammarus* in a series of disjunct caves is unique for South America (see Fig. 3.10). Particularly interesting are the relatively subtle morphological differences between the four species that, in turn, appear to be correlated with the interspecific spatial distance as well as the abundance of species per area: *S. spinilacertus* and *S. trajanoae* occur in the central part of the range of *Spelaeogammarus* and show the highest morphological resemblance. In contrast are the more obvious differences between the relatively large *S. santanensis* and the smaller *S. bahiensis*, which occur on opposite ends of the generic range.

In South America there is a second concentration of species in northern Argentina, which occur exclusively in hyporheic habitats along the Rio Grande (see Fig. 3.10). This cluster is also strictly endemic but it has a higher generic diversity, with six species in two genera and three subgenera, possibly reflecting the time of divergence from a very old freshwater precursor and the subsequent radiation into a region of isolated inland habitats. However, the distribution pattern of *Spelaeogammarus* seems to indicate quite a different historical scenario, inasmuch as the species of this genus show an exceptionally close morphological relationship with each other when compared with all other South American bogidiellids. If we assume, for the sake of argument, the same evolutionary rate for all South American bogidiellids, the cluster of species belonging to *Spelaeogammarus* appears to have originated from a common ancestor far more recently as opposed to other bogidiellids in South American freshwater habitats.

The morphological appearance of the four species as well as their distribution over a relatively wide range of disjunct karst 'islands' characterize *Spelaeogammarus* as a distinct genus within the family Bogidiellidae. The apparent isolation of these species may well reflect a sequence of allopatric (geographic) speciation events over a relatively short period of time.

Summary

Three new subterranean amphipods of the genus *Spelaeogammarus* da Silva Brum, 1975, are described from eastern Brazil, bringing the total number of species in the genus to four. Based on the examination of type-material of *Spelaeogammarus bahiensis*, a comparative diagnosis of all four species of *Spelaeogammarus*, including the new species *Spelaeogammarus spinilacertus*, *Spelaeogammarus trajanoae*, and *Spelaeogammarus santanensis*, is given and the genus is partly redescribed. The occurrence of these species in caves that are separated from each other in discontinuous karst areas is biogeographically significant for the family Bogidiellidae in continental South America.

CHAPTER IV

PHYLOGENETIC ANALYSIS OF THE AMPHIPOD CRUSTACEAN FAMILY BOGIDIELLIDAE, S. LAT., AND REVISION OF TAXA ABOVE THE SPECIES LEVEL

Introduction

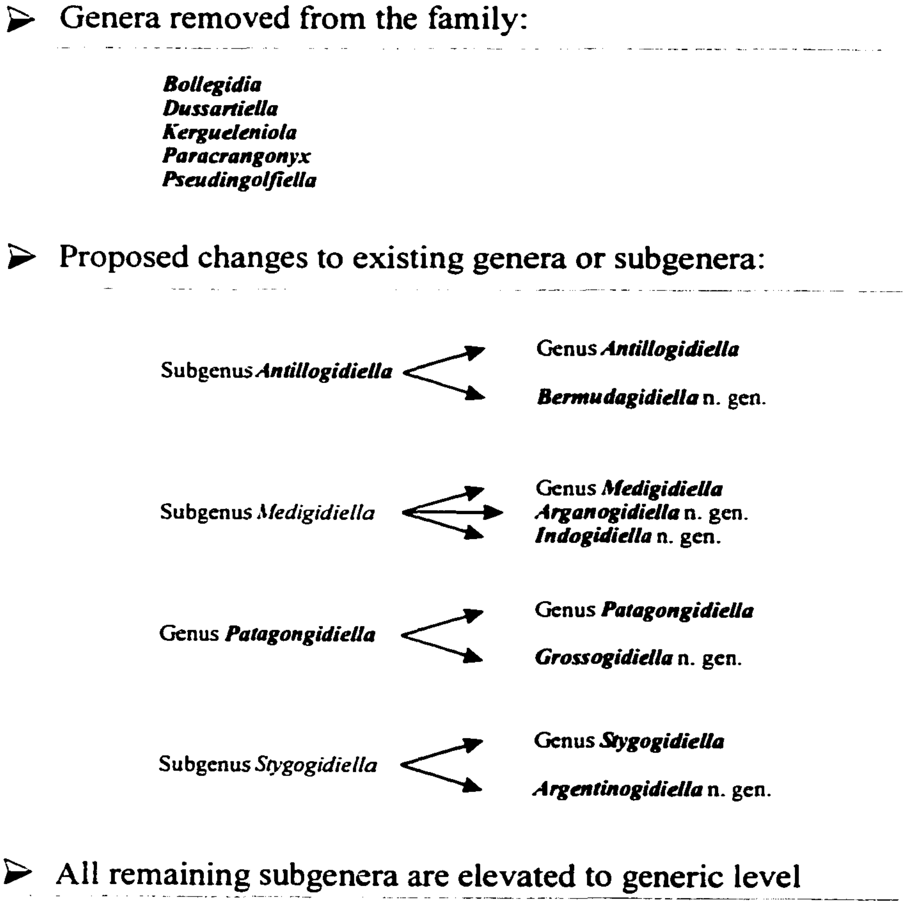
Close to 12% of the approximately 7,000 described species in the order Amphipoda inhabit subterranean groundwater environments. The vast majority of these species is distributed among 12 families, some of which are composed exclusively of hypogean species, whereas others comprise both epigean and hypogean representatives (see Holsinger, 1993, 1994). All known species of the family Bogidiellidae Hertzog, 1936, are true stygobionts. Bogidiellids are relatively small amphipods, with body lengths generally between 1 and 5 mm. They have a worldwide distribution pattern, occurring mostly in freshwater but also in brackish and marine environments. Bogidiellid habitats include caves, wells, cold mountain springs up to 2,500 m altitude, and the interstitial groundwater associated with river banks, lakes, and beaches.

The first comprehensive revision of the family was published by Ruffo (1973). Bousfield (1977), recognizing the morphological divergence within the family, introduced the concept of a superfamily Bogidielloidea, which he divided into two groups: the family Bogidiellidae and the family group of *Pseudocrangonyx-Paracrangonyx*, including the genera *Paracrangonyx*, *Procrangonyx*, *Pseudocrangonyx*, and *Sternophysinx*. Subsequent comprehensive revisions of Bogidiellidae s. lat. were made by Stock (1981) and Karaman (1981, 1982). Since then a substantial number of new, worldwide discoveries of bogidiellid amphipods has led to the introduction of taxa at both the generic and subgeneric levels. Naturally, the supplementary discoveries and records of bogidiellid amphipods help us to understand the evolutionary history of this enigmatic group. However, the addition of new taxa created some undesirable side effects, especially by increasing the uneven quality of generic diagnoses. Moreover, the familial diagnosis -- which was never very solid anyway -- gradually changed into a list of exceptions. For every diagnostic character there is at least one species that is an exception to the rule. Even the last diagnostic stronghold that defines bogidiellids as "exclusively blind stygobionts" had to be changed recently: *Bogidomma australis* Bradbury & Williams, 1996, from a cave in western Australia, despite being a perfectly typical bogidiellid, has large eyes!

Our basic criterion for a taxonomic reorganization of the Bogidiellidae s. lat. was the evaluation and comparison of significant morphological structures, hereafter referred to as diagnostic characters. In order to obtain a supplementary source of information for the taxonomic revision and also to clear up some of the unresolved questions about the evolutionary history of bogidiellid amphipods, a phylogenetic analysis was performed on the genera and subgenera of the family.

Taxonomic part

The present taxonomic composition of the family Bogidiellidae s. lat., is rather confusing. To date, the family is composed of 23 genera, 11 subgenera (all within the genus *Bogidiella*), and a total of 110 described species. In the following subsections, we will discuss several problematic aspects of bogidiellid taxonomy and propose our solution for each individual case (see Appendix A and Fig. 4.1 for overview). We believe that our proposed revision results in the recognition of a monophyletic group, therefore, eliminating the need for a superfamily Bogidielloidea. The revised family Bogidiellidae can be differentiated from all other gammaridean amphipods by a refined familial diagnosis: uropods 1-3 with 1-segmented rami of equal or near equal length, reduced pleopodal rami, and a distinct carpal lobe of gnathopod 1 form a unique combination of diagnostic characters that distinguishes all bogidiellids known to science.



➢ Genera removed from the family:

Bollegidia
Dussartiella
Kergueleniola
Paracrangonyx
Pseudingolfiella

➢ Proposed changes to existing genera or subgenera:

Subgenus ***Antillogidiella*** → Genus ***Antillogidiella***; ***Bermudagidiella*** n. gen.

Subgenus *Medigidiella* → Genus ***Medigidiella***; ***Arganogidiella*** n. gen.; ***Indogidiella*** n. gen.

Genus ***Patagongidiella*** → Genus ***Patagongidiella***; ***Grossogidiella*** n. gen.

Subgenus *Stygogidiella* → Genus ***Stygogidiella***; ***Argentinogidiella*** n. gen.

➢ All remaining subgenera are elevated to generic level

Fig. 4.1. Proposed changes to the family Bogidiellidae s. lat.

The taxonomic value of subgenera

The introduction of subgenera was made by Stock (1981) in his work on bogidiellid taxonomy and zoogeography. Stock established subgenera for several taxa that were defined by secondary sexually dimorphic characters only. He argued that "in cases in which only one of the sexes is known, the animals can at least be attributed to the 'mother genus', *Bogidiella*, without reference to a subgeneric name" (Stock, 1981: 348). In other words, as long as we have specimens of one sex only, we cannot be certain about the sexually dimorphic nature of the species, therefore, assign it to a separate subgenus within *Bogidiella*. Rather than being a helpful facilitation, this practice is disadvantageous for two reasons: (1) In case the missing sex is found, we may or may not have a sexually dimorphic species, but we unquestionably have a species with modified appendages. The genus *Bogidiella* s. str. has unmodified appendages, consequently, the designation of separate taxa for species with modifications is certainly justified. From this standpoint, a distinction between genus and subgenus becomes irrelevant. (2) The introduction of new taxa during the last two decades has obscured a clear, well-defined differentiation between genera and subgenera. The diagnostic character 'sexually dimorphic appendages' is not exclusively used for the subgenera but also became a principal feature for some of the genera, for example, *Aequigidiella* Botosaneanu & Stock, 1989, *Actogidiella* Stock, 1981, and *Marinobogidiella* Karaman, 1982. We propose to elevate all subgenera to generic level in order to rid the family of these inconsistencies.

The genus Bogidiella Hertzog, 1933

Type species by monotypy: *Bogidiella albertimagni* Hertzog, 1933.

The elevation of all subgenera to generic level entails several changes for the genus *Bogidiella*: (1) A subgenus *Bogidiella* is no longer consistent. All species in the subgenus *Bogidiella* remain in the genus *Bogidiella*. (2) The generic diagnosis of the subgenus *Bogidiella* becomes the valid diagnosis of the genus *Bogidiella* (i.e., pleopods and uropods without marked sexually dimorphic modifications).

We removed the following species from the genus *Stygogidiella* and assigned it to the genus *Bogidiella*, because the original description is apparently based on one immature female and, to our knowledge, there are no males reported or described:

Bogidiella cerberus Bou & Ruffo, 1979

Bogidiella cerberus Bou & Ruffo, 1979: 303, figs. IV-VI; type locality: Alepotrypa Cave, Peloponnesus (Greece).

Bogidiella (Stygogidiella?) cerberus, Stock, 1981: 354.

Bogidiella (Bogidiella) cerberus, Karaman, 1981: 31.

Bogidiella (Stygogidiella) cerberus, Karaman, 1982: 49.

With 38 species, *Bogidiella* is by far the largest of the bogidiellid genera. Despite the relatively high morphological variation among these species, no successful attempts have been made to separate them taxonomically (see Karaman, 1982). Apparently, the morphological divergences are not distinct enough to justify the designation of new genera. In order to give a clearer profile of *Bogidiella*, we split the genus into 4 different groups (see Appendix A). We used the status of the inner rami of the pleopods to distinguish the *albertimagni* group (A) (rami absent) from the *skopljensis* group (B) (rami present). The great majority of both groups A and B are species from southern Europe. The *niphargoides* group (C) is composed of 4 species with one or several peculiar characters, e. g., telson much longer than wide, antenna 1 with 17 or more segments, mouthparts with conspicuous modifications, etc. In group C, the inner rami of the pleopods show different states of reduction (all reduced; all absent or vestigial on pleopods 1and 2; absent on pleopod 3). We decided to place all species of which only one sex is reported or of which the sex is unknown in the *lindbergi* group (D). We separated these species mainly because their insecure taxonomic status would have biased the assessment of robust characters for the cladistic analysis (see Taxa).

Incompatible taxa

A thorough comparison of bogidiellid genera and subgenera reveals several taxa with highly reduced or modified morphological structures, indicating a distinct departure from the familial diagnosis. In our opinion, sternal gills, uniramous uropods, 2-segmented rami of the third uropods, absent or multi-segmented outer rami on pleopods 1-3, and a deeply cleft telson are examples of characters that are incompatible with our refined familial diagnosis. The development of these characters most likely followed an extensive pattern of progressive evolutionary steps, forming a sharp contrast to the overall conservative morphological evolution of bogidiellid amphipods. If bogidiellids are indeed an ancient group as some workers have suggested (see Stock, 1981), their conservative morphology, which is reflected by the absence of complex modifications, particularly implies their monophyly. We do not regard these characters as apomorphies for the bogidiellids because it is more probable that unrelated groups developed similar troglomorphic structures as a result of their adaptation to subterranean environments, for example, vermiform bodies and reduced coxal plates. Substantial morphological differences can be found in five genera only, some of which are formed by a combination of two or more atypical characters. In an attempt to establish the bogidiellids as a bona fide monophyletic group, we propose to remove the following genera from the family. Clarification of their taxonomic status and familial assignment is open for further study.

Genus *Bollegidia* Ruffo, 1974

Bollegidia Ruffo, 1974a: 405.

Type species: *Bollegidia capensis* Ruffo, 1974.

Bollegidia capensis Ruffo, 1974a: 405, figs. III-V; type locality: Blaauwberg Beach, Table Bay, Cape Town (South Africa); known only from type locality.

Second species: *Bollegidia sootai* (Coineau & Rao, 1972).

Bogidiella sootai Coineau & Rao, 1972: 85-92, figs. 11-14; type locality: Andaman Islands, Gulf of Bengal (India).

Bollegidia sootai, Ruffo, 1974a: 411; 1994: 365, fig. 4j; locality: Sabang Beach, eastern Mindoro (Philippines); also reported from Malaysia (Ruffo, 1985).

Remarks: The unusual combination of a uniramous uropod 1 and extremely reduced rami of pleopods 1-3 is not diagnostic for bogidiellids according to our concept. Excluding *Bollegidia*, bogidiellids have a biramous uropod !. The only bogidiellids with a reduced inner ramus of uropod 1 occur in the subgenus *Guagidiella* Stock, 1981. However, this modified ramus (shortened and fused with the peduncle, 1 apical spine extremely modified) is a sexually dimorphic character. It presumably is a male modification that facilitates sperm transfer. In *Bollegidia*, uniramous rami are reported for both sexes and, therefore, cannot be related to the reduction in *Guagidiella*.

Similarly, the extremely reduced pleopods of *Bollegidia* are a peculiar phenomenon, even for the family Bogidiellidae that is characterized by a trend towards a decreased number of segments of the pleopodal rami. This reduction is sexually dimorphic for the genus *Bollegidia*: the female outer rami of pleopods 1 and 2 are completely absent, and on pleopod 3 the outer ramus is reduced to a 1-segmented bud, whereas the male has a 2-segmented outer ramus on pleopod 1, a 1- or 2-segmented outer ramus on pleopod 2, and a 1-segmented outer ramus on pleopod 3. A comparable reduction of pleopodal rami segments can only be found in the genera *Pseudingolfiella* Noodt, 1965, and *Kergueleniola* Ruffo, 1974 (cf. Ruffo, 1974b), both of which we also consider incompatible with the familial diagnosis.

There are two additional, but minor morphological divergences of *Bollegidia* that do not occur in any other bogidiellid: brood plates attached to pereopods 3-4 (usually on pereopods 2-5) and a weakly lobed carpus on gnathopod 1, bearing one strong spine with several short marginal setules.

Genus *Dussartiella* Ruffo, 1979

Dussartiella Ruffo, 1979: 429.

Type species: *Dussartiella madegassa* Ruffo, 1979

Dussartiella madegassa Ruffo, 1979: 431, figs. VI-VII; type locality: spring near artificial lake Mantasoa, Manjakandriana (Madagascar); known only from type locality.

Remarks: A greatly reduced, scale-like inner ramus next to a 2-segmented outer ramus on uropod 3 and outer rami of pleopods 1-3 with 11, 8, and 5 segments, respectively, clearly mark *Dussartiella* as an atypical bogidiellid. Both characters are not unique for the family. Remarkably, however, they both occur in one of the other incompatible taxa: *Paracrangonyx* Stebbing, 1899, has an identically structured uropod 3, also combined with a set of 11-, 6-, and 3-segmented outer rami on pleopods 1-3. Pleopodal outer rami with more than (usually) 3 segments are very uncommon for bogidiellids. They are found in three taxa only: 6-segmented outer rami in *Artesia* Holsinger, 1980 and 3- to 5-segmented outer rami in both *Aurobogidiella* Karaman, 1988 (cf. Karaman, 1988c) and *Patagongidiella* Grosso & Fernández, 1993.

Minor incompatible characters are a 2-segmented, naked mandibular palp; maxilla 1 with an extremely asymmetrical palp (vestigial, 1-segmented on left and strong, 2-segmented palp on right maxilla 1) and an outer plate with 9 apical spines (usually 7 in other bogidiellids); and the absence of a carpal lobe on gnathopod 1.

Genus Kergueleniola Ruffo, 1970

Kerguelenella Ruffo, 1970b: 45.

Kergueleniola Ruffo, 1974b: 507 (emend.).

Kerguelenicola, Stock, 1981: 355 (*lapsus calami*).

Type species: *Kergueleniola macra* Ruffo, 1970

Kerguelenella macra Ruffo, 1970b: 45, figs. I-III; type locality: Kerguelen Island; known only from type locality.

Kergueleniola macra Ruffo, 1974b: 507 (emend.).

Remarks: Sex unknown; only known specimen found in the stomach of a freshwater trout. *Kergueleniola* departs significantly from the bogidiellid familial diagnosis by the following unique characters: deeply cleft telson without spines; 1-segmented outer rami and aequiramus, 1-segmented inner rami on pleopods 1-3; carpus of gnathopod 1 without distal lobe; segment 3 of mandiblular palp with row of subapical setae (C-setae).

Furthermore, there are several minor incompatible characters, for example, the unusually shaped palp of the maxilliped, the long, rounded epimeral plates, and the armature and shape of the mandibles and uropods.

Genus Paracrangonyx Stebbing, 1899

Paracrangonyx Stebbing, 1899: 422.

Type species: *Paracrangonyx compactus* (Chilton, 1882).

Crangonyx compactus Chilton, 1882: 177, pl. 10, figs. 13-19; type locality: well at Eyreton, North Canterbury (New Zealand); also reported from several other localities in New Zealand (Chilton, 1894; Karaman, 1981).

Paracrangonyx compactus Stebbing, 1899: 422.

Material examined: 1 female (broken) and 1 male (USNM 21283), both from type locality; 1 female (USNM 22810), partly dissolved, head missing, also from type locality.

Remarks: *Paracrangonyx* can be unmistakably distinguished from all bogidiellids by the following characters: head with rudimentary eye, consisting of ± 3 unpigmented cells; carpus of gnathopod

1 without lobe; mediosternal gills on pereonites 2-7; outer rami on pleopods 1-3 with 11, 6, and 3 segments, respectively (compare with *Dussartiella*); peduncles of pleopods 2-3 with several marginal spines; epimeral plates with row of strong spines along ventral margins; uropod 3 with vestigial inner ramus and 2-segmented outer ramus (compare with *Dussartiella*).

Additional, lesser divergence from the bogidiellid diagnosis includes a very long mandibular palp with an unusually high number of setae and an exceptionally asymmetrical lacinia mobilis.

Genus *Pseudingolfiella* Noodt, 1965

Pseudingolfiella Noodt, 1965: 27, fig. 1B.

Type species: *Pseudingolfiella chilensis* (Noodt, 1959).

Ingolfiella chilensis Noodt, 1959: 200, figs. 1-18; type locality: Quebrada de Córdoba, El Tabo, near San Antonio (Chile); also reported from several other localities in Chile (Noodt, 1965; Karaman, 1981).

Pseudingolfiella chilensis, Noodt 1965: 28, fig 1B.

Second species: *Pseudingolfiella soyeri* Coineau, 1977.

Pseudingolfiella soyeri Coineau, 1977: 288, figs. 1-4; type locality: Kerguelen Island.

Remarks: The following characters are the most striking morphological differences in *Pseudingolfiella*: uniramous uropod 3, with 2-segmented (or bipartite) outer ramus; uropods 1 and 2 sexually dimorphic, with distinctly modified rami in both sexes, inner ramus of uropod 2 vestigial in female (unknown in *P. chilensis*); pleopods 1-3 uniramous, eminently reduced, consisting of a single, naked stump (plp. 1-2) or an additional, vestigial segment, bearing a single seta (plp. 3), pleopod 3 sexually dimorphic (unknown in *P. chilensis*); carpus of gnathopod 1 without distal lobe.

New taxa

Another taxonomic problem of the family Bogidiellidae are taxa that contain morphologically incompatible species or species groups. This is especially the case with several of the subgenera. As pointed out above, the primary designation of these taxa is based on sexual dimorphism only. The subgenus *Medigidiella* Stock, 1981, for example, is defined by sexually dimorphic modifications of uropod 1 and/or 2, without a necessary discrimination between the two (see also Karaman, 1982 and Ruffo, 1994: 364). Furthermore, the occurrence of additional diagnostic characters has been more or less neglected (e. g., different states of reduction in pleopodal rami). With the addition of new species, the diagnostic definition of some of these taxa became increasingly unclear and blurry. We, therefore, propose to split the following subgenera and genera into more clearly defined taxa and, in doing so, elevate all subgenera concerned to generic level (see Fig. 4.1 for overview):

Proposed split of the subgenus *Antillogidiella* Stock, 1981

Genus *Antillogidiella* Stock, 1981, new status

Bogidiella (Antillogidiella) Stock, 1981: 354.

Type species by monotypy: *Bogidiella martini* Stock, 1978.

Bogidiella martini Stock, 1978: 104, figs. 1-30; type locality: Saint-Martin, Lesser Antilles.

Bogidiella (Antillogidiella) martini, Stock, 1981: 354.

Bogidiella (Bogidiella) martini (group A), Karaman 1981: 31.

Bogidiella (*Antillogidiella*) *martini*, Karaman 1982: 43.

Diagnosis: Sexual dimorphism in pleopod 2 and uropod 1: male with reduced, 1-segmented inner ramus on pleopods 1-3, segment 2 of male pleopod 2 bearing one modified spine; female pleopods without inner rami; female with dagger-shaped rami on uropod 1 (male uropod 1 normal); pars molaris strongly reduced to several small denticles, bearing one long seta; telson with 2 apical spines.

Bermudagidiella new genus

Type species by monotypy: *Bogidiella (Antillogidiella) bermudensis* Stock, Sket & Iliffe, 1987.

Bogidiella martini ssp. Sket & Iliffe, 1980: 876; type locality: anchialine caves, Bermuda.

Bogidiella (Antillogidiella.) martini ssp. Stock, 1981: 354.

Bogidiella (Antillogidiella.) bermudensis Stock, Sket & Iliffe, 1987: 55, figs. 1-16.

Diagnosis: Sexual dimorphism in pleopod 2: male with shortened segment 2 on outer ramus, bearing one modified spine; pleopods without inner rami in both sexes; female with dagger-shaped rami on uropod 1 (male uropods lacking); pars molaris strongly reduced to wide lobe with 4 spinules; telson with 2 apical and 2 subapical spines.

Etymology: The new generic name is a combination of the geographic locality and part of the generic name *Bogidiella*; the gender of the name is feminine.

Remarks: Several similar morphological reductions and modifications suggest a close relationship of *Antillogidiella* and *Bermudagidiella*. The designation of 2 distinct genera, however, is in general accordance with the diagnostic generic concept in the family. Moreover, the differentiation of 2 separate phylogenetic lines is strongly supported by the cladistic analysis.

Proposed split of the subgenus *Medigidiella* Stock, 1981

When Stock (1981) established the subgenus *Medigidiella*, he placed 5 named species into the new taxon and listed another 4 species as possibly belonging to *Medigidiella* as well. The only diagnostic character he used to define *Medigidiella* were sexually dimorphic uropods 1 and/or 2. In the last two decades, the number of medigidiellids has trebled. Accordingly, the quality of modifications in male uropods 1 and 2 has become more diverse and complex. Likewise did the occurrence of other potentially diagnostic characters. We propose to split the subgenus *Medigidiella* into the following genera:

Genus *Medigidiella* Stock, 1981, new status

Medigidiella Stock, 1981: 353.

Type species: *Bogidiella chappuisi* Ruffo, 1952.

Bogidiella chappuisi Ruffo, 1952 (in Ruffo & Delamare Deboutteville, 1952); type locality: Argelés, Pyrenees (France); reported from several localities in Southern Europe, Turkey, and North Africa.

Bogidiella (Medigidiella), chappuisi Stock, 1981: 353.

Species included: *Bogidiella (Medigidiella) antennata* Stock & Notenboom, 1988; *Bogidiella (Medigidiella) aquatica* Karaman, 1990a; *Bogidiella (Medigidiella) arista* Koenemann, Vonk & Schram, 1998; *Bogidiella (Medigidiella) dalmatina* (S. Karaman, 1953); *Bogidiella (Medigidiella) hebraea* (Ruffo, 1963); *Bogidiella (Medigidiella) minotaurus* (Ruffo & Schiecke, 1976); *Bogidiella (Medigidiella) paolii* Hovenkamp, Hovenkamp & Van der Heide, 1983; *Bogidiella (Medigidiella) paraichnusae* (Karaman, 1979); *Bogidiella (Medigidiella) uncinata* Stock & Notenboom, 1988.

Diagnosis: Sexual dimorphism in uropod 2: some male spines on uropod 2 ramus/rami modified; inner rami of pleopods 1-3 absent.

Arganogidiella new genus

Type species: *Bogidiella arganoi* Ruffo & Vigna Taglianti, 1973.

Bogidiella arganoi Ruffo & Vigna Taglianti, 1973: 115, figs. 8-9; type locality: well near Paraje Nuevo, Cordoba (Mexico).

Bogidiella (Medigidiella) arganoi, Stock, 1981: 354.

Bogidiella (Bogidiella) arganoi (group C), Karaman, 1981: 28.

Bogidiella (Guagidiella) arganoi, Karaman, 1982: 44.

Second species: *Bogidiella (Guagidiella) arganoides* Karaman, 1982

Bogidiella cfr. *arganoi*, Ruffo & Vigna Taglianti, 1977: 153, fig. 13; type locality: Etla, Oaxaca (Mexico).

Bogidiella (Guagidiella) arganoides Karaman, 1982: 44.

Diagnosis: Sexual dimorphism in uropod 1: some male spines on uropod 1 rami modified; inner rami on pleopods 1-3 absent.

Etymology: *Arganogidiella* is named in honor of Prof. Roberto Argano; the gender of the name is feminine.

.

Remarks: Karaman (1982) placed both *Arganogidiella arganoi* and *A. arganoides* in the subgenus *Guagidiella*. However, the modification of the male uropod 1 in both guagidiellid species is highly apomorphic as compared to the modified spines in *Arganogidiella* (see Incompatible taxa, genus *Bollegidia*). Moreover, *Guagidiella* can be distinguished from *Arganogidiella* by vestigial inner rami on pleopods 1 and 2 (absent in *Arganogidiella*), differently shaped, sexually dimorphic gnathopods, and a 3-segmented accessory flagellum on antenna 1 (2-segmented in *Guagidiella*).

Indogidiella **new genus**

Type species: *Bogidiella (Medigidiella) sarawacensis* Stock, 1983.

Bogidiella (Medigidiella) sarawacensis Stock, 1983: 198, figs. 1-26; type locality: caves in National parks in Sarawak (Borneo).

Second species: *Bogidiella dacordii* Ruffo, 1994.

Bogidiella dacordii Ruffo, 1994: 361, figs. 3-4; type locality: St. Paul, Palawan (Philippine Islands).

Diagnosis: Sexual dimorphism in uropods 1 and 2: some male spines on uropods 1 and 2 rami modified; pleopod 1-3 with 1-segmented, reduced inner rami.

Etymology: The new name combines the geographic distribution of the type species (East Indies) and part of the generic name *Bogidiella*; the gender of the name is feminine.

Proposed split of the subgenus *Stygogidiella* Stock, 1981

Genus *Stygogidiella* Stock, 1981, new status

Stygogidiella Stock, 1981: 354.

Type species: *Bogidiella bredini* Shoemaker, 1959.

Bogidiella bredini Shoemaker, 1959: 273, fig. 1; type locality: Dark Cave, Barbuda.

Bogidiella (Stygogidiella) bredini, Stock, 1981: 354, fig. 3.

Species included: *Bogidiella (Stygogidiella) atlantica* Sànchez, 1991; *Bogidiella (Stygogidiella) cypria* (Karaman, 1989) (cf. Karaman, 1989b); *Bogidiella (Stygogidiella) perla* Stock, 1981; *Bogidiella (Stygogidiella) purpuriae* Stock, 1988; *Bogidiella (Stygogidiella) uniramosa* Stock & Rondé-Broekhuizen, 1987; *Bogidiella (Stygogidiella) virginalis* Stock, 1981.

Diagnosis: Sexual dimorphism in pleopod 2: male with modified spine on 2nd segment of pleopod 2; telson wider than long or as wide as long.

Argentinogidiella **new genus**

Type species: *Bogidiella (Stygogidiella) hormocollensis* Grosso & Fernández, 1988.

Bogidiella (Stygogidiella) hormocollensis Grosso & Fernández, 1988: 65, figs. on pls. 1-2; type locality: Sierra de Medina, Tucamán (Argentina).

Second species: *Bogidiella (Stygogidiella) lavillai* Grosso & Claps, 1984

Bogidiella (Stygogidiella) lavillai Grosso & Claps, 1984: 224, figs. 1-32; type locality: Rio Grande near Jujuy (Argentina).

Diagnosis: Sexual dimorphism in pleopods 1 and 2 and uropod 2: male pleopod 2 bearing modified spine on 2nd segment; male pleopod 1 with initial stage of sexual dimorphism; male uropod 2 with reduced spines and reduced inner ramus; telson much longer than wide.

Etymology: *Argentinogidiella* refers to the geographic distribution of both species, combined with part of the generic name *Bogidiella*; the gender of the mane is feminine.

Remarks: Apparently, *A. hormocollensis* shows sexually dimorphic pleopods 1 and 3: the male has shortened setae on the 1st segment of pleopod 1 (normal in female) and a relatively stronger pleopod 3. The drawings of *A. lavillai* seem to indicate almost identical reductions. It would be interesting to re-examine the pleopods of *A. lavillai* in both sexes for similar structures.

Proposed split of the genus *Patagongidiella* Grosso & Fernández, 1993

Genus *Patagongidiella* Grosso & Fernández, 1993

Patagongidiella Grosso & Fernández, 1993: 340.

Type species: *Patagongidiella danieli* Grosso & Fernández, 1993.

Patagongidiella danieli Grosso & Fernández, 1993: 366, figs. on pl. IV; type locality: Del Arenal Cave, Nequén (Argentina); sympatric with *Grossogidiella mauryi*.

Diagnosis: Peduncle of male uropod 1 with highly modified spine; 2nd segment of male pleopod 2 with modified spine; large mediosternal processes on pereonites 2-5.

Grossogidiella new genus

Type species by monoitypy: *Patagongidiella mauryi* Grosso & Fernández, 1993.

Patagongidiella mauryi Grosso & Fernández, 1993: 362, figs. on pls. II-III; type locality: Del Arenal Cave, Nequén (Argentina).

Diagnosis: Appendages without sexual dimorphism; large mediosternal processes on pereonites 2-5.

Etymology: *Grossogidiella* is named in honor of Dr. Luis E. Grosso; the gender of the name is feminine.

Phylogenetic analysis

Cladistic methods

We used PAUP, version 3.0s, to perform a cladistic analysis on the bogidiellid genera and subgenera. Because our matrix contained a relatively high number of Operational Taxonomic Units (OTUs), each series of runs was started with the Heuristic Search option. For the initial run, only minimal trees were kept by random Stepwise Addition, the ancestral OTU included, TBR branch swapping, and the MULPARS option deactivated. For subsequent runs, we generally used all trees in memory from the preceding run, activating the MULPARS option and keeping all trees that were as short as or one step shorter than those loaded into memory. These steps were repeated until no shorter trees could be found. As a double-check, a second, complete Heuristic Search was performed on all shortest trees obtained from the previous Heuristic Search series. This search procedure was conducted for numerous runs with modified charactersets, i.e., unweighted and unordered versus partially weighted and ordered charactersets (for unordered and unweighted runs, the ancestral OTU was excluded from the search; see also Character assessment and choice of outgroup). From the resulting, most parsimonious trees, we calculated Strict Consensus trees. All consensus trees were finally evaluated and edited in MacClade ver. 3.0. The exclusion of doubtful or weak characters during test runs generally led to less resolved trees. For this analysis, the best results were obviously obtained using as many data available as possible.

References to taxa in the phylogenetic subsections, including Appendix B, are based on the unrevised family Bogidiellidae s. lat., and were used as follows: subgenus *Medigidiella* A = genus *Medigidiella*; subgenus *Medigidiella* B = new genus *Arganogidiella*; subgenus *Medigidiella* C = new genus *Indogidiella*; subgenus *Stygogidiella* A = genus *Stygogidiella*; subgenus *Stygogidiella* B = new genus *Argentinogidiella*. See New taxa for additional information on *Antillogidiella* and *Patagongidiella*.

Taxa

The cladistic analysis was performed on 23 genera and 10 subgenera assigned to the unrevised family Bogidiellidae s. lat. That way, we were able to directly compare the bogidiellid phylogeny with the taxonomic revision we propose for the family. Because we decided to split some of the genera, we ended up with 40 OTUs instead of 33 (Table IV.1). Basically, these splittings helped us to code characters for large, polytypic taxa like the subgenera *Bogidiella*, *Medigidiella*, and *Stygogidiella*, which contain morphologically diverse species. We also wanted to test the coherence of some of the genera and subgenera. For this reason in particular, we split the genera *Antillogidiella* and *Patagongidiella*. We arranged the species of the subgenus *Bogidiella* s. str. in 4 separate groups, of which we designated groups A-C as OTUs. We did not use group D for the analysis because of the uncertain taxonomic status of its species (see The genus *Bogidiella*).

Character assessment and choice of outgroup

With the exception of the genera *Megagidiella*, *Paracrangonyx*, and *Spelaeogammarus*, the choice of characters employed in the analyses is based on descriptions and drawings from the literature (see

Appendix B and Table IV.1). Unfortunately, the uneven quality of both species and generic descriptions obstructed or even prevented the use of several potentially contributive characters, for example, secondary sexual dimorphism in gnathopods and pereopods. The manifestation of secondary sexual dimorphism can be very subtle, with continuous interspecific changes. In several instances, assumptions about these traits could not be obtained, neither from drawings nor descriptions and, therefore, have been excluded from this analysis.

The same matrix was employed for two alternative analyses (Table IV.1): in the first run, we left all characters unweighted and unordered, so that a character has randomly reversible states (e. g., in character 24, state 5 (spines absent) can evolve directly from state 0 (4 or more spines)). For the second analysis, we ordered and weighted some of the characters differently, assuming a relatively complex evolution as opposed to more simple structures (e. g., the modification of spines and/or inner rami on male uropods have most likely evolved progressively, in linear transformation series from one state to the next, whereas the reduction of telsonic spines might occur as a spontaneous mutation within one generation). Analyses with partially weighted and ordered character sets will hereafter be referred to as 'alternative run', the unweighted and unordered run as 'default run'.

Autapomorphies, although undoubtedly important for the taxonomy of bogidiellid genera and subgenera, have also been excluded from the analyses because they are uninformative for the cladograms shown in Fig. 4.2. Distinct autapomorphies occurred as follows: sexually dimorphic pereopod 7 and uropod 3 in the subgenus *Orchestigidiella* Stock, 1981; heavily modified inner rami on pleopods 1 and 2 in the genus *Marinobogidiella* Karaman, 1982; mediosternal processes (gills?) on pereonites 2-7 in the genus *Paracrangonyx*; mandibular palp absent in the genus *Megagidiella* Koenemann & Holsinger, 1999; outer ramus of male pleopod 2 with lamellar expansions on segments 1 and/or 2 in the subgenus *Xystriogidiella* Stock, 1981; different minor sexually dimorphic reductions in pleopods 1-3 in the genera *Bollegidia* and *Pseudingolfiella*, and in the subgenus *Antillogidiella*.

In some of the genera and subgenera, the occurrence of a character showed a variable or transitional state, for example, the number of apical and subapical spines on the telson (Appendix B, characters 24 and 25). These variations were treated as separate morphological conditions, so that the varying occurrence of 2 to 3 telsonic spines is assumed to be the transitional evolutionary state between 2 and 3 spines in the alternative runs. Another problem we encountered was the pronounced degree of interspecific variation among polytypic taxa, for example, inner rami of pleopods 1-3 absent in species A, reduced in species B, and vestigial in species C and D. We used two different approaches for polytypic OTUs. For the first method, variable characters were coded as question marks. Alternatively, we coded the same characters according to the majority of their occurrences in all species of an affected taxon (see also Wiens, 1995, 1998). Because the basic difference of the resulting trees was the degree of polytomy, we chose the better resolved trees of the Majority Coding method as representative. A third solution to this problem may be obtained by an inferred groundplan character state (Exemplary Method). In this approach, the ancestral state of a polytypic OTU is determined by a partial cladistic analysis of some exemplary species (or higher taxa), investigating the variable character only (Yeates, 1995).

TABLE IV.1

Character matrix. Ancestor = outgroup. Shaded character states were subjected to different coding methods. See subsection Characters and Appendix B for a description of characters. Characters are numbered according to the list in Appendix B.

Ancestor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Actogidiella	0	1	1	5	1	0	1	0	1	0	0	0	2	0	2	2	0	2	2	0	1	1	1	4	6	0	2
Aequigidiella	0	0	0	0	0	2	0	0	0	0	0	0	1	2	1	2	0	4	2	0	0	1	0	2	2	0	2
Afridiella	0	0	1	3	0	0	0	0	0	0	0	0	2	0	0	1	0	2	2	0	1	0	2	4	3	0	2
Artesia	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	2	5	2	1	1	0	1	0	6	0	2
Aurobogidiella	?	?	0	3	?	?	1	1	?	?	0	0	1	0	1	2	0	0	2	0	0	1	2	4	6	0	2
Bogidiella A	0	0	1	5	0	0	0	0	0	0	0	0	2	1	1	1	0	1	1	0	0	1	■	3	5	0	2
Bogidiella B	0	0	1	3	0	0	0	0	0	0	0	0	2	0	1	1	0	1	2	0	0	1	1	4	5	0	2
Bogidiella C	0	0	1	■	0	0	0	0	0	0	0	0	2	0	1	2	0	0	2	0	0	1	■	4	■	0	2
Antillogidiella ber	0	1	1	5	?	?	1	0	?	?	0	0	2	0	1	2	0	2	2	0	1	1	2	4	4	0	2
Antillogidiella mar	0	1	1	■	0	0	1	0	0	0	0	0	2	0	1	2	0	2	2	0	1	1	2	4	6	0	2
Dycticogidiella	0	1	1	3	0	2	0	0	0	0	0	0	2	?	1	2	1	0	2	0	0	1	0	4	4	0	2
Guagidiella	0	0	1	■	2	0	0	0	0	0	0	0	2	0	2	2	0	2	2	0	1	1	2	4	4	0	2
Hagidiella	0	1	1	5	1	0	0	0	0	0	0	0	2	0	1	2	0	2	2	0	1	1	2	5	4	0	2
Medigidiella A	0	0	1	5	0	1	0	0	0	0	0	0	2	0	2	1	0	2	2	0	0	1	■	3	6	0	2
Medigidiella B	0	0	1	5	1	0	0	0	0	0	0	0	2	?	1	0	0	2	2	0	0	1	2	4	4	0	2
Medigidiella C	0	0	1	3	1	1	0	0	0	0	0	0	2	0	0	0	0	2	2	0	0	1	2	3	6	0	2
Mesochthongidiella	1	1	1	3	0	2	0	0	0	0	0	0	2	0	1	2	0	0	2	0	0	1	0	4	2	0	2
Mexigidiella	1	1	1	4	0	0	0	0	0	0	0	0	2	1	1	2	0	1	3	0	1	1	1	4	4	0	2
Orchestigidiella	0	0	1	4	1	0	0	0	0	0	0	0	2	0	2	2	0	2	2	0	0	1	2	3	4	0	2
Stygogidiella A	0	1	1	■	0	0	0	0	0	0	0	0	2	0	1	1	0	1	2	0	0	1	2	■	5	0	2
Stygogidiella B	1	1	1	3	0	1	0	0	0	0	0	0	2	?	1	2	1	2	2	0	0	1	0	4	3	0	2
Xystriogidiella	0	1	1	3	0	1	0	0	0	0	0	0	2	0	1	0	0	0	2	0	0	1	2	3	3	0	2
Bogidomma	?	?	1	5	?	?	0	0	?	?	0	0	2	0	1	2	0	5	2	1	1	1	0	2	6	0	0
Bollegidia	0	0	3	5	0	0	1	1	1	1	0	0	1	3	1	2	2	5	1	0	0	1	1	4	6	0	2
Cabogidiella	0	0	1	5	0	0	1	0	1	0	0	0	0	0	1	2	0	2	2	0	1	1	2	3	4	0	2
Dussartiella	0	0	0	3	0	0	?	?	1	1	1	1	0	?	0	2	1	3	0	0	0	1	0	4	6	0	2
Eobogidiella	?	?	1	3	?	?	0	0	?	?	0	0	?	?	1	2	2	0	2	0	0	1	2	4	1	0	2
Hebraegidiella	0	0	1	3	0	0	?	?	0	0	?	?	2	?	1	0	0	2	2	0	0	1	2	4	6	0	2
Kergueleniola	?	?	3	1	?	?	?	?	?	?	0	0	1	?	0	3	0	4	4	0	0	1	2	5	6	0	2
Maghrebidiella	?	?	1	4	?	?	0	0	?	?	0	0	2	0	1	2	0	5	2	0	1	1	2	2	2	0	2
Marigidiella	0	0	2	2	0	0	1	1	1	1	0	0	2	0	1	2	2	2	2	1	1	1	0	4	6	0	2
Marinobogidiella	0	0	1	2	0	0	?	?	1	1	0	0	1	?	1	2	2	0	2	1	0	1	2	4	6	0	2
Megagidiella	0	0	1	3	0	0	0	0	0	0	0	0	2	0	1	3	0	0	2	0	0	1	1	1	0	0	2
Nubigidiella	?	?	1	5	?	?	0	0	?	?	0	0	1	0	1	2	0	5	2	0	1	1	2	4	6	0	2
Patagongidiella mau.	0	1	0	2	2	0	0	0	0	0	0	0	2	?	0	0	0	0	2	0	0	1	0	2	4	1	2
Patagongidiella dan.	0	0	0	2	0	0	0	0	0	0	0	0	2	?	0	0	0	0	2	0	0	1	0	2	4	1	2
Parabogidiella	0	0	1	0	0	0	0	0	0	0	0	0	0	?	1	3	2	5	2	1	1	1	0	2	4	0	2
Paracrangonyx	0	0	0	5	0	0	0	0	0	0	1	1	0	0	2	2	0	2	2	0	1	1	0	4	6	0	1
Pseudingolfiella	0	0	3	5	0	0	1	1	1	1	2	1	0	4	2	2	0	5	2	1	1	1	0	4	6	0	2
Spelaeogammarus	0	0	1	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	3	1	0	2

Assumptions about morphological adaptations of amphipods to life in subterranean environments seem to have achieved a level of well-founded, broad consensus in the literature (Stock, 1981; Notenboom, 1991; Holsinger, 1993, 1994; Koenemann et al., 1998; see also Barnard & Barnard, 1983). Hence, we decided to treat morphological reductions attributed to hypogean adaptation as apomorphies. Accordingly, each corresponding plesiomorphic condition was coded as state 0. We think that the relatively frequent occurrences of homoplasies in stygobiont amphipods tend to impede the choice of effective outgroups and will most likely fail to produce a probable phylogenetic reconstruction of the family Bogidiellidae (see Koenemann et al., 1998). For this reason, we considered a hypothetical, 'allzero' ancestor the optimal choice for an outgroup.

Results

Because the great majority of the resulting consensus trees had the same basic structure, two exemplary trees are chosen to represent the outcome of default and alternative analyses (Fig. 4.2).

There is a distinct pattern that could be observed in all of the better resolved Strict Consensus trees obtained from partially weighted and ordered charactersets (Fig. 4.2b): (1) Several stable clades can be related to geographic regions, for example: *Artesia* and *Parabogidiella* Holsinger, 1980 (in Holsinger & Longley, 1980) from Texas and a relatively large group with mostly Argentinean taxa. (2) There is another very robust clade that cannot be related to a specific geographic range but contains all or almost all of the genera we propose to remove from the family: the incompatible taxa *Bollegidia*, *Pseudingolfiella*, *Kergueleniola*, and *Dussartiella* are accompanied by *Aurobogidiella*, *Marinobogidiella*, and *Marigidiella* Stock, 1981. Like the genera to be removed from the family, these three taxa either inhabit coastal interstitial habitats and/or also show distinct morphological modifications. (3) The clades closest to the hypothetical ancestor contain almost exclusively taxa of the New World (*Artesia*, *Parabogidiella*, *Patagongidiella*, *Spelaeogammarus*, and *Megagidiella*). (4) The New World taxa, as opposed to the Mediterranean and African groups, also show a greater tendency to form robust clades and to maintain basal topographic positions close to the hypothetical ancestor. (5) The split taxa *Medigidiella*, *Stygogidiella*, *Patagongidiella*, and *Antillogidiella* occupy clearly separate topological positions in all better resolved consensus trees.

Strict Consensus trees of unordered and equally weighted charactersets were generally poorly resolved (Fig. 4.2a). In spite of that, some of the clades from alternative runs still appeared in these more polytomous trees, e. g., a small Argentinean cluster and again the "non-geographic" clade with mostly marine, distinctly modified taxa. Also, the basal positions are again predominantly occupied by New World taxa. Interestingly, the split genus *Patagongidiella* remains separated, but still on the same clade in this equally weighted analysis.

Discussion

A look at the distribution map of bogidiellid amphipods (Fig. 4.3) reveals several particularities: (1) Bogidiellids occur worldwide, except for Boreal, Arctic, and Antarctic regions. (2) There are several major concentrations of species, the largest of which is in the Mediterranean region, and smaller ones in Central America, South America, and the West Indies. (3) Species richness is highest in the Mediterranean region (33 species in 4 genera), whereas generic diversity is greater in the New World (South America: 10 genera, 18 spp.; Central America: 5 genera, 12 spp.; West Indies: 7 genera, 10 spp.). (4) The majority of bogidiellids are located no further than ± 160 km from coastlines. In several instances, however, they live in inland, continental habitats, especially in South America but also in Europe and the Middle and Far East.

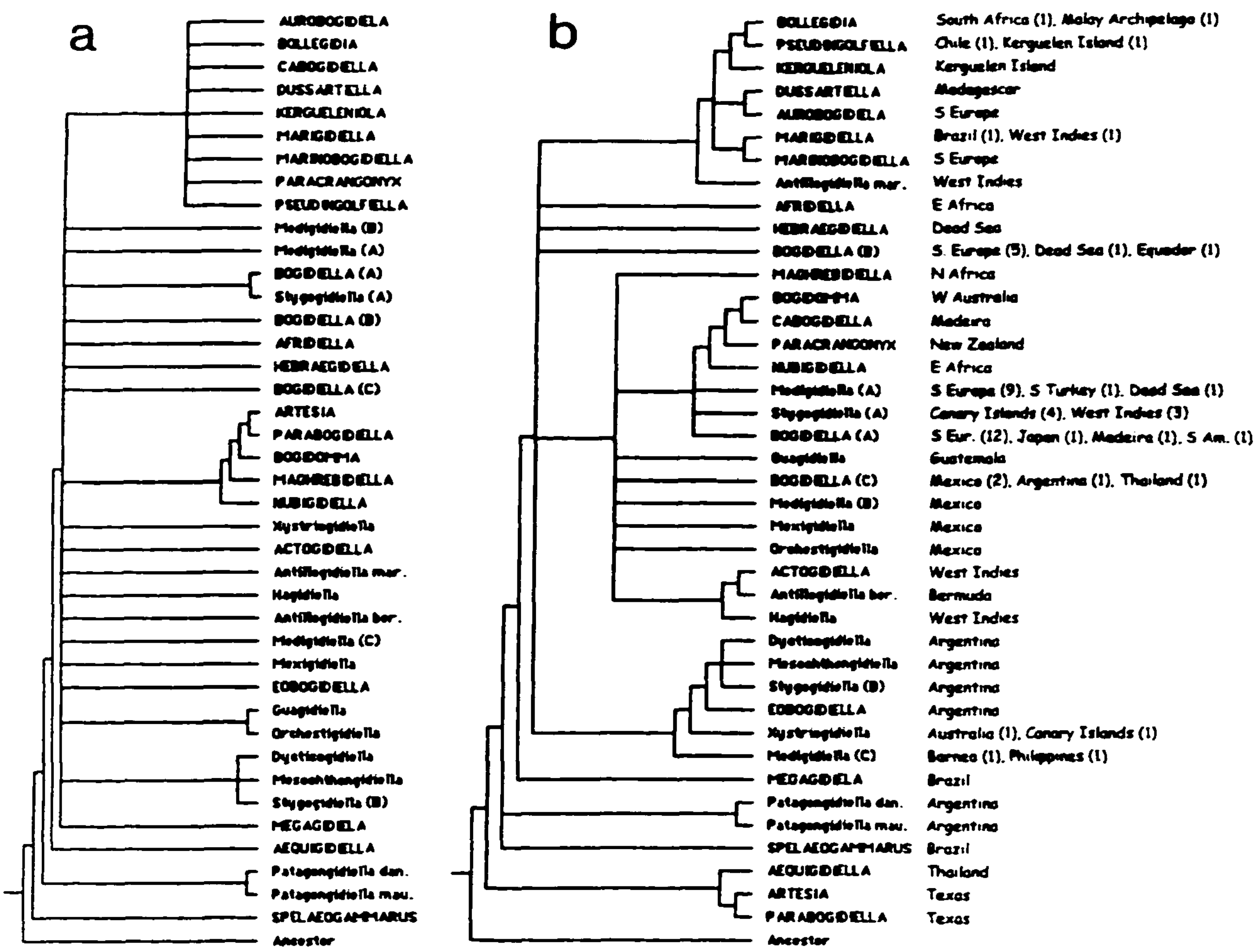


Fig. 4.2. Cladistic analysis of the family Bogidiellidae s. lat. (see Cladistic Methods for details). With the exception of *Bogidiella* and *Patagongidiella*, genera are capitalized to facilitate the discrimination between genera and subgenera. Several taxa are split as indicated by capital letters in parentheses. Numbers in parentheses refer to number of species known from each region, respectively. a) PAUP Strict Consensus tree of default run: characters unordered and equally weighted (CI: 0.29; RI: 0.29; length: 426, min. length: 124, max. length: 549); b) PAUP Strict Consensus tree of alternative run with areas related to OTUs; characters partially weighted and ordered (CI: 0.36; RI: 0.48; length: 346).

There are different points of view about the biogeographic history of bogidiellid stygobionts. Because it is assumed that some of their isolated freshwater habitats have been separated from marine waters since the break-up of Pangaea, 180-200 million years ago, and have remained isolated ever since, bogidiellids might have a very old freshwater origin. On the other hand, we also see a distribution in geographically much younger regions, like the West Indian islands, most of which emerged from the sea during tectonic uplifts and sea level regressions in the early to middle Tertiary, between 20 and 50 million years ago.

According to these two vicariant scenarios, alternative hypotheses are generally used to explain the evolution of bogidiellid amphipods. The first theory postulates an ancient freshwater origin, suggesting that bogidiellids were already adapted to freshwater habitats before (!) the break-up of Pangaea and adapted progressively to brackish and marine environments as continents drifted apart. The second model renders bogidiellids as primarily a marine group, which massively invaded continental freshwaters and subsequently adapted to subterranean inland habitats (see: Stock, 1981; Notenboom, 1991).

However, we have to be careful with final conclusions about the origin of bogidiellids. The growing number of new discoveries seems to increase the complexity of their evolutionary history and many questions still remain unanswered. There are apparently no obvious vicariant events that offer an explanation for the abundant occurrences in continental South America as opposed to an almost 'bogidiellid-free' African continent. Similarly enigmatic is the high specific radiation in the Mediterranean coastal regions. Land masses in southern Europe emerged in a complex pattern during the Miocene (Adams, 1981) and must have been subsequently inhabited by precursors of modern bogidiellids. This strongly implies that Mediterranean bogidiellids either evolved from a relatively young marine ancestor or that Bogidiellidae are generally able to re-adapt from freshwater to saline waters (and vice versa) more quickly than previously envisioned. Recent discoveries and reports of bogidiellids from marine interstitial habitats in Turkey seem to support this view (Koenemann et al., 1998; Koenemann, 1998; see also Notenboom, 1991). If bogidiellids do have an ancient freshwater origin, we would also expect to find generally more primitive taxa in isolated continental habitats and more apomorphic ones on the Caribbean islands and in the Mediterranean coastal regions. At present, the geographic distribution of known taxa does not convincingly support this idea

The results of the phylogenetic analysis characterize bogidiellids from the Western Hemisphere as the more plesiomorphic taxa. For some genera, e.g., *Spelaeogammarus* and *Artesia*, these results are not unexpected and are in general accordance with the literature (Stock, 1981; Barnard & Barnard, 1983). In other cases, however, the basal appearance of OTUs, close to the hypothetical ancestor, is less apparent. The new genus *Megagidiella* from a cave in central-western Brazil does not exhibit a particular plesiomorphic morphology after a first or even second examination. This large-sized bogidiellid, reaching a body length up to 16 mm, features short, non-overlapping coxal plates, pleopods with 3-segmented outer rami and reduced inner rami, and absence of a mandibular palp. A comparative morphological examination would not necessarily reveal a relationship with the obviously primitive *Spelaeogammarus* from caves in eastern Brazil. In the cladistic analysis, however, *Megagidiella* tends to appear close to *Spelaeogammarus* and *Patagongidiella*, indicating a possible relationship of species that inhabit disjunct caves between 1300 and 2200 km apart on the same continent. Similarly surprising is the basal position of the sympatric genera *Patagongidiella* and *Grossogidiella*. Both taxa are from the same cave system in western Argentina and show distinct apomorphic modifications (see New taxa). These results allow us to draw several hypothetical conclusions with regard to bogidiellid phylogeny:

(1) The appearance of primitive and advanced structures seems to be more complex than assumed, exhibiting various transitional, intermingled states. A conspicuous apomorphic character might not necessarily indicate an apomorphic species or genus. It may occur in primitive as well as in advanced taxa and the arbitrary use of a few discriminating characters for phylogenetic assumptions must be considered highly doubtful.

(2) Despite the extraordinary generic diversity of bogidiellids from the New World, especially South America, an explicit tendency to form robust clades could be observed for these taxa. This trend probably indicates a close relationship of taxa from several disjunct localities. The analysis also renders South American groups as the more plesiomorphic bogidiellids. Both tendencies might be correlated with the highest frequency of isolated inland habitats on the South American continent, strongly pointing towards an ancient freshwater origin of bogidiellid amphipods.

Fig. 4.3. Geographic distribution of bogidiellid amphipods. The shaded circles represent the approximate number of locality records for described species (the number of records in southern Europe is actually slightly higher than shown on the map). The white circles represent genera which we propose to remove from the family.

The results of the phylogenetic analysis also suggest the removal of five genera from the family Bogidiellidae (see Incompatible taxa). At least four of the five taxa removed formed a remarkably solid clade in all of the obtained consensus trees. Notably, most of these genera have two additional particularities in common: they inhabit marine interstitial habitats in coastal areas either outside of or marginal to the bogidiellid distribution range.

In a future project, it would be interesting to further investigate relationships within the family Bogidiellidae with molecular techniques, employing DNA sequencing or protein analyses. A conceivable objective of such an investigation could, for example, focus on the comparison of morphologically similar taxa or taxa endemic to distinct geographic regions.

Summary

The increasing number of worldwide discoveries of subterranean amphipods, especially during the last two decades, has led to additions of numerous new taxa in the stygobiont family Bogidiellidae s. lat. To date, the family is composed of 23 genera and 11 subgenera, and approximately 110 described species. However, given the uneven quality of generic and subgeneric diagnoses in the literature, there is considerable confusion regarding the status of some of the taxa at these levels. Even the family itself lacks a clear definition. In order to gain a better knowledge of the phylogeny of this group, a cladistic analysis, employing both PAUP 3.0s and MacClade, was performed on the genera and subgenera currently assigned to the Bogidiellidae s. lat. Supported by the results of this analysis, the taxonomic structure of this group is completely revised above the species level. The revision excludes 5 genera from the family, all remaining subgenera are elevated to generic level. Four taxa are split, resulting in 5 new genera. The family Bogidiellidae now consists of 33 genera.

CHAPTER V

SYSTEMATICS OF THE NORTH AMERICAN SUBTERRANEAN AMPHIPOD GENUS *BACTRURUS* (CRANGONYCTIDAE)

Introduction

The crangonyctid genus *Bactrurus* is a comparatively small stygobiont group, restricted to hypogean environments in parts of eastern and central United States. Prior to this study, only three described species were known to science. *Bactrurus* was established by Hay (1902) who proposed a new genus for *Crangonyx mucronatus* Forbes, 1876. However, a generic diagnosis was not given until 38 years later when Hubricht & Mackin (1940) introduced *B. brachycaudus* as the second species of the genus. A third species, *B. hubrichti*, was described by Shoemaker (1945). Although Holsinger (1972, 1977) discussed the morphological and biogeographic significance of *Bactrurus* as compared to its closest crangonyctid relatives and provided both familial and generic keys, the genus has lacked a comprehensive taxonomic revision until now.

In the following revision of *Bactrurus*, a total of 235 locality records, comprising approximately 2500 specimens, have been examined or re-examined. The vast majority of these records belong to specimens of the three species currently assigned to the genus. A much smaller part of the collection material, however, contained several new species. In order to obtain additional samples of potentially new species, two collection trips were made in March and May, 1999. The field trips included excursions to caves and associated groundwater habitats in Alabama, Indiana, Illinois, Ohio, Missouri, Tennessee and Virginia. Some of these localities had not been investigated for more than 50 years. The evaluation of specimens obtained during the field trips as well as older collection material led to the descriptions of four new species of *Bactrurus* given in this paper. Moreover, several new and rather unusual records enabled us to fill in some of the gaps of inter- and intraspecific distribution patterns.

To investigate the phylogeny and biogeography of the genus *Bactrurus*, a cladistic analysis was performed on seven *Bactrurus* species and 12 selected taxa of the family Crangonyctidae. Five epigean and hypogean amphipod taxa were chosen as outgroups. Based on the phylogenetic analysis, the relationship of *Bactrurus* to its potential sister-genus *Stygobromus* Cope, 1872, is reviewed and analyzed. In addition, pairwise sequence differences of the 18S (small subunit) rDNA gene are given for three *Bactrurus* species and three of the outgroup taxa (adapted from Englisch & Koenemann, in ms.). The evaluation of all data as well as a detailed discussion of biogeographic and ecological characteristics of *Bactrurus* provide new perspectives on the geographic distribution and evolutionary history of the genus in North America.

Materials, methods and definitions

Taxonomic procedures

The following redescriptions of *B. brachycaudus*, *B. hubrichti* and *B. mucronatus* are supplementary to the original descriptions. Because all species of the genus show a relatively high morphological resemblance, the description of *B. brachycaudus* was chosen to serve as the basic model with regard to a variety of minor characters that show little or no variation among the other species (e.g., number of spines and setae on mouthparts and appendages). *Bactrurus brachycaudus* is, therefore, redescribed in greater detail. If a specific character is not explicitly mentioned for one of the other species, it can be assumed to be identical with that of *B. brachycaudus*.

The defining angles of propods of the first gnathopod were measured to facilitate interspecific comparisons between differently shaped propods. For these measurements, the palmar margin and the subangular (posterior) margin of the propod were considered two sides of an angle.

Nomenclature for setal patterns on segment 3 of the mandibular palp is based on the uppercase letter system first introduced by Stock (1974) (see also Fig. 5.2g).

Because vandalism and pollution have become major threats to many cave habitats, we have endeavored to protect these sensitive environments from further destruction by providing only cave names in the 'Material examined' sections for each species. If more detailed information is needed on a particular locality, it can be obtained either from state geological surveys or from our database.

Species synonymies are complete for all references pertinent to the taxonomy and geographic distribution of taxa.

Collection methods

The following collection methods have been applied to different types of habitats:

- Caves: In small bodies of water, e.g., drip pools and small streams, the animals were collected with the aid of modified pipettes and fine-meshed hand nets. Additionally, a Cvetkov net (closing net) was used if needed for less accessible waters (larger cave streams, deep lakes). Water bodies of greater depths were sampled with the assistance of baited traps.
- Springs and wells: Animals were collected utilizing either a Cvetkov net, a hand net with extended grip, or by hand-picking from the substrate.
- Drainage outlets: Pipe outlets of drainage systems beneath farm fields were sampled with the help of a hand net or by hand-picking from substrates.

In addition, the Karaman-Chappuis method (digging method) and a Bou-Rouch pump was employed to take samples of the groundwater fauna near surface streams, springs, and wells. The Bou-Rouch pump is specifically designed to collect small groundwater invertebrates from depths of 30-120 cm.

All collection samples were preserved in 90-95% ethanol. For the drawings, specimens were first dissected and mounted on microscope slides in Faure's medium. Holotypes of the new species are deposited in the National Museum of Natural History (Smithsonian Institution) under the catalogue numbers of the former United States National Museum.

Definitions, nomenclature and abbreviations

Glacial drift: sediments deposited by Quaternary glaciers; the repeated north-south movements of the ice sheets during the Pleistocene resulted in an accumulation of deposits. In the Central Lowland Province, glacial drift is commonly composed of layers of sand, gravel, silt or clay.

Rastellate: term introduced by Holsinger (1967) to describe the comb- or brush-like spines or setae on the carpus of gnathopod 1 and/or 2.

The following abbreviations are used for the deposition of museum specimens:

AMNH:	American Museum of Natural History
JRH	John R. Holsinger collection
KBS	Kansas Biological Survey
USNM	United States National

Taxonomic part

Genus *Bactrurus* Hay, 1902

Bactrurus Hay, 1902: 430.-- Hubricht & Mackin, 1940: 200.-- Shoemaker, 1945: 24.-- Holsinger, 1972: 73; 1977: 265; 1986a: 95; 1986b: 536.-- Barnard & Barnard, 1983: 435.

Type species (by monotypy): *Crangonyx mucronatus* Forbes, 1876.

Diagnosis: Eyes absent. Body smooth, unpigmented. Uronites unfused. Coxal plates in stretched body position not overlapping; coxal plates 1 and 2 small; coxal plate 1 wider than long, coxal plates 2-4 slightly wider than long or as wide as long; plates 5-7 longer than wide; coxal plate of pereopod 5 with distinct distoanterior lobe, coxal plate of pereopod 6 with small distoanterior lobe, coxal plate of pereopod 7 ovate.

Antennae sometimes with aesthetascs on some peduncular segments and on most segments of primary flagellum First antenna 49-92 % of body length (usually 60-77%), primary flagellum longer than peduncle, with 24-59 segments in adult specimens; accessory flagellum 2-segmented, short, as long as or slightly longer than first segment of primary flagellum. Second antenna without calceoli, 31-53% of length antenna 1; flagellum approximately as long as peduncle, bearing 9-16 segments in adult specimens. Lower lip with small inner lobes. Mandibular palp 3-segmented, third palp segment falcate, as long as or slightly longer than segment 2; molar prominent, triturative. Palp of maxilla 1 symmetrical, 2-segmented (but weakly articulated); inner plate with up to 5-8 plumose setae; outer plate with 7 strong spines apically (bidentate and multidentate types). Inner plate of maxilla 2 wider than outer plate, bearing oblique row of 5-10 strong, plumose setae. Inner plate of maxilliped bearing 2-6 blade-like spines apically (one of which sometimes modified as plumose spine; see Fig. 5.6c); outer plate with 2-9 blade-like spines apically and subapically. Gnathopod 1 with expanded basis, basis of gnathopod 2 narrow; carpus of gnathopods triangular, bearing several strong, rastellate (brush-like) spines; propods of gnathopods 1 and 2 subequal (but propod of gnathopod 1 sometimes distinctly larger than that of gnathopod 2); propods with oblique palm; palmar margins with long and short, distally bifid spines; dactyls of both gnathopods with row of small setae on inner margin. Pereopods 3 and 4 subequal, with narrow bases; pereopods 5-7 gradually increasing in length, bases with posterior lobes, about as wide proximally as distally, posterior margins convex, distoposterior lobes well developed, broadly rounded; pereopod 5 as long as or slightly shorter than pereopod 4; pereopod 7 subequal to or distinctly longer than pereopod 6. Coxal gills subovate, usually present on pereopods 2-7 (on pereopods 2-6 in *B. mucronatus*). Broodplates subovate, occurring on pereopods 2-5; exceeding length and width of bases on pereopods 2-4, greatly reduced on pereopod 5. Paired sternal processes proximolaterally on pereonites 6 and 7 and sometimes also on pleonite 1 (processes simple, non-bifurcate); sometimes single, median sternal (mediosternal) processes on pereonites 2 and 3 (absent in most species). Pleopods unmodified, biramous, with 5-18 segments per ramus (number of segments decreasing from pleopod 1 to 3; large specimens with up to 30 segments); outer ramus slightly shorter than inner ramus. Epimeral plates with rounded distoposterior corners, each of which bearing several short setae. Uropods unmodified, biramous, 1-segmented; peduncles of uropod 1 and 2 without basiofacial (ventrolateral) spines; rami of uropod 3 reduced: inner ramus vestigial. Telson typically longer than wide (about 10% wider than long in *B. hubrichti*), apex with shallow excavation or V-shaped cleft (up to 23% cleft), bearing only apical spines (sometimes 1-3 small, subapical spines in *B. brachycaudus*) Several species with secondary sexual dimorphism of the male as follows: antenna 1 with longer flagellum; peduncle of uropod 1 distally with serrate process; telson slightly to extremely elongated (up to 3 times longer than female telson); telson with relatively short apical spines.

Remarks: Bactrurus is morphologically closely related to the crangonyctid genus *Stygobromus*. However, unlike *Bactrurus*, *Stygobromus* has a uniramous uropod 3, with a more greatly reduced outer ramus. In addition, most species of *Stygobromus* can also be differentiated from *Bactrurus* by absence of bladelike spines on the inner (apical and subapical) margin of the outer plate of the maxilliped, and in some species by bifurcate lateral sternal processes.

Key to the species of *Bactrurus*

1. Telson of male distinctly elongated, at least twice as long as uropod 3; female telson distinctly tapered, about twice as long as broad (width less than 54% of length) 2

   Telson of male not distinctly elongated, shorter or slightly longer than uropod 3; female telson not tapered (or slightly tapered), usually not twice as long as broad (width in *B. brachycaudus* up to 58% of length) 3
2. Coxal gill absent from pereopod 7; lateral sternal processes on pereopods 6 and 7 and on pleonite 1 (sometimes absent on pleonite 1); median sternal processes absent .... *B. mucronatus*

Reduced coxal gill present on pereopod 7; lateral sternal processes on pereopods 6 and 7; median sternal processes on pereonites 2 and 3 .... *B. pseudomucronatus* n. sp.

3. Lateral sternal processes on pereopods 6 and 7 and on pleonite 1; telson as wide as or wider than long, distinctly cleft (18-25%); merus of gnathopod 1 with plumose spine(s) .... *B. hubrichti*

   Lateral sternal processes on pereopods 6 and 7 (sometimes also on pleonite 1 in *B. brachycaudus*); telson longer than wide, cleft usually 1-12% of length of telson (sometimes up to 23%); merus of gnathopod 1 without plumose spine(s) 4

4. Peduncle of male uropod 1 with serrate distal process; defining angle of gnathopod 1 propod distinct (120-140°); armature of telson sexually dimorphic 5

   Peduncle of male uropod 1 without serrate distal process; defining angle of gnathopod 1 shallow; armature of telson not sexually dimorphic .... *B. brachycaudus*

5. Coxal gill of pereopod 7 only slightly smaller than coxal gill on pereopod 6; distal medial margin of carpus on gnathopod 2 with ca. 3 plumose setae; spines on anterior margin of basis of pereopod 5 relatively long .... *B. wilsoni* n. sp.

   Coxal gill of pereopod 7 less than half the size of coxal gill on pereopod 6; distal medial margin of carpus on gnathopod 2 with 6-7 plumose setae; spines on anterior margin of basis of pereopod 5 short 6

6. Palmar angle of propod of gnathopod 1 distinctly defined (ca. 120°);. inner plate of maxilla 2 with oblique row of 7 plumose setae; outer ramus of third uropod < 80% of length peduncle, bearing 2-5 subapical spines on both margins; peduncle of uropod 3 armed with 1-2 distal spines .... *B. angulus* n. sp.

   Palmar angle of propod of gnathopod 1 less distinct (135-140°) ; inner plate of maxilla 2 with oblique row of 10 plumose setae; outer ramus of third uropod > 80% of length peduncle, bearing 4-8 subapical spines on both margins; peduncle of uropod 3 armed with 3 distal spines ....*B. cellulanus* n. sp.

Bactrurus brachycaudus **Hubricht & Mackin, 1940**

Figs. 5.1-5

Bactrurus brachycaudus Hubricht & Mackin, 1940: 201-202, Figs. 8A-L.; type locality: walled spring on Keifer Creek, 0.6 miles NW of Fern Glen (St. Louis County, Missouri).-- Hubricht, 1943: 693; 1959: 878.-- Barnard, 1958: 44.-- Nicholas, 1960: 127. -- Holsinger, 1972: 73, Fig. 30c; 1977: 277; 1981: 93; 1986a: 95, Fig. 6; 1986b: 542.-- Peck & Lewis, 1977: 45.-- Barnard & Barnard, 1983: map 12.-- Fitzpatrick, 1983: 44.-- Gardner, 1986: 15.

Material examined.-- ILLINOIS: Adams Co.: Well with pump, 10 mi. S of Quincy, 2 males, 6 females (JRH), J. G. Weise, 17 Sept. 1957. **Greene Co.:** Spring on west edge of S14, 2.5 mi. W of Hillview, 2 males, 2 females (JRH), S. Peck, 22 Apr. 1966. Spring at base of bluff, 3 mi W of Eldred, 5 males, 3 females (USNM), L. Hubricht, 8 Sept. 1940. **JacksonCo.:** Giant City State Park, 1 male, 3 females (JRH), J. A. Beatty, 27 Apr. 1971. **Johnson Co.:** Firestone Creek Cave (formerly called Vienna City Dump Cave), 1 female (JRH), J. L.ewis, 3 Mar. 1973; 1 juvenile (JRH), 15 Dec 1973. Belknap Cave, 3 males, 3 females, 1 juvenile (JRH), J. Lewis, 19 May 1974. **Monroe Co.:** Camp Vandeventer Cave, 1 male (JRH), S. Peck, 27 Nov. 1965. Dane's Cave, 1 juvenile (JRH), J. Lewis and P. Moss, 5 Sept. 1999. Fogelpole Cave, 2 males, 3 females (JRH), S. Peck, 25 Jun. 1965; 1 female (JRH), 16 Aug. 1968. Frog Cave, 1 female (JRH), J. Lewis, S. Rafail, P. Moss and D. Tecic, 4 Jul. 1999. Fults Creek Cave, 1 female (JRH), S. Peck, 17 Aug. 1968. Morrison (Burkesville) Cave, 1 female (USNM), L. Hubricht, 6 Nov. 1937; 2 males, 1 female (JRH), J. R. Holsinger and R Norton, 13 Jun. 1965. Pautler Cave, 1 male (JRH), J. Lewis, S. Rafail, P. Moss and D. Tecic, 6 Jul. 1999. Rose Hole, 3 mi. WSW of Waterloo, 5 specimens (JRH), J. Lewis, S. Rafail, P. Moss and D. Tecic, 5 Jul. 1999. Schipp's Well, 0.25 mi. SW of New Hanover, 1 male (JRH), J. Lewis, S. Rafail, P. Moss and D. Tecic, 7 Jul. 1999. Seep, 2.8 mi. S of Valmeyer, 4 males, 5 females, 3 juveniles (USNM), L. Hubricht, 30 Jan. 1943. Small cave, 1 male (USNM), L. Hubricht, 30 Jan. 1943. Terry Spring (Long Slash) Cave, 1 male (JRH), S. Peck, 27 Nov. 1965. **Montgomery Co.:** Drain tile into concrete catchment basin, 5.2 mi. NW of Harvell, 3 specimens (JRH), S. Koenemann and U. Englisch, 15 May 1999. Drain pipe, ca. 5 mi. E of Morrisonville, 9 specimens (JRH), S. Koenemann and U. Englisch, 15 May 1999. **Pike Co.:** Croxville Cave, 3 females (JRH), S. Peck, 15 Aug. 1968. Small spring, 1 mi SE of Pearl, 4 juveniles (USNM), L. Hubricht, 28 Apr. 1940. Twin Culvert Cave, 2 males, 2 females (JRH), S. Peck, 25 Sept. 1965. **Sangamon Co.:** Drain pipe into Panther Creek, ca. 0.5 mi. W of Auburn, 14 specimens (JRH), S. Koenemann and U. Englisch, 16 May 1999. **St. Clair Co.:** Dashed Hopes Pit, 3.25 mi. (?) ENE of Columbia, 1 male (JRH), J. Lewis, S. Rafail, P. Moss and D. Tecic, 3 Jul. 1999. Small spring, 1 mi. S of Falling Spring, 7 males, 8 females (USNM), L. Hubricht, 18 April, 1937. Stemmlers Cave, 1 female (USNM), L. Hubricht, 16 Jan. 1938. **Union Co.:** Seep near McCann School, 2 females (USNM), L. Hubricht, 14 Apr. 1940. Ditch along creek, 4 juveniles (JRH), J. Weise, 8 Jan. 1952. **MISSOURI: Bollinger Co:** Blue Pond Natural Area, SW part of county, 1 male (JRH), W. C. Pflieger, 23 Mar. 1988. **Boone Co.:** Devils Icebox Cave, J. and T. Lewis, 2 juveniles (JRH), 4 Aug. 1979; 4 males, 3 females (JRH), S. W. Schulte, Dec. 1980; 4 males, 4 females (JRH), J. E. Gardner, 29 Jan, 1981. Hunted Cave, 26 specimens (USNM), L. Hubricht, 4 Aug. 1940. Pollys Pot (Cave), 6 specimens (JRH), J. E. Gardner, 28 Jan. 1981. Seep near small creek, 4 mi. SE of Ashland, 9 specimens (USNM), L. Hubricht, 30 Apr. 1938. Spring 4 mi. E of Ashland, on Brushy Creek, 5 specimens (USNM), L. Hubricht, 14 May 1936. **Crawford Co.:** Jagged Canyon Cave, 1 juvenile (JRH), J. L. Craig, 16 Dec. 1973; 1 male (JRH), 5 females, 12 Apr. 1974. Nameless Cave, 5 females (JRH), B. Harris, 3 Jul. 1976; 1 male (JRH), J. Craig, 16 Feb. 1974. Narrows Cave, 1 male, 1 female (JRH), J. E. Gardner, 7 Aug. 1980. **Dent Co.:** Bounds Branch Cave, 2 males, 2 females (JRH), J. E. Gardner, 20 Jun. 1980. **Franklin Co.:** Fisher Cave, 11 specimens (JRH), J. E. Gardner, 11 Mar. 1982. Meramec Caverns, 1 male, 7 females (USNM), L. Hubricht, 16 Dec. 1939. Sheep Cave, 1 female (JRH), J. E. Gardner, 17 Mar. 1981. **Iron Co.:** Boulder Cave, 3 males (JRH), J. E. Gardner, 1 Jun. 1982. Cave Hollow Cave, 1 male, 2 females (JRH), J. E. Gardner, 2 Jun. 1982. **Jefferson Co.:** Anderson Cave, >25 specimens (USNM), L. Hubricht, 24 Sept. 1939. Becker's spring, 0.5 mi. E of

Seckman, 1 male (USNM), L. Hubricht, 6 Jun. 1937. Hoffarth's well, Antonia, 4 females (USNM), collected by Hoffarth, 16 Oct. 1938. Pleasant Valley Cave, 1 male (JRH), D. and M. Bechler, 20 Mar. 1977. Rice's Cave, 5 specimens (USNM), L. Hubricht, 31 Oct. 1937; 1 female (JRH), D. Bechler and M. Harder, 30 Jan 1977. Sims Cave, 3 males, 3 females, 1 juvenile (JRH), T. Marsh, 27 Mar. 1966. Small spring on bluff, 0.5 mi. NW of Selma, 52 specimens (USNM, in 2 vials), L. Hubricht, 10 May 1936. Small spring at foot of hill, on tributary of Selma Creek, 0.7 mi. NNW of Selma, 16 specimens (USNM), L. Hubricht, 23 Jan. 1938. Small spring on bluff, 1 mi. W of Selma, 9 specimens (USNM), L. Hubricht, 2 Jun. 1937. Spring house on hillside, 2.2 mi. E of Antonia, 4 females (USNM), L. Hubricht, 6 Jun. 1937. Small spring on bluff, 2 mi. E of Antonia, 1 female (USNM), L. Hubricht, 6 Jun. 1937. Seep, 5 mi. S of Antonia, 26 specimens (USNM), L. Hubricht, 2 Jun. 1940. Small spring, 5 mi. N of Antonia, 11 female (USNM), L. Hubricht, 6 Jun. 1937. Seep near large spring, 2 mi. S of Platt, 3 specimens (USNM), L. Hubricht, 14 Jan. 1942. Spring on Antire Creek, 3.5 mi. E of Eureka, 2 females (USNM), L. Hubricht, 30 Apr. 1939. **Lincoln Co.**: Aker's Cave, 3 males, 2 females (USNM), L. Hubricht, 24 Jan. 1943. Creech Cave, 1 female (JRH), J. E. Gardner, 15 Apr. 1982. **Madison Co.**: Small intermittent stream (tributary of Twelvemile Creek), 7 mi. S of Fredericktown, 1 male, 1 female, 1 juvenile (USNM), L. Hubricht, 21 Apr. 1938. **Perry Co.**: Crevice Cave, 1 male (JRH), S. Peck, 23 Sept. 1961; 1 male, 2 females (JRH), J. R. Holsinger, 9 Jun. 1964. Mystery Cave (north upper passage, stream entrance to Spider Pit), 2 males (JRH), J. Lewis, 20 May 1972, and 1 juvenile (JRH), 31 Mar. 1974. Tom/Berome Moore Cave, 1 male (USNM), L. Hubricht, 14 Sept. 1969; 1 male, 1 female (JRH), J. Lewis, 30 Nov. 1973; 2 males, 1 female, 1 juvenile (JRH), T. L. Saberton and S. Trimbley (donated by D. L. Bechler), date not given. **Pike Co.**: Cave N of Frankford, 9 specimens (USNM), L. Hubricht, 21 Jun. 1941. **Ralls Co.**: Fisher's Cave, 1 male, 4 females (USNM), L. Hubricht, 21 Jun. 1941; 2 females (JRH), J. G. Weise (?), 21 Apr. 1953; 1 male, 1 female (JRH), J. G. Weise, 16 Oct. 1954. **Reynolds Co.**: Spring, 3 mi. WSW of Ellington, ca. 95 specimens (USNM), L. Hubricht, 3 May 1942. **Scott Co.**: Spring seeps along gravel road, 1.75 mi. NW of Illmo (T30N, R14E, S29), 6 males, 11 females (JRH), W. L. Pflieger and G. McDonald, 27 Feb. 1976. **St. Charles Co.**: Dingledine Cave, 1 male, 5 females (JRH), J. Holsinger, 9 Jun. 1964. **St. Francois Co.**: Shaver Cave, 2 juveniles (USNM), L. Hubricht, 20 Jul. 1941. **St. Genevieve Co.**: Kohm's Cave, 6 males, 2 females (USNM), L. Hubricht, 24 Aug. 1941; 1 male, 1 female (JRH), D. and M. Bechler, 6 Feb. 1977. Saltpeter Cave, 9 specimens (USNM), L Hubricht, 24 Aug. 1941. Small spring, 4.6 mi. SW of St. Genevieve, 14 specimens (USNM), L. Hubricht, 9 Mar. 1941. **St. Louis Co.**: Biffle's bunker spring, Tyson Research Center, 1 male (JRH), J. C. Walker, 21 Feb. 1996; 1 male, 14 Mar. 1996; 4 males (JRH), E. C. Biffle, Apr.-May 1996; 12 specimens (JRH), S. Koenemann and U. Englisch, 17 May, 1999. Crystal Springs, Babler State Park, 1 male (JRH), S. Koenemann and U. Englisch, 17 May 1999. Cherokee Cave, 1 male (JRH), A. R. Templeton, 19 Jan. 1984. Basement of Biology Dept., St. Louis University, 1 male (JRH), D. L. Bechler, 1 male, 1977; 1 male (JRH), 12 Aug. 1977; 1 male, 1 female (JRH), D. L. Bechler and J. Rice, 16 Feb. 1978. Cliff Cave, 1 male (USNM), L. Hubricht, 25 Apr. 1938. Cave, Kirkwood, juvenile (USNM), L. Hubricht, 13 Jun. 1937. Small cave, Fern Glen, 1 male (USNM), L. Hubricht, 23 Apr. 1939. Small cave (10-15 m long), NW of Eureka, 3 males, 1 female (JRH), J. and T. Lewis, 9 Aug. 1979. Woods Cave, 12 specimens (JRH), J. E. Gardner, 17 Jul. 1979; spring, Monarch, 4 females (USNM), L. Hubricht, 23 May 1937. Walled spring on Keifer Creek, 0.6 mi. NW of Fern Glen, 1 female and 5 male **SYNTYPES** (AMNH 9158), 23 male, 44 female and 5 juvenile **SYNTYPES** (USNM 74846) (2 specimens mounted on permanent slides in JRH collection), L. Hubricht, 17 Apr. 1937; ca. 50 **topotypes** (USNM), L. Hubricht, 22 Mar. 1942. Small spring on bluff, 0.7 mi. S of Grimsby, 30 specimens (USNM), L. Hubricht, 29 Feb. 1936. Outlet of drain, Osage Hills Country Club, Kirkwood, 4 specimens (USNM), L. Hubricht, Dec. 1931. Small spring near old quarry, Kirkwood , Osage Hills, 1 male (USNM), L. Hubricht, 23 Apr. 1939. **Texas Co.:** Bat Cave, 5 males, 13 females, 2 juveniles (USNM), L. Hubricht, 5 Jul. 1940; 1 female (JRH), W. R. Elliott, 14 Dec. 1999.

Diagnosis: A medium-sized to very large species without marked secondary sexual dimorphism. Propod of gnathopod 1 slightly shorter than propod of gnathopod 2. Pereopod 5 slightly longer than pereopod 4 (about 8%). Coxal gills on pereopods 2-7 (reduced on pereopod 7). Lateral sternal processes on pereonites 6 and 7; median sternal (mediosternal) processes usually absent. Telson entire or slightly emarginate, longer than wide. Largest male 31 mm, largest females up to 24 mm.

Description: The description is based on several adult specimens of both sexes (19.9 mm female, Fig.5.1); 23.5 mm female; 19.0 mm male; 20.5 mm male; 13.0 mm male).

Antenna 1 (Fig. 5.2a) up to 75% length of body. Second peduncular segment 74% length of peduncular segment 1; third peduncular segment 42% length of peduncular segment 2; several plumose setules dorsoproximally on peduncular segment 1. Aesthetascs sometimes on peduncular segments 2 and 3 and on most segments of main flagellum. Primary flagellum with up to 59 segments; accessory flagellum 2-segmented.

Antenna 2 (Fig. 5.2b) about 40% length of antenna 1. Third peduncular segment 96% length of peduncular segment 2. Aesthetascs and several plumose setules on peduncular segment 4. Flagellum with up to 15 segments.

Upper lip (Fig. 5.2c) rounded apically, with small setae along distal margin.

Mandibles (Figs. 5.2d-g) subequal. Molar strong, triturative, with row of 10-12 small plumose setae on anterior outer margin. Row of 8-10 plumose spines between molar and lacinia mobilis. Left lacinia mobilis 4-dentate (Fig. 5.2f); right lacinia irregular, with serrate distal margin (Fig. 5.2e). Palp segments 2 and 3 subequal in length; palp segment 2 with 5-8 short naked setae on lateral margin and 7-12 plumose setae on medial margin (2-4 of which can be slender spines in some males; see Fig. 5.2g); palp segment 3 bearing apically 3-4 long, plumose E-setae, a row of about 22 short, plumose D-setae, 5-6 long, plumose B-setae, 4-5 long, plumose A-setae, and faint, small setae on medial surface. Because there are two types of plumose setae on mouthparts and gnathopods the plumose setae of the mandibular palp are referred to as comb-type setae (Fig. 5.3b).

Maxilla 1 (Fig. 5.3a): Palp 2-segmented, articulation of segments faint; palp segment 2 bearing apically and subapically up to 14 naked setae and 1 or 2 long plumose setae (comb-type, Fig. 5.3b). Outer plate with 7 strong spines, 3-4 of which bidentate and 3-4 multidentate (Fig. 5.3d). Inner plate with 6-8 apical, plumose setae (Fig. 5.3c).

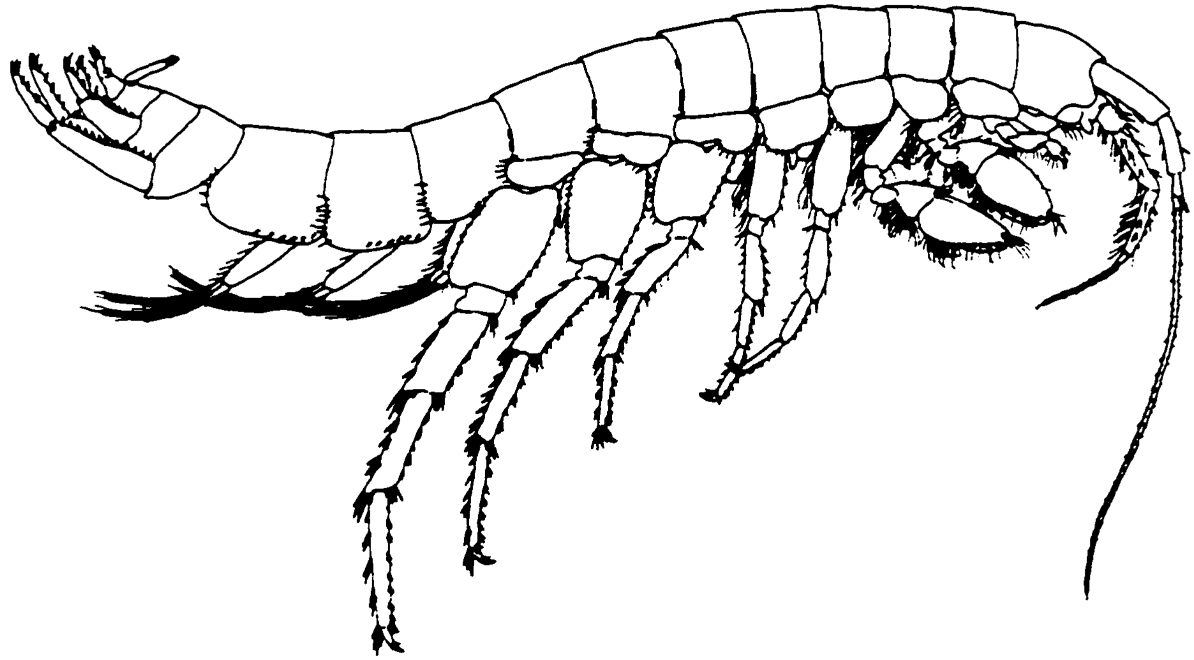


Fig. 5.1. *Bactrurus brachycaudus*, syntype female (19.0 mm) from Keifer Creek (St. Louis County, Missouri).

Maxilla 2 (Fig. 5.3e): Outer plate bearing apically up to 18 setae, some of which faintly plumose (comb-type, Fig. 5.3b). Inner plate with oblique row of 8-10 strong, plumose setae on inner margin and 20-26 plumose, apical and subapical setae (all plumose setae of same type, Fig. 5.3c).

Lower lip (Fig. 5.3f) bearing small setae on margins of outer lobes and on inner lobes; inner lobes very small; mandibular lobes short, with slightly rounded, pointed corners.

Maxilliped (Figs. 5.3g-i): Outer plate with row of 6-9 bladelike spines and up to 23 naked setae on inner margin. Inner plate with 2-4 naked, bladelike spines (one of which sometimes modified as plumose spine; see Fig. 5.6c) and 4-8 plumose setae on apical and subapical inner margin (brush-type setae, Fig. 5.3c; compare with mandible).

Gnathopod 1 (Figs. 5.4a-d): Basis bearing up to 29 long, naked setae on posterior margin, 2-6 plumose setae on distoposterior corner, 4-5 long, naked setae on proximoanterior margin, 3-4 setae on distoanterior margin and 6 or 7 short setae on medial surface. Carpus triangular, with 12-13 plumose setae on distal margin and up to 9 (usually 3-4) rastellate spines on distoposterior corner (Fig. 5.4b). Propod slightly shorter than propod of gnathopod 2; palm almost even, short part of distal margin finely serrate, armed with up to 17 spines on lateral margin and 7 spines on medial margin; defining angle rounded, shallow, with 6 short spines medially and 5 spines (long and short) on lateral corner (Fig. 5.4d); posterior (subangular) margin about 31% length of propod, with 3-6 sets of plumose setae (naked at defining angle);

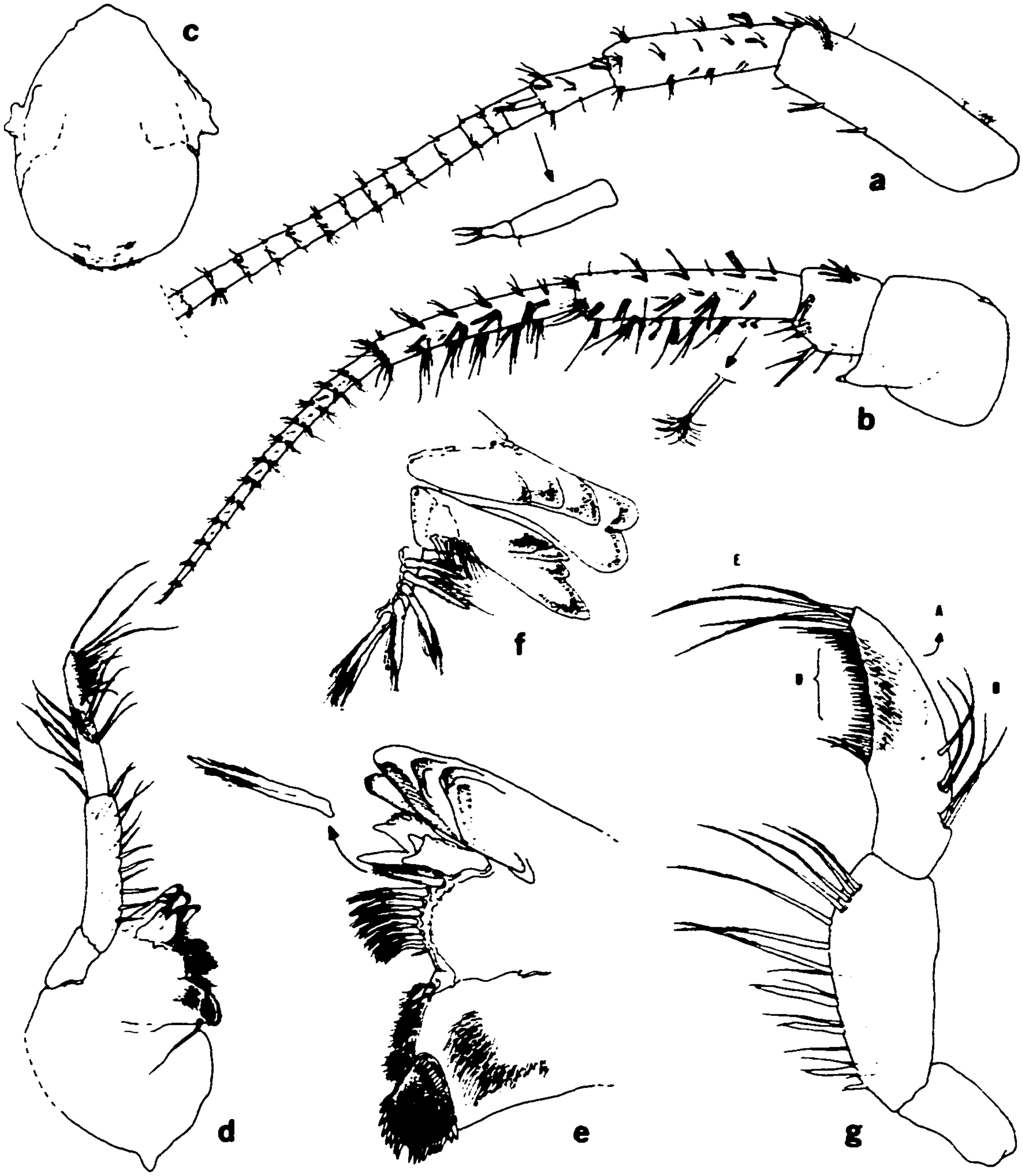


Fig. 5.2. *Bactrurus brachycaudus*, female (19.9 mm) from Keifer Creek (St. Louis County, Missouri): a) antenna 1 (accessory flagellum enlarged), b) antenna 2 (plumose setule enlarged), c) upper lip, d) left mandible, e) detail of right mandible, with 1 spine enlarged. Male (19.0 mm) from same locality: f) dentate part of left mandible, g) palp of left mandible; note that setal types on segment 3 are designated by uppercase letters in accordance with Stock (1974).

anteromedial surface bearing 5 rows of plumose setae (with 2-5 setae per row). Dactyl reaching about 79 % length of propod. Coxal plate wider than long, with 6-7 setae. All plumose setae of gnathopod 1 belong to comb-type (Fig. 5.3b).

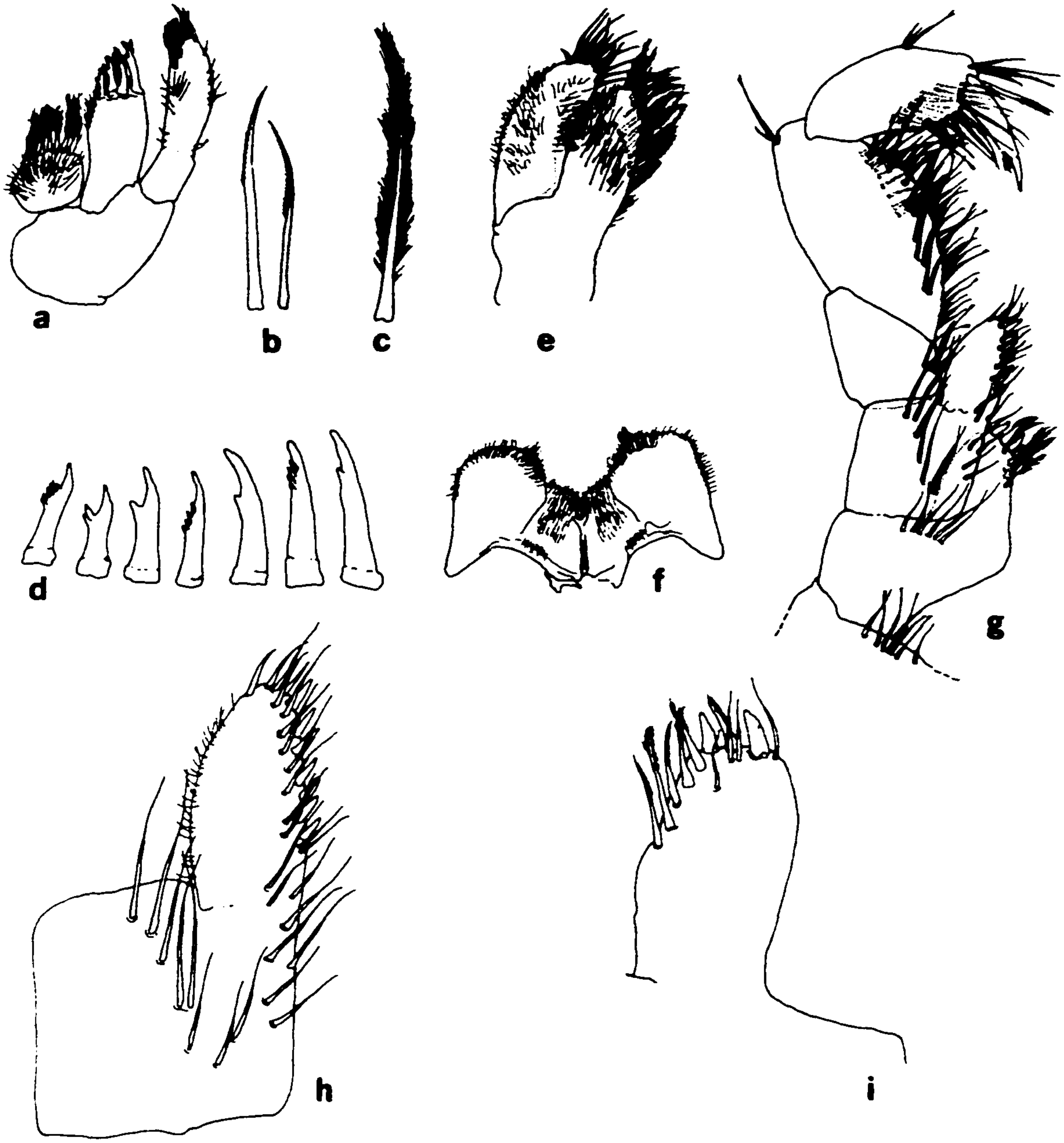


Fig. 5.3. *Bactrurus brachycaudus*, female (19.9 mm) from Keifer Creek (St. Louis County, Missouri): a) maxilla 1, b) comb-like setae of mouthparts and gnathopods, c) plumose seta of mouthparts, d) spine types of outer plate of maxilla 1, e) maxilla 2, f) lower lip, g) maxilliped, h) outer plate of maxilliped, i) inner plate of maxilliped.

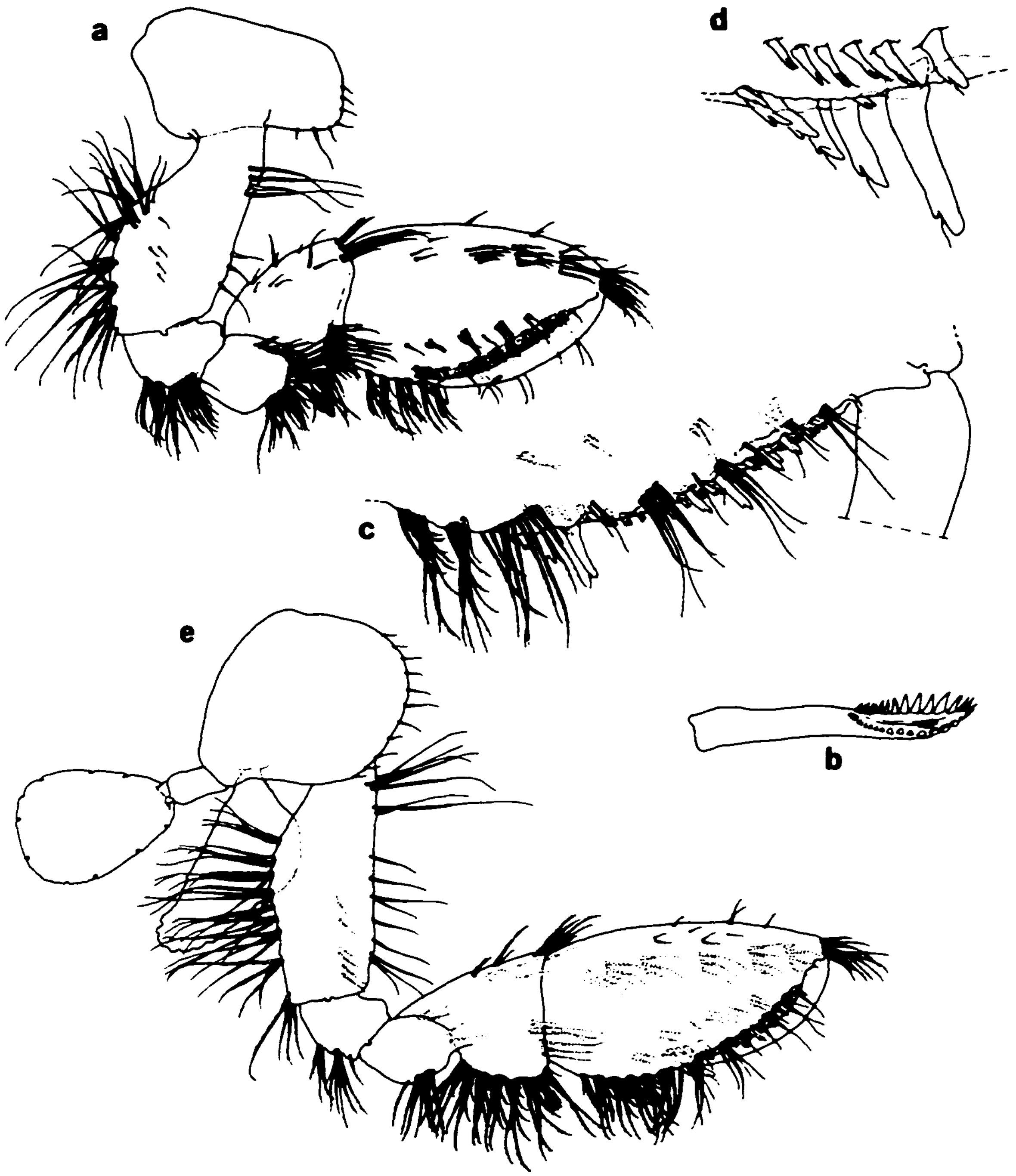


Fig. 5.4. *Bactrurus brachycaudus*, female (19.9 mm) from Keifer Creek (St. Louis County, Missouri): a) gnathopod 1 (medial view), b) rastellate spine on carpus of gnathopod 1, c) palm of gnathopod 1 (lateral view), d) defining angle (corner) spines of propod (gnathopod 1, medial view), e) gnathopod 2 (lateral view).

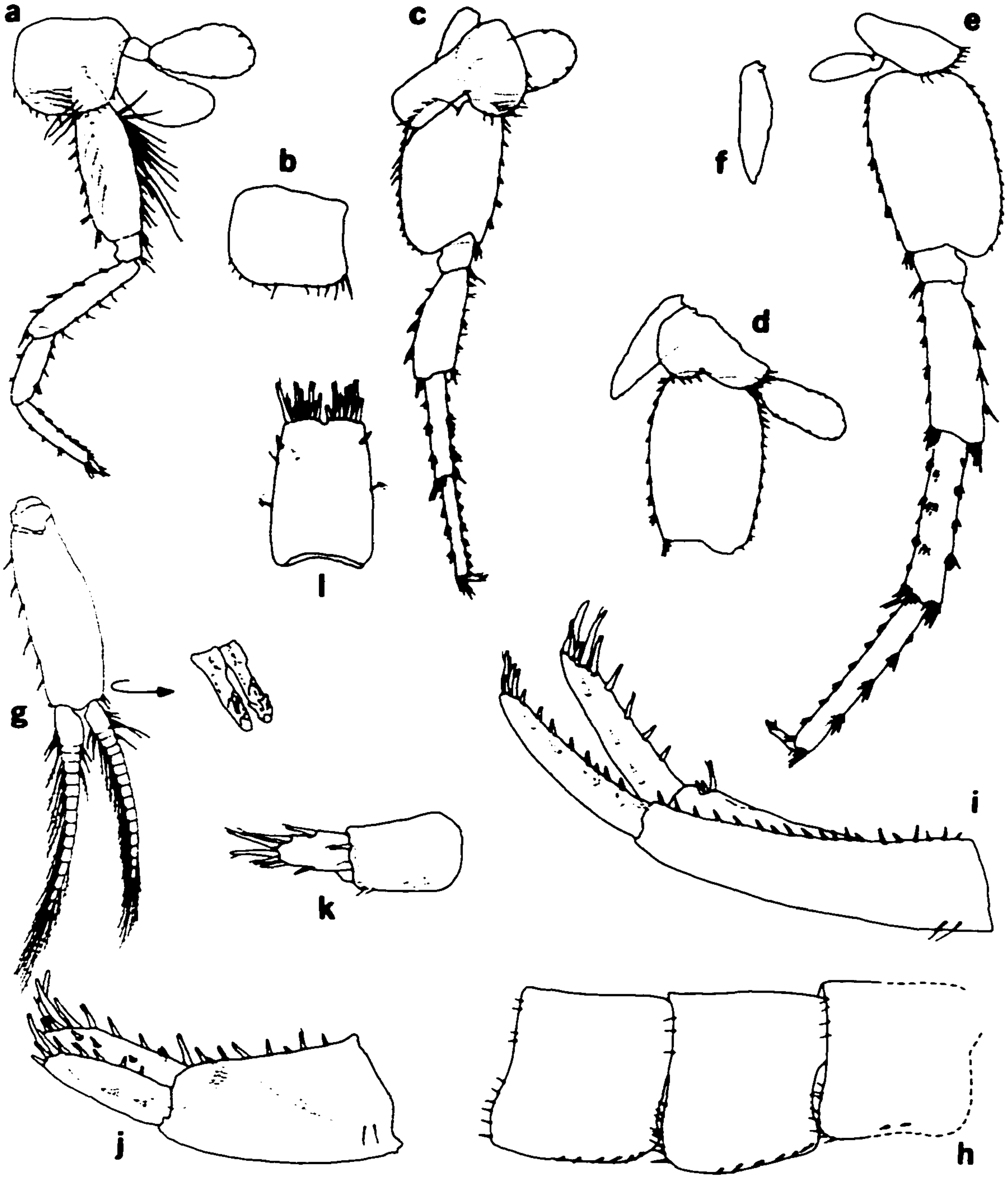


Fig. 5.5. *Bactrurus brachycaudus*, female (19.9 mm) from Keifer Creek (St. Louis County, Missouri): a) left pereopod 3, b) coxal plate of pereopod 4, c) right pereopod 5, d) proximal part of left pereopod 6, e) left pereopod 7, f) lateral sternal process of pereonite 7, g) pleopod 1 (retinaculae enlarged), h) epimeral plates 1-3, i-k) uropods 1-3, l) telson.

Gnathopod 2 (Fig. 5.4e): Basis with 9 sets of 2-4 long setae on posterior margin (all naked, except 2 distal sets with plumose setae), up to 7 long, naked setae on proximoanterior margin, up to 9 setae on distoanterior margin and 6-7 short setae on medial surface. Carpus triangular, with 7-10 sets of plumose setae on posterior margin, ca. 7 plumose setae on distal margin and up to 9 (usually 5) rastellate spines on distoposterior corner (Fig. 5.4b). Palm of propod slightly rounded, short part of distal margin finely serrate, bearing 18-22 spines on lateral margin and up to 24 spines on medial margin; defining angle rounded, very shallow, with 4-5 short, spines medially and 3-5 spines (long and short) on lateral corner; proximal (subangular) margin about 35% length of propod, with 8-9 sets of plumose setae (naked at defining angle); anteromedial surface bearing 7 rows of plumose setae. Dactyl reaching about 70 % length of propod. Coxal plate round, as wide as long, bearing 9 or 10 setae on distal margin. All plumose setae of gnathopod 1 belong to comb-type (Fig. 5.3b).

Pereopod 3 (Fig. 5.5a): Basis with up to 10 long setae on proximoposterior margin (excluding distoposterior corner), 2-6 long setae on proximoanterior margin and 6-10 long setae on medial surface. Coxal plate slightly wider than long, distal margin with to up to 14 setae.

Pereopod 4 subequal to pereopod 3. Coxal plate slightly wider than long, distal margin bearing up to 11 setae and 1 slender spine (Fig. 5.5b).

Pereopods 5-7: Plumose setae absent. Dactyls relatively short, typically about 15% percent length of corresponding propod. Anterior lobe of coxal plate of pereopod 5 bearing 4-7 setae; distoposterior corner with 1 slender spine (Fig. 5.5c). Pereopods 6 and 7 subequal, but pereopod 6 reaching only 69% length of pereopod 7. Anterior lobe of coxal plate of pereopod 6 with 2-4 setae; posterior margin bearing 2-4 setae and 1 slender spine (Fig. 5.5d). Coxal plate of pereopod 7 ovate, with 6-9 setae on posterior margin (Fig. 5.5e).

Coxal gills subovate, present on pereopods 2-7 (Figs. 5.4e; 5.5a, c-e), greatly reduced on pereopod 7. Two pairs of pointed sternal processes present on pereonites 6 and 7 (Figs. 5.5d, f).

Brood plates on pereopods 2-5 (Figs. 5.4e; 5.5a, c; not fully developed in specimen illustrated), distinctly smaller on pereopod 5.

Pleopods biramous (Fig. 5.5g); pleopod 3 slightly shorter than pleopods 1 and 2, which are subequal in length. Peduncle of pleopod 1 shorter than peduncles of pleopods 2 and 3; width of peduncles gradually increasing from pleopod 1 to 3. Inner rami about 12% longer than outer rami; first 3-6 proximal segments of both rami fused to form a single segment; both rami with subequal number of segments; number of unfused segments slightly decreasing from pleopods 1-3 (average number of rami segments: 16 in pleopod 1, 14 in pleopod 2 and 12 in pleopod 3; can be as many as 30 in large specimens).

Epimeral (pleonal) plates (Fig. 5.5h): Distoposterior margins bearing 4-8 setae (1-3 of which can be small spines on corner); ventral margins with 2 spines on plate 1 and 4-7 spines on plate 2 and 3, respectively.

Uropod 1 (Fig. 5.5i): Peduncle with up to 21 spines on dorsolateral margin and 3-4 spines on distomedial corner. Rami subequal, about 57% length of peduncle; inner ramus with 5 apical spines, and 7-

9 spines on dorsal surface. Outer ramus with 4-6 apical spines and 8-12 spines on dorsal surface.

Uropod 2 (Fig. 5.5j): Peduncle with up to 9 spines on dorsolateral margin, 1 or 2 spines on dorsomedial margin (sometimes absent), and a row of 5-6 spines near distomedial corner. Inner ramus slightly longer than outer ramus, about 68% length of peduncle, with 5-6 apical spines, and 7-9 spines on dorsal surface. Outer ramus with 4 apical spines and 5-7 spines on dorsal surface.

Uropod 3 (Fig. 5.5k): Peduncle bearing 1 spine on distal margin (sometimes 2 spines). Inner ramus vestigial, unarmed, about 37% length of outer ramus. Outer ramus short (approximately 71% length of peduncle), bearing up to 6 (usually 3-4) apical spines and 1-5 subapical spines.

Telson slightly tapered apically (Fig. 5.5l); width 58-73% of length (usually about 68%); lateral margins with 1 small plumose seta each (see enlarged seta of Fig. 5.2b); apical margin entire or slightly emarginate (1-2% cleft), bearing 16-20 spines.

Sexual dimorphism and variation: As already noted, the majority of specimens did not show any marked secondary sexual dimorphism. However, the propods of both gnathopods appeared to have somewhat sinusoid palmar margins in some of the larger males (> 25 mm).The propod of gnathopod 1 was noticeably larger than the propod of gnathopod 2 in some large males. One 26 mm male (with sinusoid palmar margins on propods of gnathopod 1 and 2) had a double (U-shaped) row of 12 rastellate spines on the carpus of gnathopod 2.

No marked morphological variation could be observed in the majority of the specimens examined. Minor variation occurred in the number of aesthetascs on both antennae, which were strongly reduced or absent in some specimens. Palp segments 1 and 2 on maxilla 1 were weakly articulated in most specimens, apparently indicating an early stage of fusion. The strong apical, plumose spine on the inner plate of the maxilliped appears to be a plumose seta (brush-type, Fig. 5.3c) in some specimens. Variation was also found in the number of subapical, lateral spines on the telson (some specimens had 1-3 subapical spines) and the width/length ratio of the telson.

Marked variation, however, was found only in two populations at opposite edges of the distribution range of *B. brachycaudus*: 39 specimens, collected from drain pipe outlets in central Illinois (Montgomery and Sangamon Co.), showed sexual dimorphism in the width/length ratio of the telson. The majority of adult males had telsons that were about 20% longer than those of the female. The same sexually dimorphic width/length ratio could be observed in a cavernicolous population from central Missouri (Texas Co.). In addition, these specimens had median sternal (mediosternal) processes on pereonites 2 and 3. Both populations were characterized by a relatively small average body size of adult specimens (9-13 mm), resulting in an proportionate reduction of segments and armature of some appendages, for example, the number of flagellar segments in antennae 1 and 2 and the number of spines and setae on mouthparts and pereopods. Most of diagnostic characters, however, left no doubt that both populations were geographic variants and belonged to *B. brachycaudus*. This assumption was supported by relative differences of DNA sequence data (Table V.2).

Remarks: With some large specimens reaching up to 31 mm in length, *B. brachycaudus* is not only one of the largest subterranean amphipod species known to science but is also the largest freshwater amphipod on the North American continent. *Bactrurus brachycaudus* is reported from various subterranean habitats in Illinois and Missouri, including mostly caves, but also springs and seeps, and, as the May 1999 collection trip revealed, in drainage systems in glaciated areas (see Fig. 5.28). To date, formerly glaciated areas in Ohio, Indiana, Illinois, Iowa and Michigan were assumed to be inhabited by only one species of the genus, *B. mucronatus*. Remarkably, these are the first recorded occurrences of *B. brachycaudus* from drain pipe outlets in glacial drift areas. In one locality (Montgomery Co., Illinois), *B. mucronatus* and *B. brachycaudus* were found together in the same drain pipe catchment basin, marking first collection of these species from the same locality.

Bactrurus mucronatus and the new species *B. pseudomucronatus* can be differentiated from *B. brachycaudus* by an exceptionally long telson and a tapered outer ramus on uropod 3, bearing only a few spines. *Bactrurus hubrichti* differs from *B. brachycaudus* by having a distinctly emarginate telson with relatively long apical spines and the presence of plumose spines on the merus of gnathopod 1. The presence of a serrate peduncular process on the male uropod 1 distinguishes *B. brachycaudus* from the new species *B. wilsoni*, *B. angulus* and *B. cellulanus*.

Aquatic crustacean fauna associated with *B. brachycaudus* includes the amphipods *Gammarus troglophilus*, *Crangonyx forbesi*, *Stygobromus* n. sp. (Holsinger, in ms.), *B. mucronatus*, *Crangonyx packardi*, *G. pseudolimnaeus*, and the isopods *Caecidotea* spp.

Sex ratios: The sex ratio of 72 specimens of *B. brachycaudus* from Fern Glen (St. Louis County, Missouri) was female biased (61%). Approximately 3/4 of other *Bactrurus* species with sample sizes of 10 or more specimens also showed a differential sex ratio favoring females. A study of sex ratios in 12 species of *Stygobromus* yielded similar data (Culver & Holsinger, 1969).

A female biased sex ratio may be related to the unique properties of subterranean habitats, which are typically characterized by limited food resources and restricted habitat space. Species with low dispersal abilities are more easily subjected to inbreeding, which in turn leads to 'local mate competition' (Krebs & Davies, 1993): If habitat space is limited, a female will increase her reproductive success if she produces just as many sons as needed to fertilize her daughters. From an energy economy point of view, any additional sons are wasted since they compete with their brothers for mating opportunities with available females from the same brood. As a result, populations affected in this way tend to develop female biased sex ratios.

Bactrurus hubrichti **Shoemaker, 1945**

Figs. 5.6-8

Bactrurus hubrichti Shoemaker, 1945: 27, Fig. 2; type locality: well at Topeka (Shawnee County, Kansas).-- Barnard, 1958: 44.-- Hubricht, 1959: 828.-- Nicholas, 1960: 127.-- Holsinger, 1972: 74, Fig. 30d; 1977: 277, Figs. 8c, 14j, 15d; 1986a: 95, Fig. 6; 1986b: 542.-- Barnard & Barnard, 1983: Figs. 18f, 20d, 21b, map 12.-- Fitzpatrick, 1983: 144.

Material examined: **KANSAS: Bourbon Co.:** Seep, 2.3 mi. S of Bronson, 3 males, 2 females (USNM), L. Hubricht, 17 May 1942. **Butler Co.:** Hourglass Cave, 4 males, 6 females (JRH and KBS), W. H. Busby and J. J. Young, 7 Sept. 1991; 1 male (JRH), W. H. Busby and D. Figg, 3 May 1989. **Chautaugua Co.:** Mill's Cave, 1 male (JRH), W. H. Busby and J. J. Young, 30 Jun., 1991. Dry Crawl Cave, 3 males, 4 females (JRH and KBS), W. H. Busby and J. J. Young, 30 Jun., 1991. **Clay Co.:** Spring, ca. 1 mi. NNW of rt. 82 and Knollwood in Wakefield, 1 male (JRH), N. W. Youngsteadt, 20 May 1980. **Cowley Co.:** Shallow, hand-dug well, ca. 8 mi. SE of Dexter (on Metcalf farm), 13 males, 5 females , 7 juveniles (JRH), A. C. Metcalf, 1968-1970. Abandoned well, 4 mi. S of Burden, 4 males, 6 females (JRH and KBS), W. A. Busby and J. J. Young, 30 Jun. 1991. **Neosho Co.:** Hand-dug well, 1 mi. N of Parson's Reservoir dam, 1 female (JRH), J. Danoff-Burg, 13 Jul. 1991. **Riley Co.:** Endler Spring, Konza Prairie Research Natural Area, 1 male, 1 female (JRH), W. Dobbs, 8 Jun. 1995. Spring, north fork of Kings Creek, Konza Prairie Research Natural Area, 9 specimens (KBS), M. Moffett, 28 May, 1981. Shallow well flow, Konza Prairie Research Natural Area (in Flints Hills area), 1 female (JRH), M. Whiles and K. Hooker, 20 Jun. 1986; 3 females (JRH), 23 Jul. 1986. **Shawnee Co.:** Well at Topeka, 12 specimens (**paratypes** in USNM) collected by A. Popenoe as follows: 1 female, 9 Jun. 1911; 1 male, 15 Feb. 1912; 1 male, 11 Feb. 1912; 1 female, 23 Feb. 1912; 2 males, 1 female, 25 Feb. 1912 (2 samples); 2 females, 5 Apr. 1912; 1 female, 9 Apr. 1912; 1 male, 4 May 1912; 1 male, 25 Sept. 1913. **MISSOURI: Miller Co.:** Klugs Cave, 2 males, 3 females, 2 juveniles (USNM), L. Hubricht, 24 Aug. 1940. **OKLAHOMA: Muskogee Co.:** Well, near Connors State College, Warner, 1 female (USNM), A. Seamster, 6 Jan. 1939. **Rogers Co.:** Unnamed spring, ca. 3.5 mi. S of Oolagah, 1 male, 1 juvenile (JRH), J. J. Hoover and W. B. Milstead, 1 Jun. 1981.

Diagnosis: *Bactrurus hubrichti* is a medium-sized to large species without marked secondary sexual dimorphism. Propod of gnathopod 1 distinctly larger than propod of gnathopod 2. Pereopod 5 as long as or slightly longer than pereopod 4 (5-14%). Coxal gills on pereopods 2-7; coxal gill on pereopod 7 only slightly reduced. Three pairs of lateral sternal processes on pereonites 6 and 7 and pleonite 1; median sternal processes absent. Telson cleft, wider than long. Largest male 22.0 mm, largest female 20.5 mm.

Description: *Bactrurus hubrichti* is morphologically closely allied with *B. brachycaudus*. The following description is based on an adult female (19.5 mm) and adult male (17.5 mm).

Antenna 1 72-92% length of body (sometimes exceeding length of body). Primary flagellum with up to 40 segments.

Antenna 2 about 37% length of antenna 1. Flagellum with up to 14 segments.

Lower lip subequal to that of *B. brachycaudus* (Fig. 5.6a).

Mandibles: Spine row with 7-9 plumose spines. Right lacinia mobilis with row of irregular, relatively long denticles on distal margin (Fig. 5.6b). Palp segment 2 without spines, bearing up to 17 setae on lateral and medial margins; palp segment 3 bearing up to 33 short, plumose D-setae, about 3 long, plumose B-setae and 2-4 long, plumose A-setae.

Maxilla 1 subequal to that of *B. brachycaudus*. Inner plate with 5-6 apical, plumose setae.

Maxilla 2 subequal to that of *B. brachycaudus*. Inner plate with oblique row of 6-7 strong, plumose setae.

Maxilliped: Outer plate with 5-7 bladelike spines on inner margin. Inner plate with 3-6 naked, bladelike spines (one of which sometimes modified as plumose spine; Fig. 5.6c) and 3-4 plumose setae on apical and subapical inner margin.

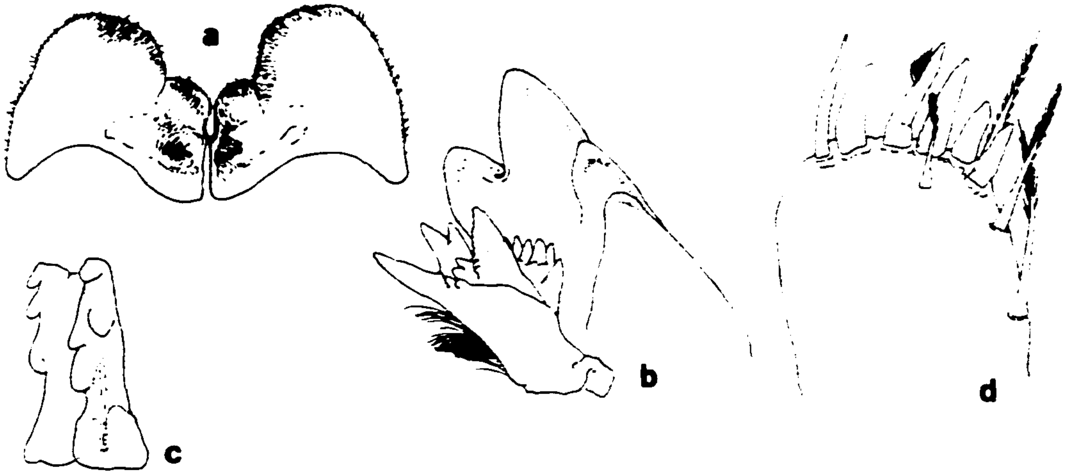


Fig. 5.6. *Bactrurus hubrichti*, female (19.5 mm) from an abandoned well (Cowley County, Kansas): a) lower lip, b) dentate part of right mandible, c) retinaculae of pleopods. Male (17.5 mm) from same locality: d) distal part of inner plate of maxilliped.

Gnathopod 1 (Fig. 5.7a): Basis with 6-8 sets of long, naked setae on posterior margin (with up to 17 setae in total), about 4 plumose setae on distoposterior corner, 5-6 long, naked setae on proximoanterior margin, 3 shorter setae on distoanterior margin and up to 8 short setae on medial surface. Merus with 2-3 plumose spines (Fig. 5.7c). Carpus bearing 11 plumose setae on distal margin and 2-3 rastellate spines on distoposterior margin (Fig. 5.7d). Propod wider and longer than propod of gnathopod 2; palm slightly convex, at least 2/3 of palmar margin finely serrate, bearing 17-20 spines on lateral margin and 11-15 spines on medial margin; defining angle distinct (ca. 135°), with 2-3 spines medially and 3-6 spines on lateral corner; posterior (subangular) margin 24% length of propod, with 4 sets of plumose setae; anteromedial surface bearing 5 rows of plumose setae. Dactyl reaching about 77 % length of propod. Coxal plate bearing 6-8 setae.

Gnathopod 2 (Fig. 5.7b): Basis with 5-7 sets of 2-3 long setae on posterior margin, ca. 4 plumose setae on distoposterior corner, about 4 long, naked setae on proximoanterior margin, 6-7 (mostly short) setae on distoanterior margin and a row of 7 (mostly) short setae on medial surface. Carpus with 6-7 sets of plumose setae on posterior margin, about 5 plumose setae on distal margin and up to 4-5 rastellate spines on distoposterior corner (Fig. 5.7d). Palm of propod slightly rounded, finely serrate along whole margin, with 13-17 spines on lateral margin and 12-14 spines on medial margin; defining angle rounded, very shallow, with row of 5-6 spines on medial corner and a row of 4 spines on lateral corner; proximal (subangular) margin ca. 42% length of propod, with 5 sets of plumose setae; anteromedial surface bearing 5 rows of plumose setae. Dactyl 70 % length of propod. Coxal plate round, as wide as long, bearing 11-15 setae on distal margin.

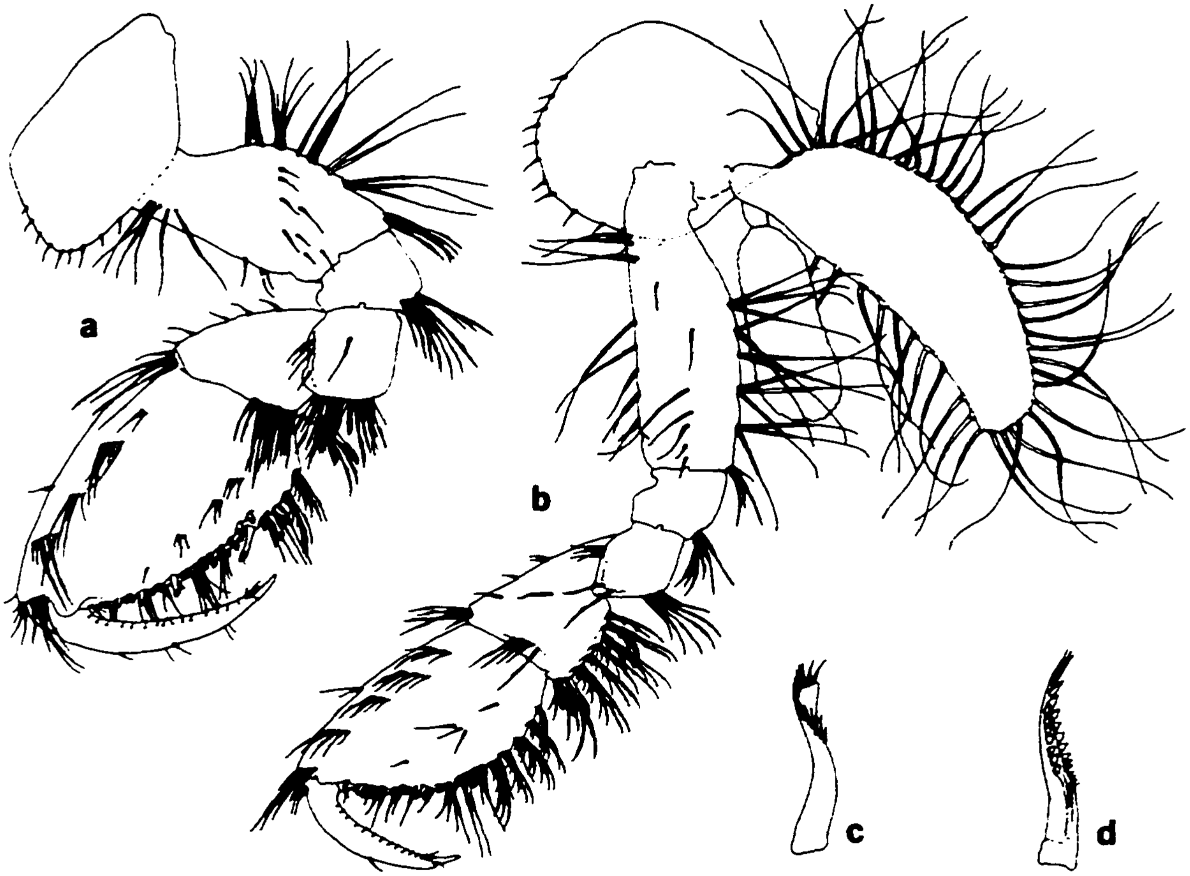


Fig. 5.7. *Bactrurus hubrichti*, female (19.5 mm) from an abandoned well (Cowley County, Kansas): a) gnathopod 1 (medial view), b) gnathopod 2 (medial view), c) dentate spine of merus of gnathopod 1, d) rastellate spine of carpus of gnathopods 1 and 2.

Pereopod 3 (Fig. 5.8a): basis with up to 8 long setae and 2 short setae on proximoposterior margin, 6 short setae on anterior margin and 11 long setae on medial surface. Coxal plate with to up to 11 setae.

Pereopod 4: Coxal plate with 10 setae and 1 slender spine (Fig. 5.8b).

Pereopods 5-7: Coxal plate of pereopod 5 with 5 setae on distoanterior lobe, distoposterior corner with 1 slender spine and 1 seta (Fig. 5.8c). Coxal plate of pereopod 6 with 3 setae on distoanterior lobe, posterior margin with 4 setae (Fig. 5.8d). Coxal plate of pereopod 7 with 5 setae on posterior margin (Fig. 5.8e).

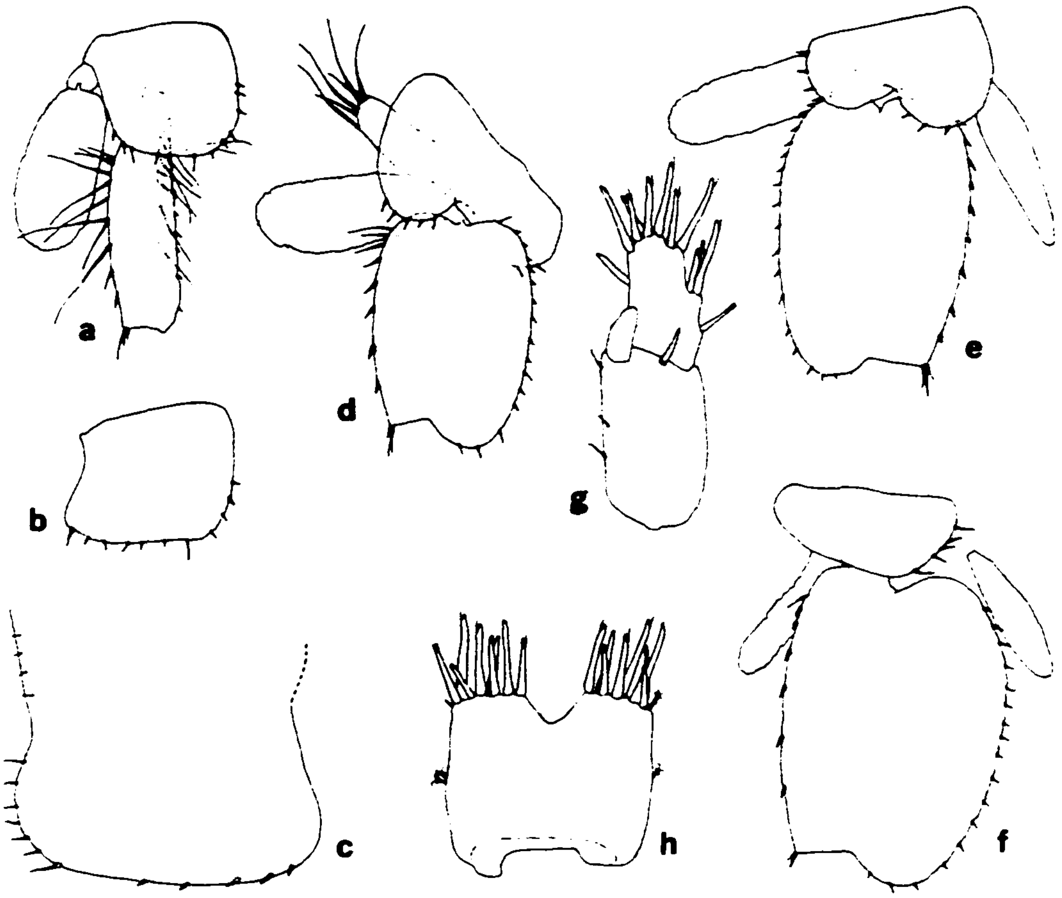


Fig. 5.8. *Bactrurus hubrichti*, male (17.5 mm) from an abandoned well (Cowley County, Kansas): a) right pereopod 3, b) coxal plate of pereopod 4, c) epimeral plate 2. Female (19.5 mm) from same locality: d) left pereopod 5, e) right pereopod 6, f) left pereopod 7, g) uropod 3, h) telson.

Coxal gill on pereopod 7 only slightly reduced (Fig. 5.8e). Three pairs of pointed, lateral sternal processes present on pereonites 6 and 7 and pleonite 1 (Figs. 5.8d, e).

Pleopods Number of unfused segments of outer and inner ramus as follows: pleopod 1 with 15-18 segments, pleopod 2 with 15 segments and pleopod 3 with 10 segments. Retinaculae subequal to those of *B. brachycaudus* (Fig. 5.6d).

Epimeral plates: Distoposterior margins bearing 6-8 short setae; ventral margins: plate 1 without spines, plates 2 and 3 with 4-5 spines, respectively (Fig. 5.8f).

Uropod 1: Peduncle with about 18 spines along dorsolateral margin and row of 4 spines on distomedial corner. Inner ramus with 3-4 apical spines and about 11 spines on dorsal surface. Outer ramus 59% of length peduncle, with 3-4 apical spines and up to 17 spines on dorsal surface.

Uropod 2: Peduncle with ca. 6 spines on dorsolateral margin, and a row of 3-5 spines near distomedial corner. Inner ramus slightly longer than outer ramus, 74% of length peduncle, bearing 4 apical spines and 9-11 spines on dorsal surface. Outer ramus with 3-4 apical spines and 5-11 spines along dorsal surface.

Uropod 3 (Fig. 5.8g): Peduncle with 1 spine on distal margin (sometimes absent). Inner ramus about 45% length of outer ramus. Outer ramus ca. 73% length of peduncle, bearing 4-7 apical spines, 2-8 subapical spines on lateral margin and 1 spine on medial margin (sometimes absent).

Telson subquadrate, usually 11-14% wider than long (sometimes as long as wide), cleft 18-25% length of telson; apical lobes with 6-10 spines each.

Sexual dimorphism and variation: The specimens examined did not show any secondary sexual dimorphism or marked morphological variation.

Remarks: *Bactrurus hubrichti* occurs in caves, springs, seeps and wells in eastern Kansas, central Missouri and northeastern Oklahoma. It can be easily distinguished from all other *Bactrurus* species by a distinctly cleft telson, which is usually wider than long, and the presence of plumose spines on the merus of gnathopod 1.

Aquatic crustacean fauna associated with B. hubrichti includes the amphipods *Stygobromus clantoni*, *Crangonyx packardi*, and the isopod *Caecidotea* sp.

A single, 9.2 mm male specimen (USNM 81545) was found in a vial with a label that read: "well at Fairview, Loudon Co., VA, ca. 6 mi. from Bull Run Mountain, Aug. 5, 1924, Mr. March". The specimen was in a bad condition, but it perfectly matched the description of *B. hubrichti*. Because *B. hubrichti* occurs in the middle-western US without any known occurrence east of the Mississippi River, we have concluded that the Virginia collection was mislabeled.

***Bactrurus mucronatus* (Forbes, 1876)**

Figs. 5.9-13

Crangonyx mucronatus Forbes, 1876: pp. 6, p. 21; Figs. 1-7; type locality: well, Normal (McLean County, Illinois).-- O. P. Hay, 1882: 241.-- Schellenberg, 1936: 34.

Eucrangonyx mucronatus (Forbes).-- Stebbing, 1899: 423; 1906: 388.-- Weckel, 1907: 29, Fig. 2.

Bactrurus mucronatus (Forbes).-- W. P. Hay, 1902: 430.-- Hubricht & Mackin, 1940: pp. 200.-- Hubricht, 1943: 693.-- Shoemaker, 1945: p. 24, Fig. 1 (redescription).-- Barnard, 1958: 44.-- Hubricht, 1959: 878.-- Nicholas, 1960: 127 [in part].-- Holsinger, 1972: 75, Fig. 30d; 1977: 265, Figs. 8c, 14j, 15d; 1981: 93; 1986a: 95-96, Fig. 6; 1986b: 542.-- Peck & Lewis, 1978: 45.-- Barnard & Barnard, 1983: map 12.-- Fitzpatrick, 1983: 144.-- Lewis, 1983: 36.

Material examined: **ILLINOIS**: **Champaign Co.**: Big concrete drain pipe into drainage ditch, 3.2 mi. N of Mayview, 7 specimens (JRH), S. Koenemann and U. Englisch, 13 May 1999. Old well, Champaign, 1 male **NEOTYPE** (USNM 81546) 3 males, 7 females and 1 juveniles (USNM), collector not given, 29 Mar.1902. Drainage ditch, Savoy; 2 females (USNM), H. J. Van Cleave, 9 May 1942. Old well, Urbana, 4 males, 12 females (USNM), J. G. Mackin, date not given. Drain tile discharge (at T20N, R10E, 529+35), 15 males, 25 females, 2 juveniles (JRH), A. Seidenberg, 28 Feb. 1967. **Coles Co.:** Drain pipes into ditch or little stream, ca. 6 mi. N of Charleston, 15 specimens (JRH), S. Koenemann and U. Englisch, 13 May 1999. **Fulton Co.:** Outlet of drain, 1.4 mi. S of Avon, 13 specimens (USNM), L. Hubricht, 4 May 1941. **Gallatin Co.:** Drain pipes, ca. 5 mi. S of Omaha, 1 female (JRH), S. Koenemann and U. Englisch, 15 May 1999. **Henderson Co.:** Outlet of drain, 3.0 mi. E of Biggsville, over 10 specimens (USNM), L. Hubricht, 25 Apr. 1942. **Knox Co.:** Outlet of drain, 2 mi. N of Abingdon, 1 male (USNM), L. Hubricht, 4 May 1941. Outlet of drain, 3.1 mi. N of St. Augustine, ca. 50 specimens (USNM), L. Hubricht, 4 May 1941. Outlet of drain, 1.5 mi. SE of Williamsfield, >50 specimens (USNM), L. Hubricht, 3 May 1941. **La Salle Co.:** Outlet of drain, Gustave Engelhaupt farm, just N of Peru, >50 specimens (USNM), L. Hubricht, 4 May 1941. **McDonough Co.:** Outlet of drain, 4.8 mi. N of Macomb, >100 specimens (USNM), L. Hubricht, 4 May 1941. Outlet of drain, 3.0 mi. S of Colmar, >125 specimens (in 2 lots) (USNM), L. Hubricht, 4 May 1941. **Montgomery Co.:** Big iron drain pipe into catchment basin (high volume output), ca. 5 mi. W of Morrisonville, 1 male (JRH), S. Koenemann and U. Englisch, 15 May 1999. **Moultrie Co.:** Old concrete drainage catchment basin, ca. 5 mi. WSW of Mattoon, 8 specimens (JRH), S. Koenemann and U. Englisch, 13 May 1999. **Peoria Co.:** Outlet of drain, 1.5 mi. E of Laura, 7 specimens (USNM), L. Hubricht, 4 May 1941. **Saline Co.:** Equality Cave (Cave Hill Cave), 26 specimens (USNM), L. Hubricht, 22 Jun. 1940. 3 males, 6 females (JRH), J. Holsinger and R. Norton, 14 Jun. 1965; 1 female (JRH), S. Peck, 23 Oct. 1965; 3 males, 2 females (JRH), D. A. Hubbard, Jr., 8 Aug. 1992; 8 specimens (JRH), S. Koenemann and U. Englisch, 14 May 1999. **Vermillion Co.:** Outlet of drain, 1.0 mi. W of Fairmont, >50 specimens (USNM), R. W. Larimare, 11 Apr. 1952. **Warren Co.:** Outlet of drain, 5.2 mi. E of Biggsville, >25 specimens (USNM), L. Hubricht, 25 Apr. 1942. Outlet of drain, 2 mi. SE of Cameron, 5 females (USNM), L. Hubricht, 25 Apr. 1942. **INDIANA: Grant Co.:** Outlet of drain, 5 mi. W of Jonesboro, 4 specimens (USNM), L. Hubricht, 27 Apr. 1941. Drain pipes at little bridge, ca. 5 mi. W of Jonesboro, ca. 40 specimens (JRH), S. Koenemann, U. Englisch and J. Lewis, 10 May 1999. **Henry Co.:** Drain tiles, 3.2 mi. W of Knightstown, 11 specimens (JRH), S. Koenemann, U. Englisch and J. Lewis, 10 May 1999. Outlet of drain, 3.8 mi. W of Knightstown, >25 specimens (USNM), L. Hubricht, 17 Apr. 1942. Outlet of drain, 3.5 mi. W of Knightstown, 4 males, 4 females (in 2 lots) (USNM), L. Hubricht, 17 Apr. 1942. **Marion Co.:** Well or swamp (?), Irvington, 4 males, 12 females (USNM), W. P. Hay, date not given. **Montgomery Co.:** Drain tiles, ca. 1/2 mi. E of jct. 47/234, ca. 75 specimens (JRH), S. Koenemann and U. Englisch, 11 May 1999. **Noble Co.:** Outlet of drain, 1.4 mi. W of Wawaka, 1 female (USNM), L. Hubricht, 19 Apr. 1942. **Shelby Co.:** Drain tile, 3-4 mi. N of Hope, 1 male (JRH), S. Koenemann, U. Englisch and J. Lewis, 10 May 1999. **IOWA: Des Moines Co.:** Outlet of drain, 0.2 mi. NW of Danville, 4 males, 3 females (USNM), L. Hubricht, 24 Apr. 1942. **Henry Co.:** Outlet of drain, 1.7 mi. S of Swedesburg, >100 specimens (in 2 lots) (USNM), L. Hubricht, 24 Apr. 1942. Outlet of drain, 1.4 mi. S of New London, 23 specimens (USNM), L. Hubricht, 24 Apr. 1942. **Washington Co.:** Outlet of drain, 0.5 mi. S of Haskins, 12 specimens (USNM), L. Hubricht, 24 Apr.1942. Outlet of drain, 1.0 mi. S of Haskins, >10 specimens (USNM), L. Hubricht, 24 Apr. 1942. **MICHIGAN: Berrien Co.:** Bear Cave, just N of Buchanan, 1 male, 2 females (JRH), D. A. Hubbard, Jr., 28 Dec. 1993. **Monroe Co.:** Outlet of drain, 0.5 mi. N of Ottawa Lake, >25 specimens (USNM), L. Hubricht, 18 Apr. 1942. Outlet of drain, 1.5 mi. WSW of Dundee, 18 specimens (USNM), L. Hubricht, 18 Apr. 1942. **OHIO: Butler Co.:** Dug well, Francis farm at Shandon, 1 male, 1 female (USNM), S. R. Williams, date not given. **Clinton Co.:** 15 foot-deep well, Starbucktown, Liberty Turnpike, 1 male (USNM), collector not given, 19 Oct. 1939. **Logan Co.:** Drain pipe outlet, 2.4 mi. SW of Middlesboro, 2 males, 1 female (USNM), L. Hubricht, 17 Apr. 1942. **Lucas Co.:** Drain pipe outlet, 1.3 mi. S of Reynolds Corner, >10 specimens (USNM), L. Hubricht, 18 Apr. 1942. **Marion Co.:** High pressure groundwater pumps at sewage construction site, ca. 2-3 mi. S of Marion, 2 males, 1 female, 3 juveniles (JRH), S. Koenemann and U. Englisch, 6 May 1999. Drain pipe outlet, 8.6 mi. SSW of Marion, 2 males (USNM), L. Hubricht, 18 Apr. 1942. **Montgomery Co.:** Driven well, Phillipsburg, 1 male (USNM), J. S. Hauser, Feb. 1930. Drain pipe outlet, 4.4 mi. E of New Lebanon, 9 specimens (USNM), L. Hubricht, 17 Apr. 1942. **Preble Co.:** Drain pipe, 5 mi. W of Eaton, 13 specimens (JRH), S. Koenemann and U. Englisch, 8 May 1999; >20 specimens (USNM), drain pipe outlet, 0.2 mi. NW of New Hope, L. Hubricht, 17 Apr. 1942. **Wayne Co.:** Crayfish burrow, Wooster, (at R14W, T20N, Sec. 25), 1 male, 1 female (JRH), A. Weaver, 21 Apr. 1960. **Wood Co.:** Drain pipe outlet, 1.3 mi. SE of Perrysburg, ca. 100 specimens (USNM), L. Hubricht, 18 Apr. 1942. **County ?** (not given): Drilled well, 2 females (USNM), collector and date not given.

Diagnosis: *Bactrurus mucronatus* is a medium-sized species with conspicuous secondary sexual dimorphism in the telson: Telson in adult males distinctly elongate, reaching 34% length of body (up to 3 times longer than female telson). Propods of gnathopod 1 and 2 subequal in size. Pereopod 5 slightly shorter than pereopod 4 (8-9%). Coxal gills on pereopods 2-6. Three pairs of lateral sternal processes on pereonites 6, 7 and pleonite 1 (sometimes absent on pleonite 1); median sternal processes absent. Telson entire or slightly emarginate (2%); width of female telson ca. 54% of length. Most adult specimens 6.0-9.0 mm; largest male 15.5 mm, largest female 12.0 mm.

Description: Based on adult female (12.0 mm) and adult male (15.5 mm; Fig. 5.9).

Antenna 1 73-75% length of body (Fig. 5.10a). Primary flagellum with up to 39 segments.

Antenna 2 about 41-50% length of antenna 1 (Fig. 5.10b). Flagellum with up to 12 segments.

Lower lip with short mandibular lobes (Fig. 5.11c)

Mandibles: Row of 8 plumose spines between molar and lacinia mobilis; distal margin of right lacinia mobilis irregularly serrate (Fig. 5.10c). Palp segment 2 usually without spines, bearing 9-11 setae on lateral and medial margins (1 female with 2 spines and 9 setae); palp segment 3 with ca. 28 short, plumose D-setae, about 3 long, plumose B-setae and 2-4 long, plumose A-setae.

Maxilla 1 (Fig. 5.10d, e): Palp segment 2 bearing apically and subapically 8-12 naked setae and 1 or 2 stronger, plumose setae (comb-type, Fig. 3b). Inner plate with 6-8 apical, plumose setae.

Maxilla 2 (Fig. 5.10f): Inner plate with oblique row of 6-7 strong, plumose setae.

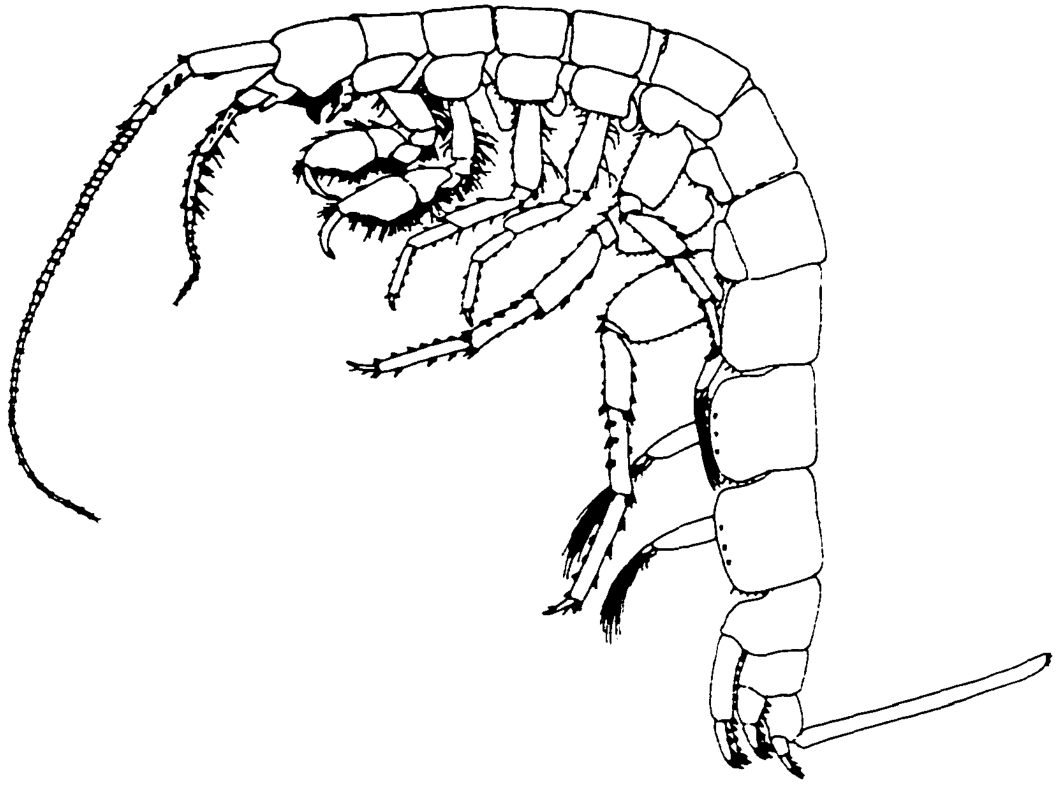


Fig. 5.9. *Bactrurus mucronatus*, male (15.5 mm) from an old well near Champaign (Champaign County, Illinois).

Maxilliped: Outer plate armed with 5-8 bladelike spines on inner margin. Inner plate apically with 3-6 naked, bladelike spines (one of which sometimes modified as plumose spine; see Fig. 5.6c) and 3-7 plumose setae apically and subapically.

Gnathopod 1 (Figs. 5.11b): Basis with 5-9 sets of long, naked setae on posterior margin (with up to 20 setae in total), ca. 4 plumose setae on distoposterior corner, 3-4 long, naked setae on proximoanterior margin, 2 shorter setae on distoanterior margin and 6-7 short setae on medial surface. Carpus with up to 4 rastellate spines on distoposterior margin and 9 plumose setae on distal margin (see Fig. 5.11b). Propod subequal in size to propod of gnathopod 2; palmar margin slightly concave, finely serrate at proximal and distal ends, bearing 12-14 spines on lateral margin and approximately 10 spines on medial margin; defining angle rounded, shallow (sometimes distinct in adult males: 140°), with 6 spines medially, 5-6 short spines on lateral corner and 3 strong spines between medial and lateral corner spines; posterior (subangular) margin 28% length of propod, with about 17 plumose setae (naked at defining angle); anteromedial surface bearing 5-8 rows of plumose setae. Dactyl ca. 81% length of propod. Coxal plate with 6-7 setae.

Gnathopod 2 (Fig. 5.11a): Basis with 5-7 sets of long setae on posterior margin (with 13-18 setae in total), ca. 4 plumose setae on distoposterior corner, 3-4 long, naked setae on proximoanterior margin, 1-4 naked setae on distoanterior margin (sometimes absent) and a row of 6 short setae on medial surface.

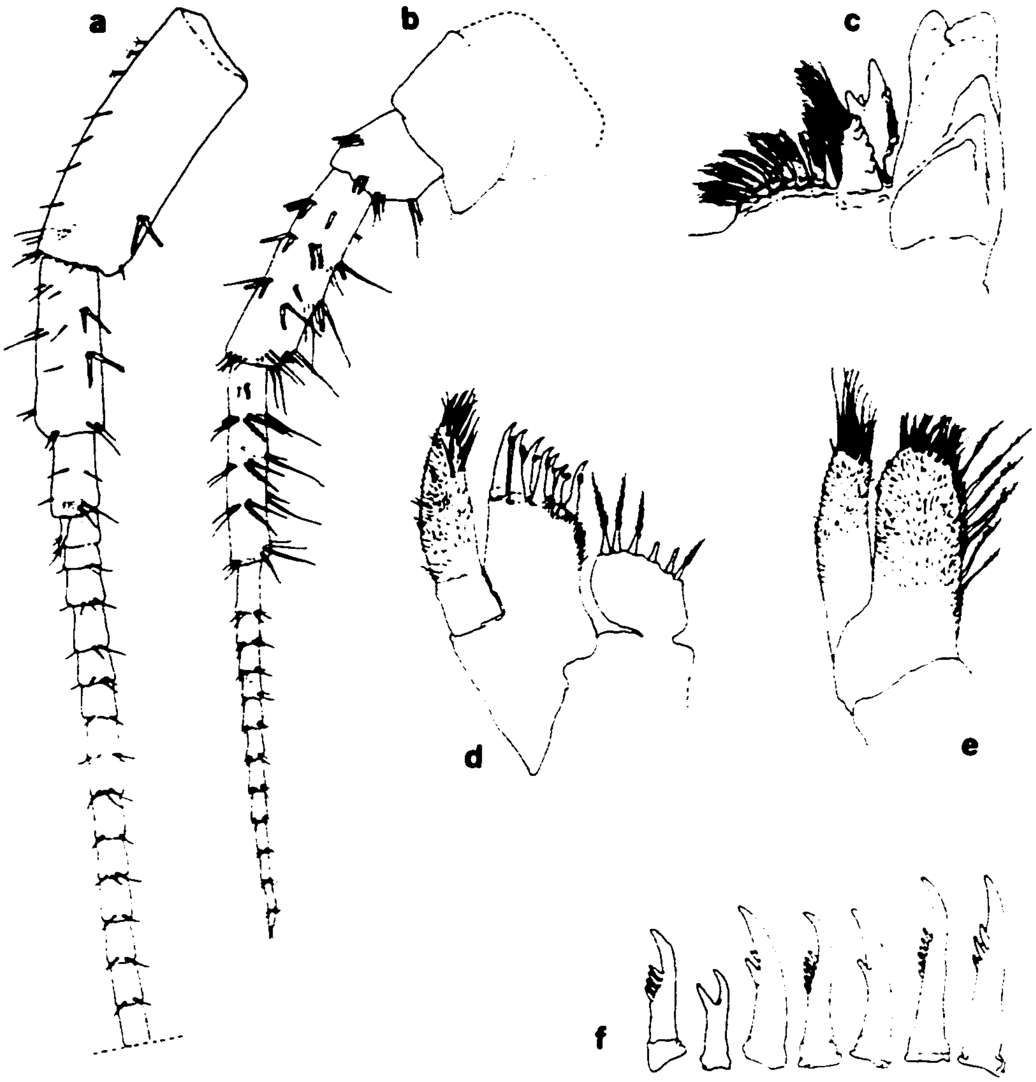


Fig. 5.10. *Bactrurus mucronatus*, female (12 mm) from Equality Cave (Saline County, Illinois): a) antenna 1, b) antenna 2, c) dentate part of right mandible, d) maxilla 1, e) maxilla 2; 14 mm male; f) spine types of inner plate of maxilla 1.

Carpus with approximately 5 sets of plumose setae on posterior margin, 3-7 plumose setae on distal margin and 3-4 rastellate spines on distoposterior corner (see enlargement of Fig. 5.11b). Palm of propod almost even (sometimes slightly concave), finely serrate at distal and proximal ends, armed with ca. 9 spines on lateral margin and 7-9 spines on medial margin; defining angle rounded, with row of 4-5 spines on medial corner, 2-3 spines on lateral corner (long and short spines) and 2-4 spines between medial and lateral corner spines; proximal (subangular) margin ca. 33% length of propod, bearing 7-9 sets of plumose setae; anteromedial surface bearing 7 rows of plumose setae. Dactyl 69 % length of propod. Coxal plate round, as wide as long, bearing 9-12 setae.

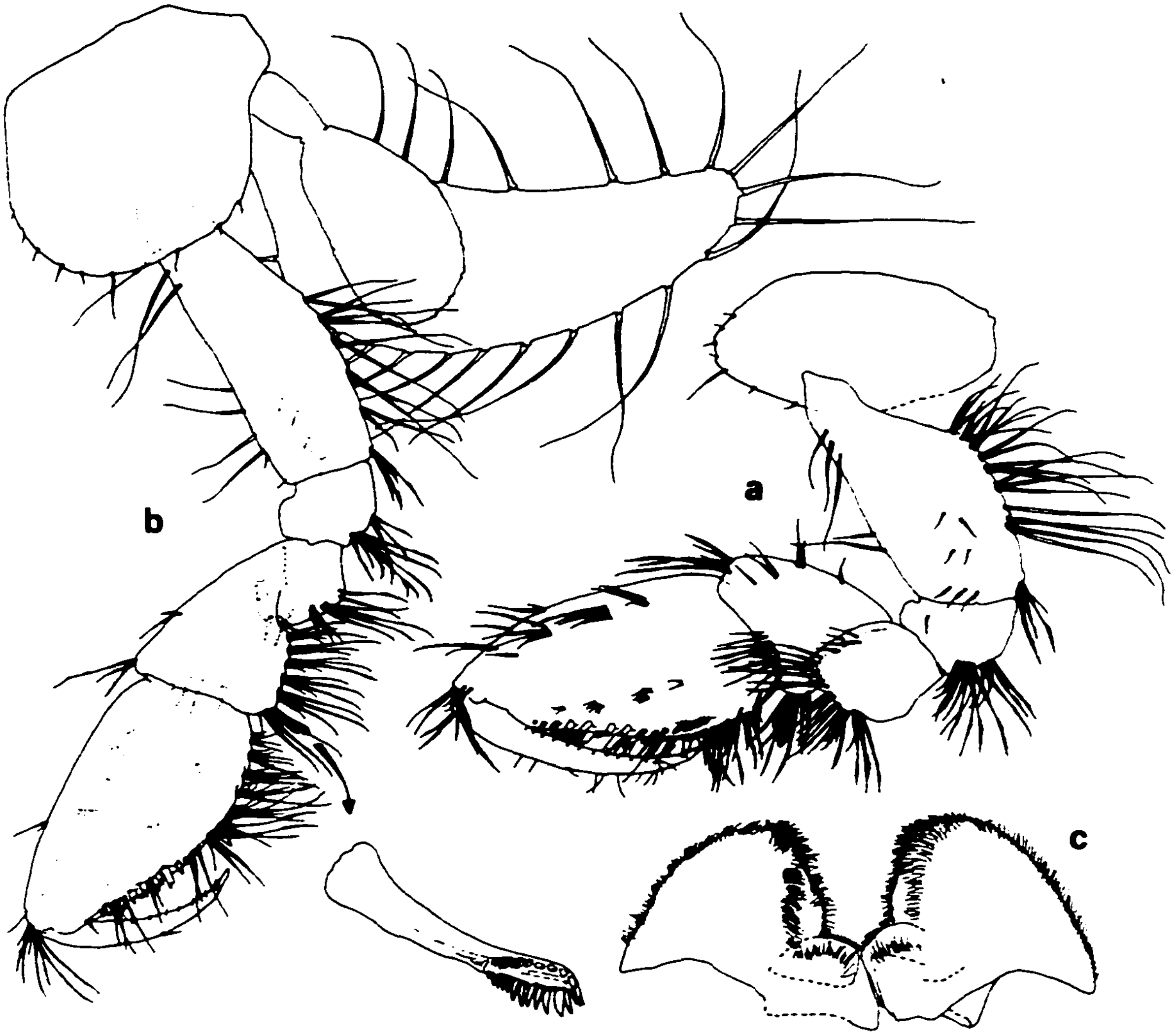


Fig. 5.11. *Bactrurus mucronatus*, female (12 mm) from Equality Cave (Saline County, Illinois): a) gnathopod 1 (medial view), b) gnathopod 2 (lateral view, with rastellate spine of carpus enlarged), c) lower lip.

Pereopod 3 (Fig. 5.12a): Basis with ca. 5 long setae and 2 short setae on proximoposterior margin, 5 short setae on anterior margin and 5 long setae on medial surface (sometimes additionally 1-3 long setae on proximoanterior margin). Coxal plate with to 9-10 setae.

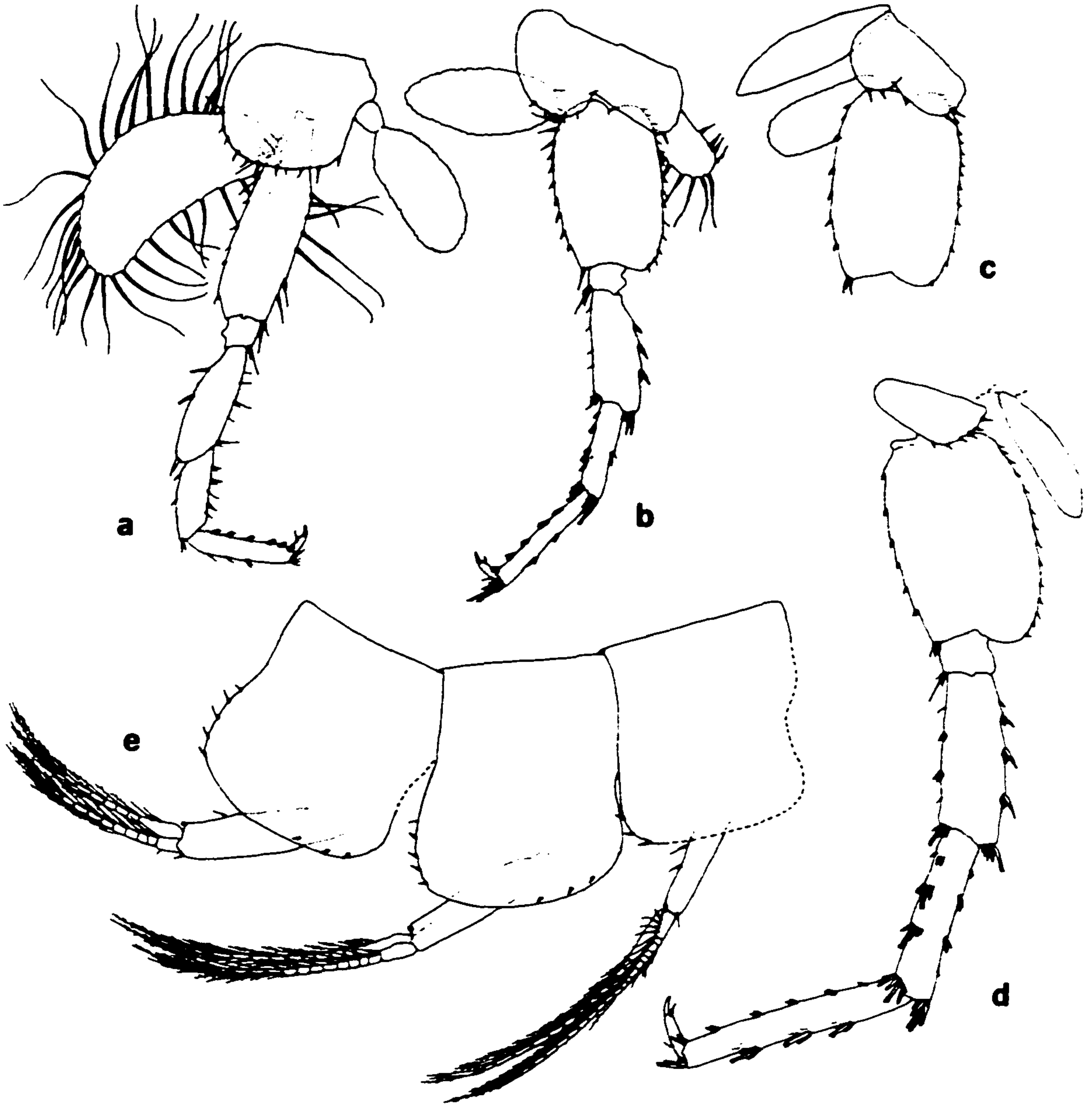


Fig. 5.12. *Bactrurus mucronatus*, female (12 mm) from Equality Cave (Saline County, Illinois): a) left pereopod 3, b) left pereopod 5, c) proximal part of left pereopod 6, d) left pereopod 7; 14 mm male: e) pleonites 1-3.

Pereopod 4: subequal to pereopod 3.

Pereopods 5-7: Coxal plate of pereopod 5 with 5 setae on distoanterior lobe and 1 seta (or slender spine) on distoposterior corner (Fig. 5.12b). Coxal plate of pereopod 6 bearing 2-3 setae on distoanterior lobe and 2 setae on posterior margin (Fig. 5.12c). Coxal plate of pereopod 7 with 4-5 setae on posterior margin (Fig. 5.12d).

Coxal gill absent from pereopod 7. Most specimens with paired sternal processes present on pereonites 6 and 7 and pleonite 1 (Figs. 5.12c, d).

Pleopods (Fig. 5.12e): Peduncles subequal to those of *B. brachycaudus*. Number of unfused rami segments: pleopod 1 with 12-16 segments, pleopod 2 with 11-14 segments and pleopod 3 with 8 segments. Retinaculae with unilateral row of hooks (Fig. 5.13e).

Epimeral plates (Fig. 5.12e): Distoposterior margins bearing 5-7 short setae; ventral margins: plate 1 without spines, plates 2 and 3 with 2-5 spines, respectively.

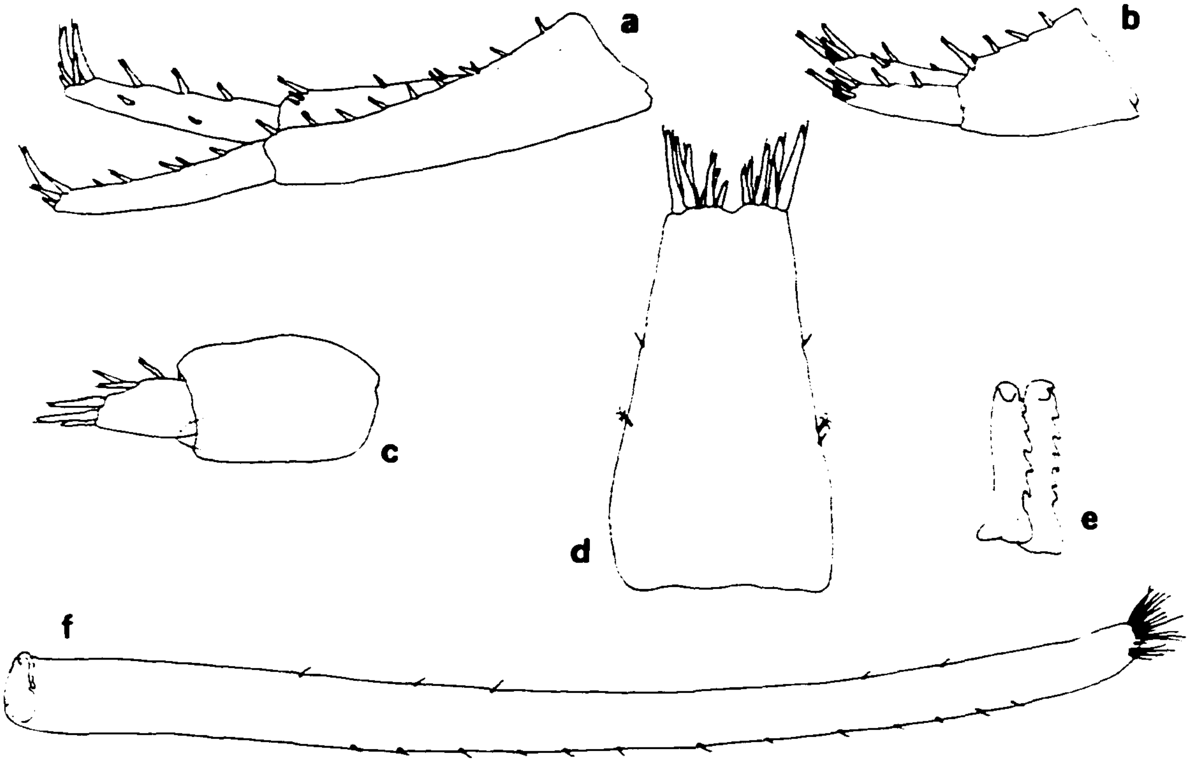


Fig. 5.13. *Bactrurus mucronatus*, female (12 mm) from Equality Cave (Saline County, Illinois): a-c) uropod 1-3, d) telson, e) retinaculae of pleopods. Male (14 mm) from same locality: f) telson.

Uropod 1 (Fig. 5.13a): Peduncle with 9-12 spines on dorsolateral margin, 2-4 slender spines on dorsomedial margin and 2-3 spines on distomedial corner. Rami subequal, about 61% length of peduncle; inner ramus with 4-5 apical spines and 5-7 spines on dorsal surface. Outer ramus with 4-5 apical spines and 6-7 spines on dorsal surface.

Uropod 2 (Fig. 5.13b): Peduncle with 4 spines on dorsolateral margin, 1 spine on dorsomedial margin (sometimes absent) and 2-3 spines near distomedial corner. Inner ramus slightly longer than outer ramus, ca. 79% of length peduncle, bearing 4-5 apical spines and 9-11 spines on dorsal surface. Outer ramus with 4 apical spines and 2-4 spines along dorsal surface.

Uropod 3 (Fig. 5.13c): Peduncle without spines. Inner ramus vestigial, bud-like, about 32% length of outer ramus. Outer ramus distinctly reduced, only about 54% length of peduncle, with 3-4 apical spines and 1-3 subapical spines on lateral margin.

Telson sexually dimorphic. Female telson entire or slightly emarginate (2%), tapered, width about 54% of length, armed with approximately 14 apical spines. Male telson extremely elongated, reaching 34% length of body in adult specimens (about 3 times longer than female telson), with ca. 22 apical setae and few small setae on lateral margins.

Sexual dimorphism and variation: Apart from the telson and a longer antenna 1 in most male specimens, the propods of gnathopod 1 and 2 had concave palmar margins in some adult males (opposed to almost even or slightly concave palms in females).

The majority of specimens examined (86%) had two pairs of lateral sternal processes on pereopods 6 and 7 and a smaller pair on pleonite 1. Seven records, exclusively from Illinois, consisted of specimens with paired sternal processes on pereonites 6 and 7 but no processes on pleonite 1. Although three of these seven records occurred in an isolated cluster in southern Illinois (two records from Equality Cave, Saline Co., and one record from a drain pipe in Gallatin Co.), the remaining four collections seemed to be randomly distributed in north-central Illinois. Their localities were in close proximity to samples with specimens that had sternal processes on pereonites 6 and 7, and pleonite 1. Thus, no apparent geographic separation into morphologically different subpopulations (three pairs vs. two pairs of sternal processes) could be observed.

Remarks: *Bactrurus mucronatus* lives in groundwater habitats associated with glacial drift areas of Ohio, Indiana, Illinois, Iowa and Michigan. Although the majority of recorded localities of this species are mainly outlets of farm field drainage systems, a few are dug or drilled wells. The only exceptions are Equality Cave in southeastern Illinois (specimens collected from a small, vadose stream), and Bear Cave in southwestern Michigan. The latter, located near Lake Michigan, has about 100 m of passage developed in Kansian-aged travertine deposits. The specimens were collected from epikarstic drip pools (D. A. Hubbard, pers. com.). In almost all of the drain pipe samples, *B. mucronatus* was accompanied by the stygobiont isopod *Caecidotea kendeighi*. In one instance, *B. mucronatus* was collected together with *B. brachycaudus* from the same drainage catchment basin (see 'Sexual dimorphism and variation' and 'Remarks' under *B. brachycaudus*).

Bactrurus mucronatus is easily distinguished from most other *Bactrurus* species by the absence of coxal gills on pereopod 7 and the extremely elongate male telson. However, even in female specimens, the width length ratio of the apically tapered telson is a unique, distinguishing character.

Aquatic crustacean fauna associated with *B. mucronatus* includes the amphipods *Crangonyx forbesi*, *Crangonyx packardi*, Synurella dentata and the isopods *Caecidotea kendeighi*, *Caecidotea.* sp. and *Lirceus* sp.

***Bactrurus pseudomucronatus* new species**

Figs. 5.14-15

Bactrurus subsp.(?).-- Holsinger, 1972: 75-76.

Bactrurus n. sp. or subsp.-- Gardner, 1986: 15.

Bactrurus sp.-- Holsinger, 1986a: 95.

Type locality: Mansell Cave (Randolph County, Arkansas).

Material examined: **ARKANSAS: Lawrence Co.**: Deep cistern, 5.5 mi. S of Imboden, 1 male (USNM), B. C. Marshall, 16 Sep. 1940. **Randolph Co.**: Mansell Cave, **HOLOTYPE** male (8.5 mm, on 2 slide mounts; USNM), **allotype** female (8.5 mm; USNM acc. nr. 330764) and 102 **paratypes** (USNM), D. Barnett, 5 Jan. 1970. **MISSOURI: Carter Co.**: Jayco Hole Cave, 6 specimens (JRH), J. E. Gardner, 16 Sept. 1980. Norris Cave, 4 females (JRH), J. E. Gardner, 29 Jan. 1979. Secesh Cave, 2 males, 4 females (JRH), J. E. Gardner, 23 Jul. 1979. Spout Spring Cave, 2 males, 1 female (JRH), J. E. Gardner, 10 Nov. 1972. Seeps along bluff of Current River, at mouth of Mill Creek, 4 mi. NW of Van Buren, >25 specimens (USNM), L. Hubricht, 3 May 1942. **Douglas Co.**: Seep, 1.5 mi. W of Roosevelt, 7 males, 24 females, 1 juvenile (USNM), L. Hubricht, 1 May 1940. **Oregon Co.**: Bluehole Cave, 1 male (JRH), J. E. Gardner, 7 Jul. 1981. Kelly Hollow Cave, 3 females (JRH), J. Craig, 10 Feb. 1973; 2 males, 1 female (JRH), M. Sutton, 20 Jan. 1991; 5 specimens (JRH), S. Koenemann and U. Englisch, 20 May 1999. New Liberty Cave, 1 male (JRH), M. Sutton, 5 Jan. 1991. **Shannon Co.**: Benton Stretch Cave, 3 males, 9 females (JRH), J. E. Gardner, 22 Mar. 1984. Bootlegger Cave, 1 male (JRH), J. E. Gardner, 20 Apr. 1983. Douglas Hollow Cave, 2 males, 1 juvenile (JRH), J. E. Gardner, 15 Mar. 1983. Fillchew Cave, 1 female (JRH), J. E. Gardner, 20 Mar. 1984. Forester Cave (formerly known as Bloom Hollow Cave?), 17 specimens (JRH), S. Koenemann and U. Englisch, 19 May 1999. Jam-Up Cave, 1 female (USNM), L. Hubricht, 1 Sept. 1940. Packrat Cave, 3 females (JRH), J. E. Gardner, 18 Apr. 1984. Cave in Powder Mill Hollow, 23 specimens (USNM), L. Hubricht, 2 Aug. 1942. Small cave in Searey Hollow, 2 males, 1 female (USNM), L. Hubricht, 1 Sept. 1940.

Diagnosis: *Bactrurus pseudomucronatus* is a medium-sized species that shows a high superficial morphological resemblance to *B. mucronatus*. Like the latter, the male telson of *B. pseudomucronatus* is extremely elongate, although at 16-28% of body length, it is proportionally not quite as long as in *B. mucronatus*. Propods of gnathopods 1 and 2 subequal in size. Coxal plate of gnathopod 2 wider than long. Coxal gills on pereopods 2-7 (distinctly reduced on pereopod 7). Two pairs of lateral sternal processes on pereonites 6 and 7; single median sternal processes on pereonites 2 and 3. Telson entire or slightly emarginate (2%); width of female telson 52% of length. Most adult specimens 5.0-10.0 mm; largest male 13.5 mm, largest female 12.0 mm.

Description: Based on an allotype female (8.5 mm) and holotype male (8.5 mm).

Antenna 1 49-73% length of body (Fig. 5.14a). Primary flagellum with up to 24 segments.

Antenna 2 about 43-53% length of antenna 1 (Fig. 5.14b). Flagellum with 9-11 segments.

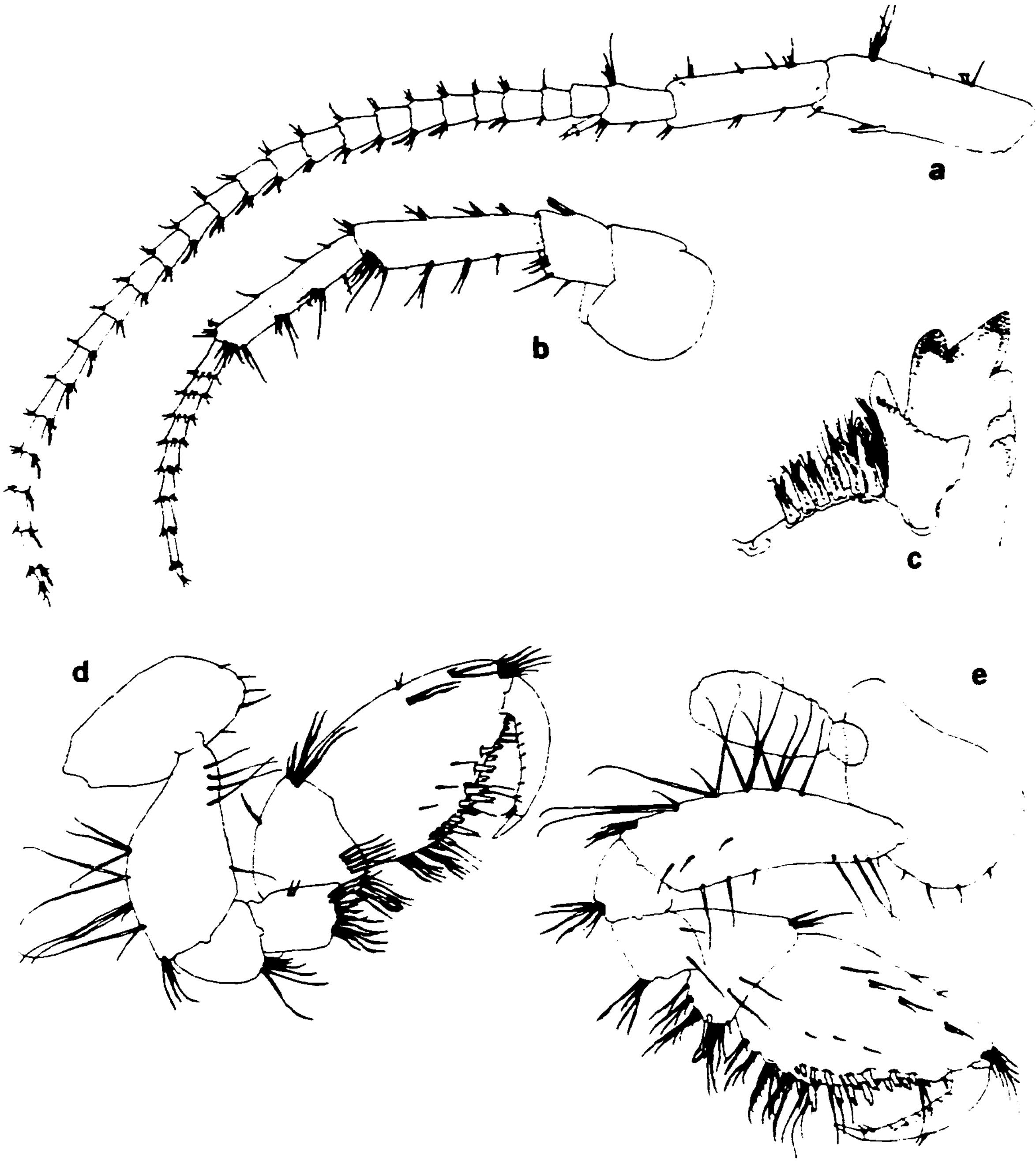


Fig. 5.14. *Bactrurus pseudomucronatus* n. sp., allotype female (8.5 mm) from Mansell Cave (Randolph County, Arkansas): a) antenna 1, b) antenna 2, c) dentate part of right mandible, d) gnathopod 1 (medial view), e) gnathopod 2 (medial view).

Lower lip subequal to that of *B. mucronatus*.

Mandibles: Row of 6 plumose spines between molar and lacinia mobilis; distal margin of right lacinia mobilis weakly serrate (Fig. 5.14c). Palp segment 2 with about 8 setae on lateral and medial margins; palp segment 3 with approximately. 21 short, plumose D-setae, 4 long, plumose B-setae and 2 long, plumose A-setae.

Maxilla 1 subequal to that of *B. mucronatus*. Segment 2 of palp with 9 naked spines apically (2 of which are strong and plumose).

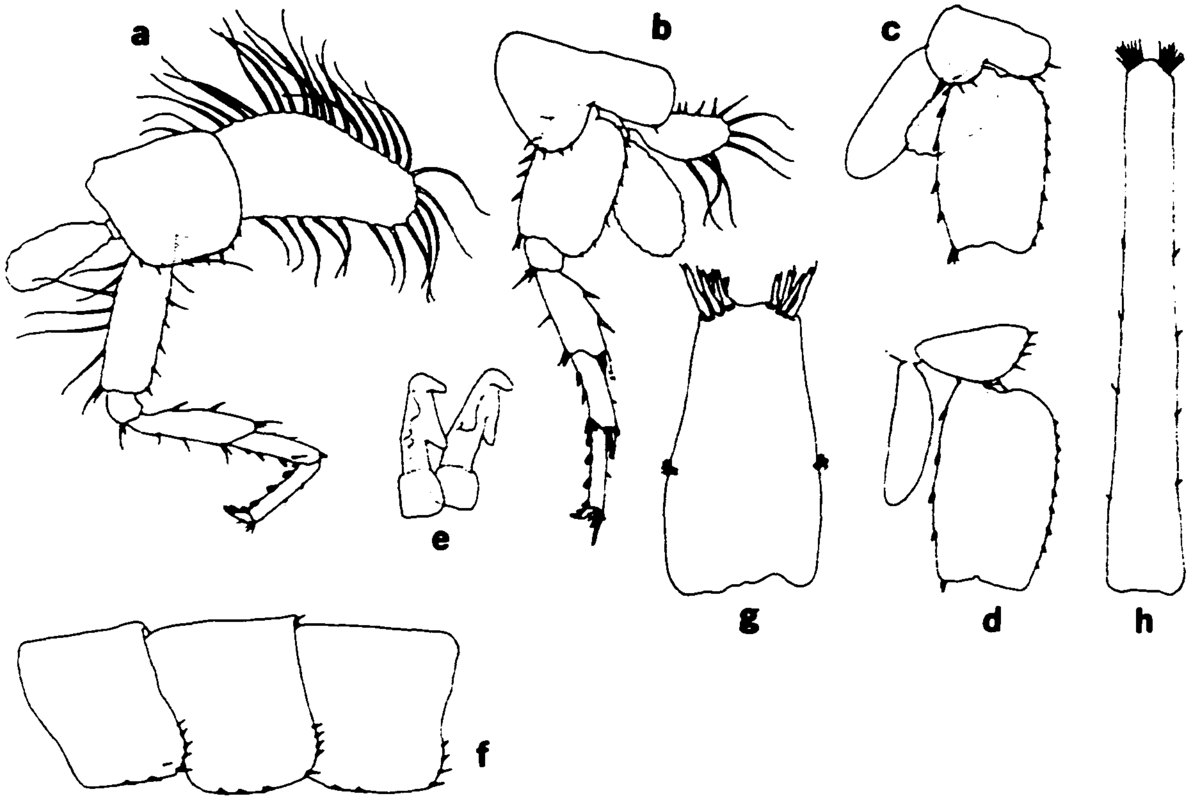


Fig. 5.15. *Bactrurus pseudomucronatus* n. sp., allotype female (8.5 mm) from Mansell Cave (Randolph County, Arkansas): a) left pereopod 3, b) left pereopod 5, c, d) proximal parts of pereopods 6 and 7, e) retinaculae of pleopods, f) epimeral plates 1-3, g) telson. Holotype male (8.5 mm) from same locality: h) telson.

Maxilla 2 subequal to that of *B. mucronatus*. Inner plate with oblique row of 5-7 strong, plumose setae.

Maxilliped: Outer plate armed with 5-6 bladelike spines on inner margin. Inner plate with 2-5 naked, bladelike spines (one of which sometimes modified as plumose spine or spine with jagged margins) and 4-6 plumose setae.

Gnathopod 1 (Fig. 5.14d): Basis with 4 sets of long, naked setae on posterior margin (with about 9 long setae in total), 4 plumose setae on distoposterior corner, 3-4 long, naked setae on proximoanterior margin, 1 seta on distoanterior margin and 2-3 setae on medial surface. Carpus with 2 rastellate spines on distoposterior margin and 5 plumose setae on distal margin. Palmar margin of propod slightly concave, with faint serrations on proximal and distal ends, bearing 7-9 spines on lateral margin and 5-8 spines on medial margin; defining angle rounded, with 4-5 spines medially and a row of 3-4 short spines on lateral corner; posterior (subangular) margin 30% length of propod, with about 9 plumose setae (naked at defining angle); anteromedial surface with 3 rows of plumose setae. Dactyl ca. 78% length of propod. Coxal plate with 4-5 setae.

Gnathopod 2 (Fig. 5.14e): Basis with 5 sets of long setae on posterior margin (with about 11 long setae in total), ca. 3 plumose setae on distoposterior corner, 3 long, naked setae on proximoanterior margin, 3 naked setae on distoanterior margin and 3-4 short setae on medial surface. Carpus with 3 sets of plumose setae on posterior margin, 2 plumose setae on distal margin and 3 rastellate spines on distoposterior corner. Palm of propod slightly concave, with faint serrations at distal and proximal ends, with 8-10 spines on lateral margin and 4-5 spines on medial margin; defining angle rounded, shallow, bearing 3-4 spines on medial corner and 2 spines on lateral corner; proximal (subangular) margin ca. 39% length of propod, bearing 4 sets of plumose setae; anteromedial surface bearing 5 rows of plumose setae. Dactyl 71 % length of propod. Coxal plate wider than long, with 5-7 setae.

Pereopod 3 (Fig. 5.15a): Basis with 6-8 long setae on proximoposterior margin (excluding setae on distoposterior corner), 5 short setae on anterior margin, 2-3 long setae on proximoanterior margin and 2-4 long setae on medial surface. Coxal plate with to 9-11 setae.

Pereopod 4: subequal to pereopod 3.

Pereopods 5-7: Anterior margins of bases with only 5-7 short spines. Coxal plate of pereopod 5 with 3-4 setae on distoanterior lobe and 1 seta on distoposterior corner (Fig. 5.15b). Coxal plate of pereopod 6 subequal to that of *B. mucronatus* (Fig. 5.15c). Coxal plate of pereopod 7 with 4 setae on posterior margin (Fig. 5.15d).

Coxal gills on pereopods 2-7, distinctly reduced on pereopod 7 (less than half the size of coxal gill on pereopod 6). Lateral sternal processes present on pereonites 6 and 7 (Figs. 5.15c, d).

Pleopods: Number of unfused rami segments (for outer and inner rami): pleopod 1 with 9-12 segments, pleopod 2 with 7-9 segments and pleopod 3 with 5 segments. Retinaculae subequal to those of *B. brachycaudus* (Fig. 5.15e).

Epimeral plates (Fig. 5.15f): Distoposterior margins bearing 2-5 short setae each; ventral margins: plate 1 with 2-3 spines, plate 2 with 3 spines and plate 3 with 2-4 spines.

Uropod 1: Peduncle with 7-9 spines on dorsolateral margin, 2 setae on dorsomedial margin and 2 spines on distomedial corner. Length peduncle/outer ramus and armature of inner ramus subequal to that of *B. mucronatus*. Outer ramus with 4-5 spines on dorsal surface.

Uropod 2: Peduncle with 2-3 spines on dorsolateral margin and 3 spines near distomedial corner. Length peduncle/outer ramus subequal to that of *B. mucronatus*. Inner ramus with 3-4 spines on dorsal surface. Outer ramus bearing 2-5 spines along dorsal surface.

Uropod 3: Subequal to that of *B. mucronatus*. Outer ramus bearing 2-3 apical spines and 1-2 subapical spines on lateral margin.

Telson sexually dimorphic. Female telson entire or slightly emarginate (2%), slightly tapered, width about. 52% of length, bearing 10 apical spines. Male telson extremely elongate, reaching 16-22% length of body in adult specimens, with about 20 apical setae (in 2 clusters) and few small setae on lateral margins.

Sexual dimorphism and variation: As in *B. mucronatus*, the only noticeable secondary sexual dimorphism was observed in the telson and in the length of the male antenna 1. No marked morphological variation was noted otherwise.

Remarks: The range of *B. pseudomucronatus* is restricted to karst areas in the Salem Upland section of the Ozark Plateaus. The species typically occurs in caves and seeps in southern Missouri (several caves, a few seeps) and northern Arkansas (1 cave, 1 deep cistern).

The type locality, located in Eleven Point River Valley (White River drainage), is developed in Cotter limestone of Ordovician age. The type series consisted of 109 specimens collected from a small, shallow pool approximately 10 m from the entrance (D. E. Barnett, pers. com.).

Bactrurus pseudomucronatus can be distinguished from *B. mucronatus* by the characters given in the diagnosis (see also: *B. mucronatus*, 'Remarks').

Aquatic crustacean fauna associated with *B. pseudomucronatus* includes the amphipod *Stygobromus* n. sp. (Holsinger, in ms.) and the isopod: *Caecidotea tridentata*.

Etymology: The epithet *pseudomucronatus*, meaning "false *mucronatus*", alludes to the morphological similarity of *B. pseudomucronatus* to *B. mucronatus* (*B. pseudomucronatus* was sometimes falsely identified as *B. mucronatus*).

Bactrurus wilsoni new species

Figs. 5.16-19

Bactrurus sp.-- Holsinger, 1986a: 95.

Type locality: well in Culwell residence, Hayden (Blount County, Alabama).

Material examined: **ALABAMA: Blount Co.**: Well in kitchen of Wilson residence (presently owned by Charlie and Jane Culver), 2.1 mi. NE of Hayden, 1 male paratype, Jimmy Wilson, J. R. Wilson and L. M. Ferguson, 12 Aug. 1982; **allotype** female (11.5 mm; JRH 3901), J. R. Wilson, May 1983; 1 male, 1 female, paratypes (JRH), J. R. Wilson, Jul.-Aug. 1983 (specimens donated to present study by L. M. Ferguson); **HOLOTYPE** male (16.0 mm; USNM), J. Culwell, 9 Jun. 1999, and 1 male paratype (JRH), 12 Jun. 1999.

Diagnosis: A medium-sized species distinguished by sexual dimorphism in uropod 1 and telson. Distal margin of right lacinia mobilis with large, irregular denticles. Propod of gnathopod 1 distinctly wider and shorter than that of gnathopod 2. Pereopod 5 about as long as pereopod 4. Coxal gills on pereopods 2-7. Two pairs of lateral sternal processes on pereonites 6 and 7; median sternal processes absent. Peduncle of male uropod 1 with serrate distal process. Telson of adult male slightly longer than telson of female, apical spines shorter than those of female; telson entire or with V-shaped excavation (12-15% cleft), width 75-77% of length. Largest male 16.0 mm, largest female 15.5 mm.

Description: Based on holotype male (16.0 mm), paratype male (15.5 mm) and paratype female (10.5 mm).

Antenna 1 subequal to that of *B. mucronatus*, reaching 58-78% length of body. Primary flagellum with up to 44 segments.

Antenna 2 about 31-47% length of antenna 1 (Fig. 5.16a). Flagellum with up to 12 segments.

Lower lip with relatively well-developed outer lobes (Fig. 5.16d).

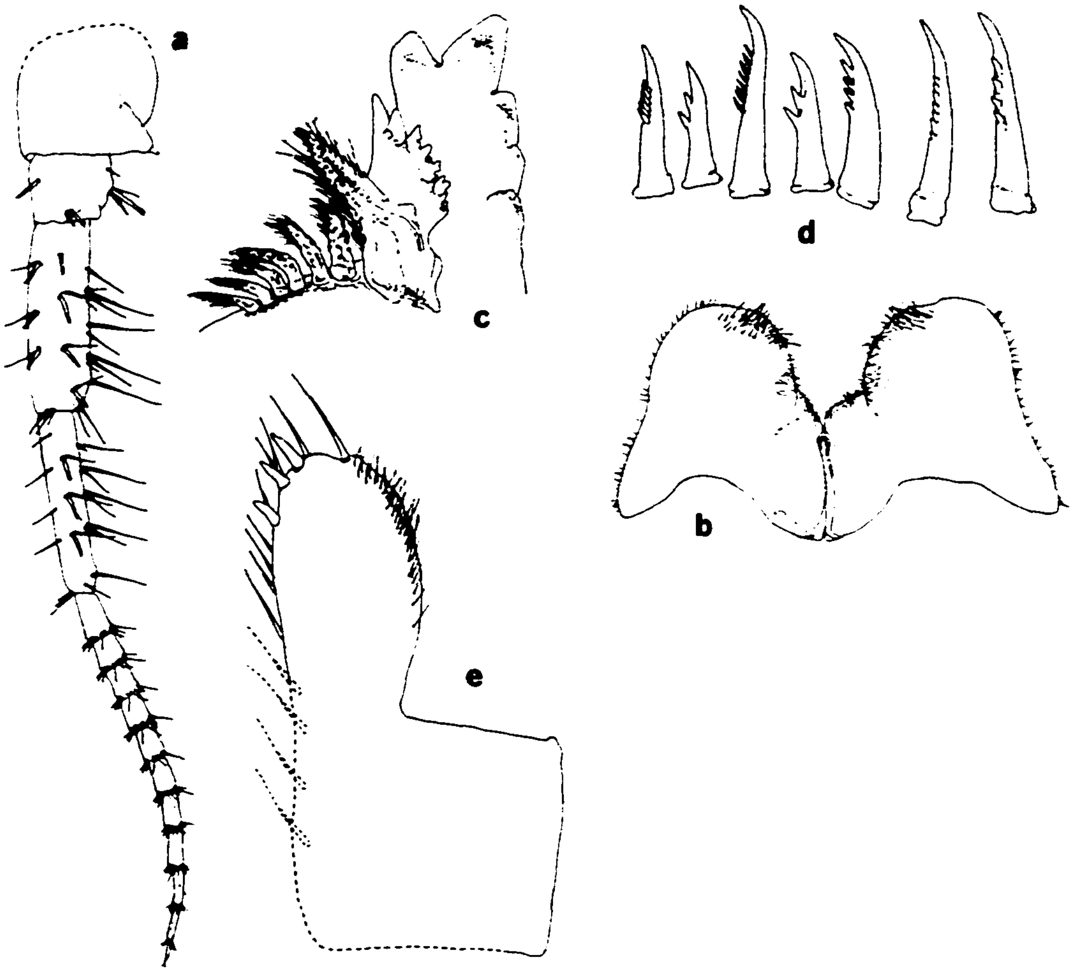


Fig. 5.16. *Bactrurus wilsoni* n. sp., paratype male (15.5 mm) from well in Hayden (Blount County, Alabama): a) antenna 2, b) lower lip. Paratype female (10.5 mm) from same locality: c) dentate part of right mandible, d) spine types of inner plate of maxilliped, e) outer plate of maxilliped.

Mandibles: Row of 5-7 plumose spines between molar and lacinia mobilis; distal margin of right lacinia mobilis with large, irregular denticles (Fig. 5.16b). Palp segment 2 with 10-11 setae on lateral and medial margins; palp segment 3 with 19-22 short, plumose D-setae, about 4 long, plumose B-setae and 1 long, plumose A-seta (sometimes absent).

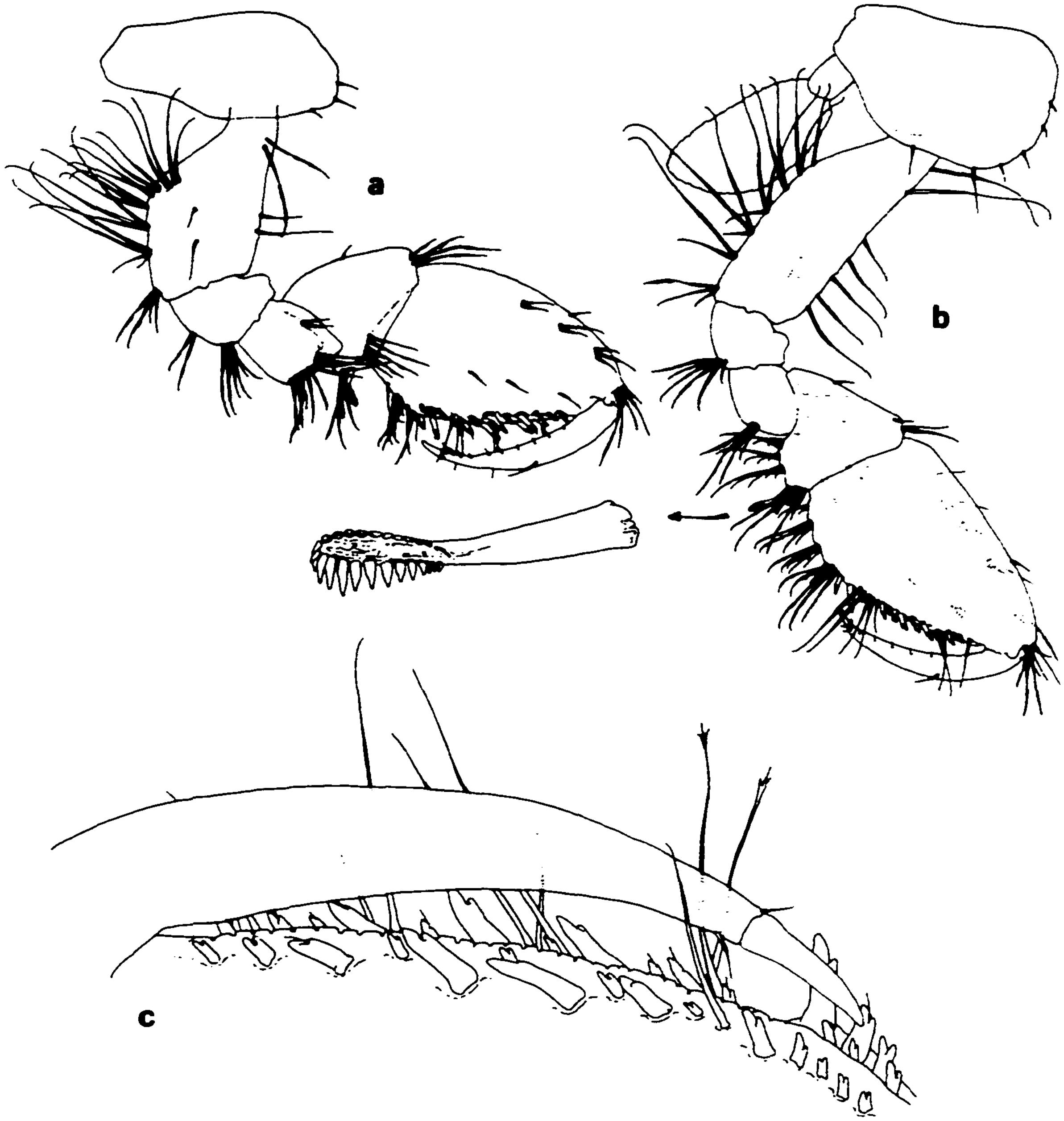


Fig. 5.17. *Bactrurus wilsoni* n. sp., paratype male (15.5 mm) from well in Hayden (Blount County, Alabama): a) gnathopod 1 (medial view), b) gnathopod 2 (lateral view, with rastellate spine of carpus enlarged). Paratype female (10.5 mm) from same locality: c) palm of propod of gnathopod 1 (medial view).

Maxilla 1: Palp segment 2 bearing apically and subapically 6-7 naked setae. Outer plate with 7 bidentate and multidentate spines (bidentate spines sometimes absent; Fig. 5.16c). Inner plate with 5-6 apical, plumose setae.

Maxilla 2: Outer plate bearing apically 10-11 mostly naked setae. Inner plate with oblique row of 7 strong, plumose setae on inner margin and 17 plumose, apical and subapical setae.

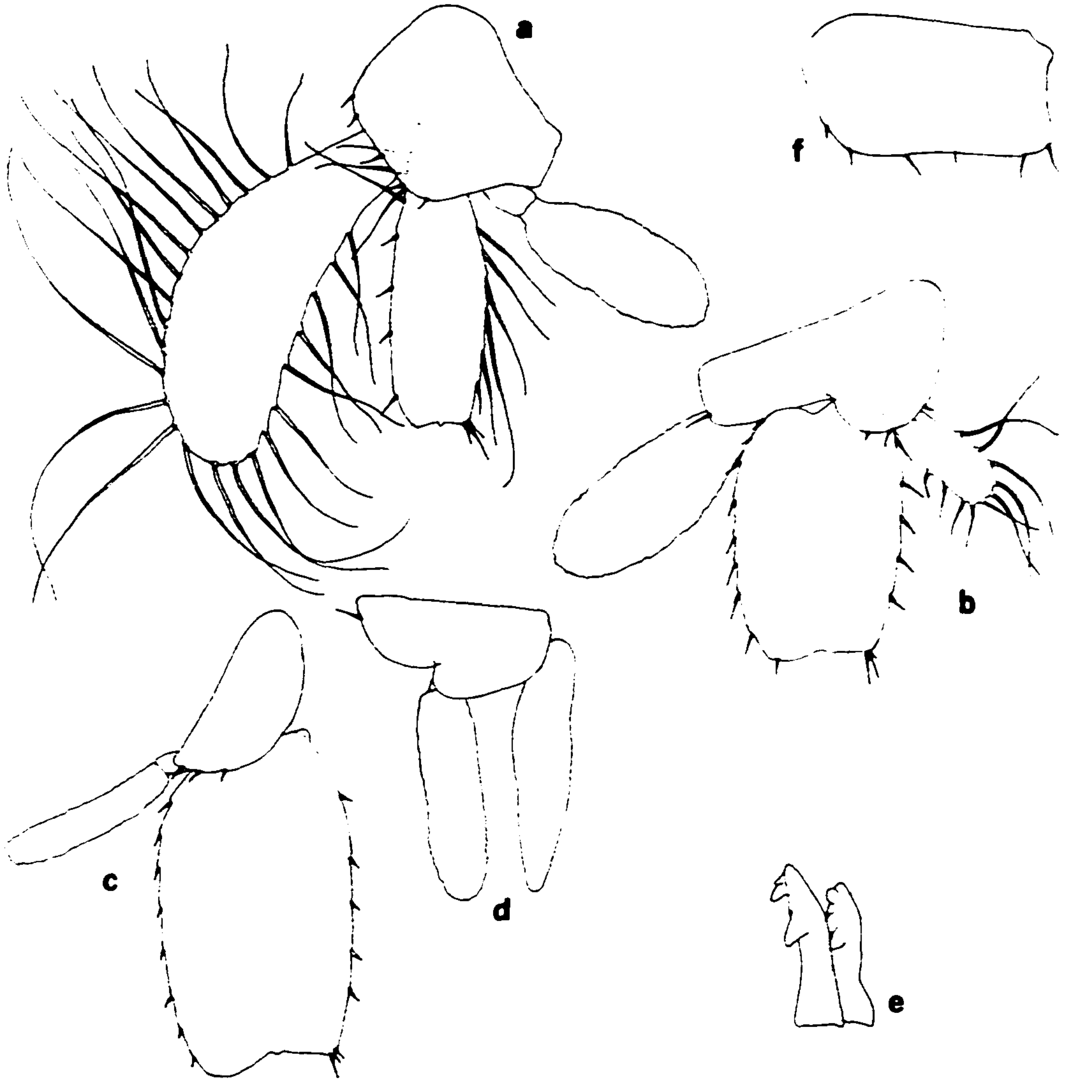


Fig. 5.18. *Bactrurus wilsoni* n. sp., paratype female (10.5 mm) from well in Hayden (Blount County, Alabama): a-c) proximal parts of pereopods 3, 5 and 7. d) coxal plate, with coxal gill and lateral sternal process of pereonite 6. e) retinaculae of pleopods. Paratype male (15.5 mm) from same locality: f) coxal plate of pereopod 4.

Maxilliped: Outer plate armed with 2-3 bladelike spines and 10-19 naked setae on inner margin (Fig. 5.16e). Inner plate apically with about 3 bladelike spines and 5 plumose setae.

Gnathopod 1 (Fig. 5.17a): Basis with 5-7 sets of long, naked setae on posterior margin plus row of 4-6 long, naked setae on proximoposterior margin (with about 16 setae in total), 4 plumose setae on

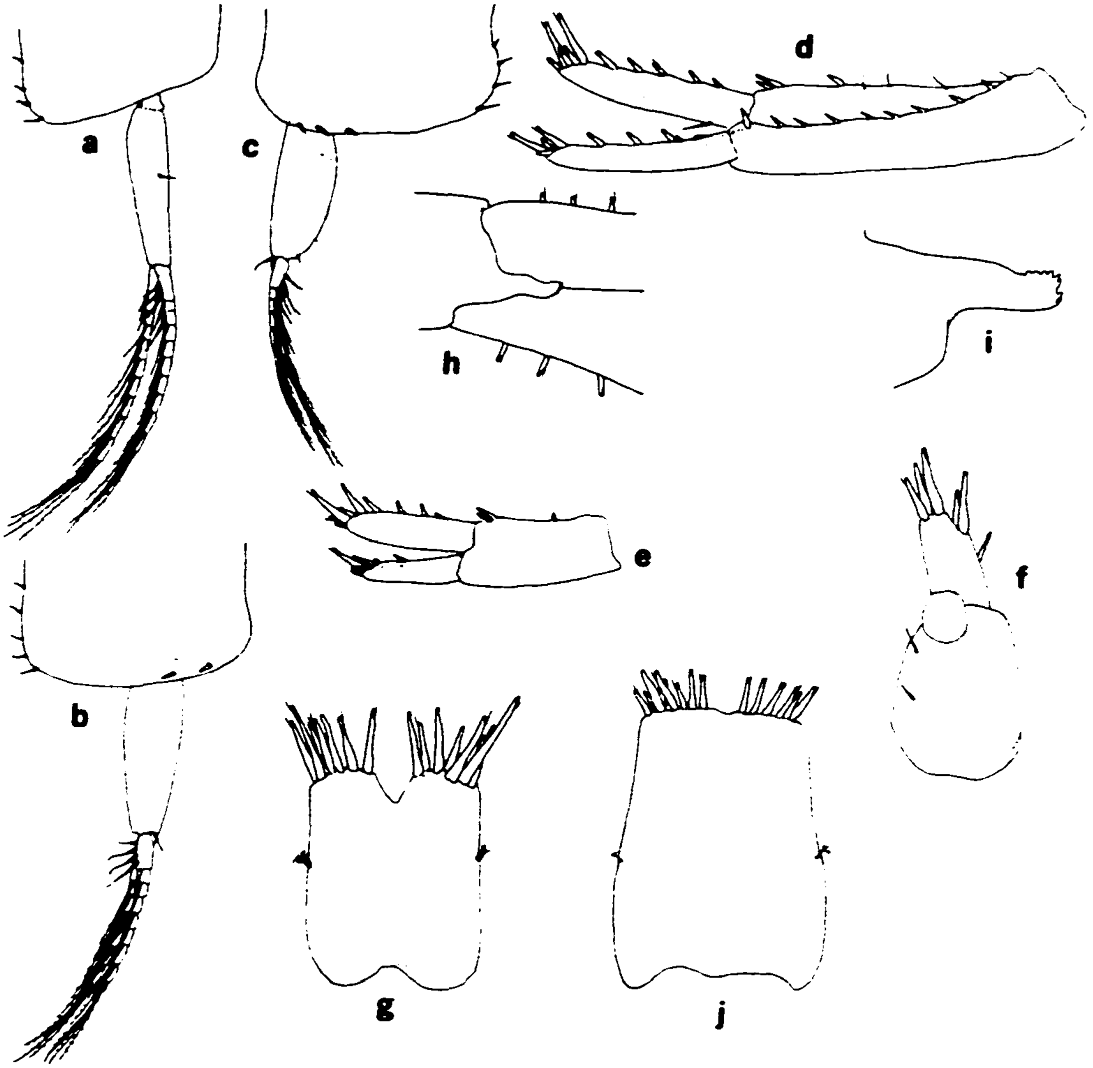


Fig. 5.19. *Bactrurus wilsoni* n. sp., paratype male (15.5 mm) from well in Hayden (Blount County, Alabama): a-c) pleopods 1-3. d-f) uropod 1-3. Paratype female (10.5 mm) from same locality: g) telson; 16.0 mm male: h, i), peduncular process of uropod, j) telson.

distoposterior corner, 2-3 long, plumose setae on proximoanterior margin, 2-3 setae on distoanterior margin and 2 short setae on medial surface. Carpus with 2 rastellate spines on distoposterior margin and 5-8 plumose setae on distal margin. Propod slightly shorter and wider than propod of gnathopod 2; palmar margin almost even, crenulated, bearing 10-14 spines on lateral margin and 7-10 spines on medial margin; angle well-defined (ca. 135°), with 4 spines medially and 4-5 spines on lateral corner; posterior (subangular) margin 20% length of propod, with 2-3 sets of plumose setae; anteromedial surface bearing 4 rows of plumose setae. Dactyl ca. 86% length of propod. Coxal plate with 3 setae.

Gnathopod 2 (Fig. 5.17b): Basis with 6-7 sets of long setae on posterior margin (with about 13 setae in total), 3 plumose setae on distoposterior corner, 2 long, naked setae on proximoanterior margin, 5 naked setae on distoanterior margin and 2 short setae on medial surface. Carpus with 3-4 sets of plumose setae on posterior margin, 3 plumose setae on distal margin and 4-5 rastellate spines on distoposterior corner (see enlargement of Fig. 5.17b). Palm of propod almost even, surface crenulated, bearing 8-12 spines on lateral margin and 10-12 spines on medial margin (no distinct separation between corner spines and palmar spines on medial margin); defining angle ca. 135°, with about 2 spines on lateral corner; proximal (subangular) margin ca. 36% length of propod, bearing 4 sets of plumose setae; anteromedial surface bearing 6 rows of plumose setae. Dactyl 74 % length of propod. Coxal plate wider than long, bearing 5-6 setae.

Pereopod 3 (Fig. 5.18a): Basis with ca. 5 long setae and 1-2 short setae on posterior margin, 4-5 short setae on anterior margin, 2-3 long setae on proximoanterior margin and 1-2 long setae on proximomedial surface. Coxal plate with 4-6 marginal setae.

Pereopod 4: subequal to pereopod 3. Coxal plate with 6 marginal setae (Fig. 5.18b).

Pereopod 5 (Fig. 5.18c): Anterior margin of basis with 6 relatively long, slender spines; posterior margin with 7-9 setae. Coxal plate with 3 setae on distoanterior lobe and 1 slender spine on distoposterior corner.

Pereopods 6 and 7: Anterior margins of bases with 4-5 short spines; posterior margins bearing 8-12 short setae. Coxal plate of pereopod 6 with 1 seta on distoposterior lobe (Fig. 5.18d). Coxal plate of pereopod 7 with 3 setae on posterior margin (Fig. 5.18e).

Coxal gills on pereopods 2-7, only slightly reduced on pereopod 7. Lateral sternal processes present on pereonites 6 and 7 (Fig. 5.18d).

Pleopods (Figs. 5.19a-c): Width of peduncles gradually increasing from pleopod 1 to 3. Number of unfused rami segments: pleopod 1 with 8-10 segments, pleopod 2 with 8 segments and pleopod 3 with 6-7 segments. Retinaculae subequal to those of *B. brachycaudus* (Fig. 5.18f).

Epimeral plates (Fig. 5.19a-c): Distoposterior margins bearing 3-5 short setae; plate 1 without spines, plates 2 and 3 with 2-3 spines, respectively.

Uropod 1 (Fig. 5.19d): Peduncle with 8-9 spines on dorsolateral margin, 1 spine on dorsomedial margin (sometimes absent) and 2-3 spines on distomedial corner. Rami about 56% length of peduncle; inner ramus with 5 apical spines and 7-8 spines on dorsal surface. Outer ramus with 4-5 apical spines and about 7 spines on dorsal surface. Peduncle of male with distoventral, serrate process (Figs. 5.19e, f).

Uropod 2 (Fig. 5.19g): Peduncle with 3-4 spines on dorsolateral margin and 2-4 spines near distomedial corner. Inner ramus longer than outer ramus, about 83% of length peduncle, bearing 5 apical spines and 4-5 spines on dorsal surface. Outer ramus with 5 apical spines and 3-4 spines on dorsal surface.

Uropod 3 (Fig. 5.19h): Peduncle with 1 spine on distal margin. Inner ramus vestigial, about 48% length of outer ramus. Outer ramus reduced, about 64% length of peduncle, with 3-4 apical spines and 1-5 subapical spines on lateral margin.

Telson sexually dimorphic; apical margin entire or with V-shaped excavation (12-15% cleft), armed with 6-9 apical spines per lobe; width about 75% of length in examined males and 77% of length in females; spines of female telson distinctly longer than spines of male telson (Figs. 5.19i, j).

Sexual dimorphism and variation: The telson of the holotype male had an entire distal margin, whereas those of all other specimens had a V-shaped excavation (12-15% cleft). Secondary sexual dimorphism occurs in both uropod 1 and telson.

Remarks: To date, *B. wilsoni* is known only from a single groundwater aquifer on the Cumberland Plateau in northern Alabama. The type locality, a drilled well approximately 17 m deep, is apparently in Bangor limestone of Mississippian age. Specimens from the well were collected with a bucket-and-rope system through an opening in the kitchen floor of the Culver house. One of the first samples (May 1983) also contained three specimens of the stygobiont amphipod *Stygobromus* n. sp. (Holsinger, in ms.).

Bactrurus wilsoni is morphologically very similar to the new species *B. angulus* and *B. cellulanus*, both of which have a serrate peduncular process on male uropod 1.

Etymology: The species is named in honor of Mr. Johnny R. Wilson, who helped collecting the first specimens in 1982 and also assisted with the field work in the spring of 1999.

Bactrurus angulus **new species**

Figs. 5.20-22

Bactrurus sp.-- Holsinger, 1986a: 95; 1986b: 540, Fig. 2-- Holsinger & Culver, 1988: 25-26.

Type locality: Saur Kraut Cave, (Claiborne Co., Tennessee).

Material examined: **TENNESSEE: Claiborne Co.**: Kings Saltpeter Cave, 2 female paratypes (JRH), J. R. Holsinger, 24 Nov. 1973. Saur Kraut Cave, **HOLOTYPE** male (16.7 mm; USNM), J. R. Holsinger and D. C. Culver, 4 Aug. 1977. **VIRGINIA: Lee Co.**: Cumberland Gap Saltpeter Cave, **allotype** female (11.2 mm; JRH 1994), 1 male and 2 female paratypes (JRH), J. R. Holsinger, V. M. Dalton (Tipton), et al., 15 Jul. 1979.

Diagnosis: A medium-sized species morphologically closely allied with *B. wilsoni*. It can be distinguished by the following characters: Propod of gnathopod 1 noticeably wider and slightly shorter than that of gnathopod 2; defining angle of gnathopod 1 very distinct (ca. 120°). Pereopod 5 about as long as pereopod 4. Coxal gills on pereopods 2-7. Two pairs of lateral sternal processes on pereonites 6 and 7; median sternal processes absent. Peduncle of male uropod 1 with serrate distal process. Telson of adult males sometimes slightly longer than telson of female, apical spines shorter than those of female; telson with shallow notch or V-shaped excavation (5-19% cleft). Largest male 16.7 mm, largest female 14.0 mm.

Description: Based on holotype male (16.7 mm), allotype female (11.2 mm) and paratype male (13.5 mm).

Antenna 1 subequal to that of *B. mucronatus*, about 66% length of body (Fig. 5.20a). Primary flagellum with up to 41 segments.

Antenna 2 approximately 43-47% length of antenna 1 (Fig. 5.20b). Flagellum with 11-12 segments.

Lower lip subequal to that of *B. wilsoni* (Fig. 5.20c).

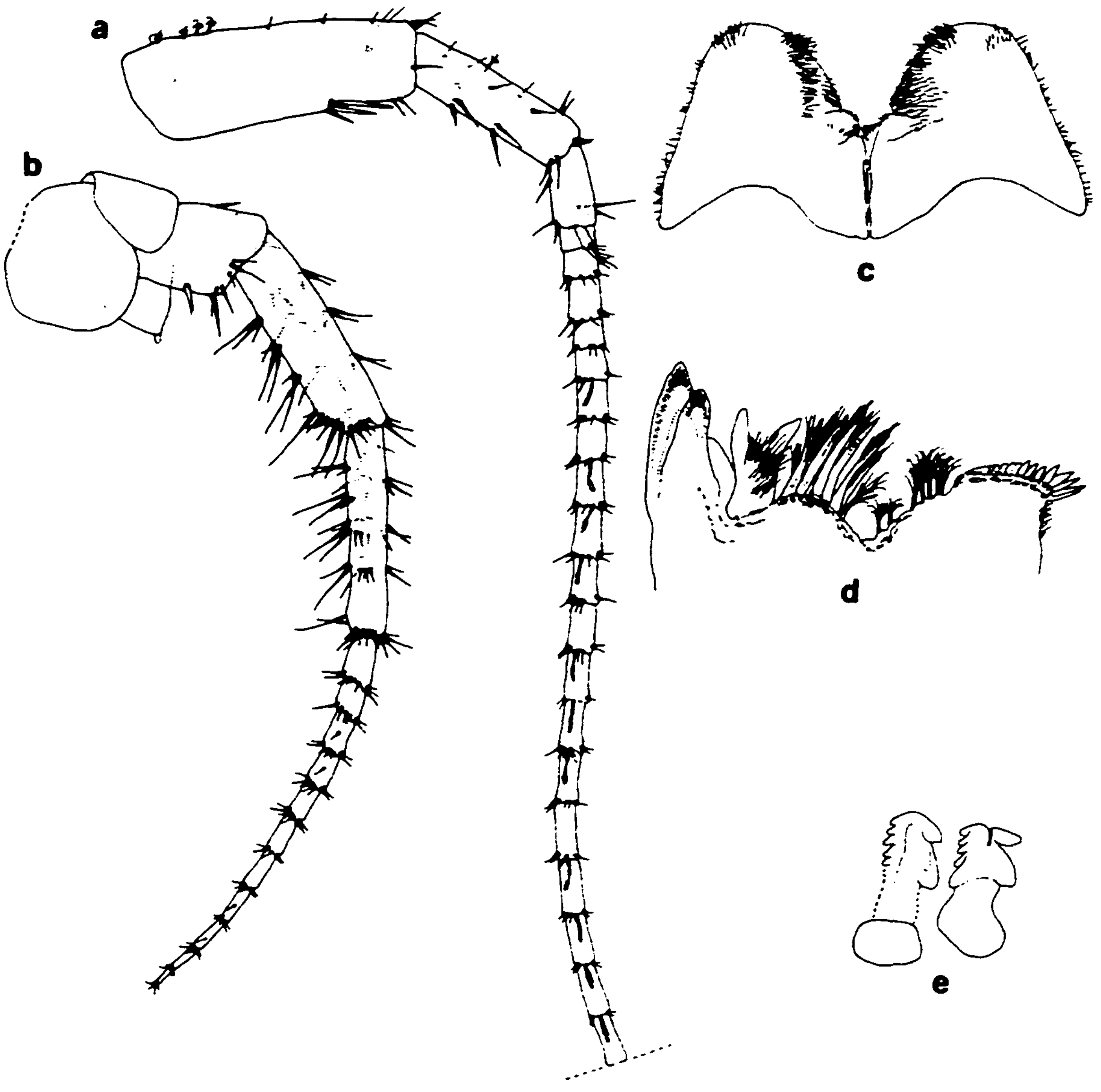


Fig. 5.20. *Bactrurus angulus* n. sp., allotype female (11.2 mm) from Cumberland Gap Saltpeter Cave (Lee County, Virginia): a) antenna 1, b) antenna 2, c) lower lip, d) dentate part of right mandible, e) retinaculae of pleopods.

Mandibles: Row of 6-7 plumose spines between molar and lacinia mobilis; distal margin of right lacinia mobilis with irregular serrations (Fig. 5.20d). Palp segment 2 with 12-13 setae on lateral and medial margins; palp segment 3 with 24-25 short, plumose D-setae, 2-3 long, plumose B-setae and 2-3 long, plumose A-seta.

Maxilla 1: Palp segment 2 bearing apically and subapically 7-8 naked setae, 1-2 of which are stronger and plumose. Outer plate with 7 bidentate and multidentate spines. Inner plate bearing 5 apical, plumose setae.

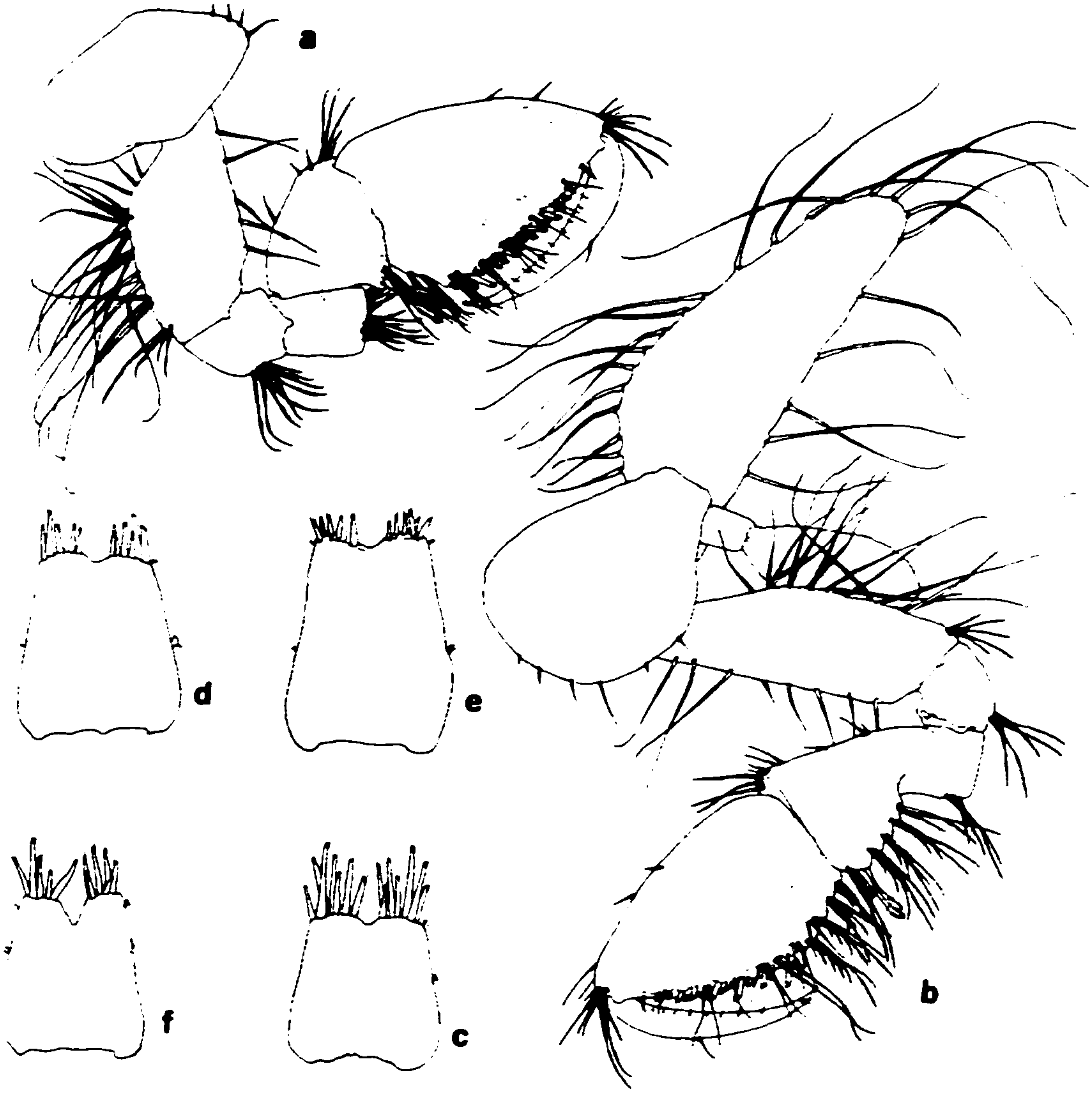


Fig. 5.21. *Bactrurus angulus* n. sp., allotype female (11.2 mm) from Cumberland Gap Saltpeter Cave (Lee County, Virginia): a) gnathopod 1 (lateral view), b) gnathopod 2 (lateral view), c) telson; 13.5 mm male: d) telson. Holotype male (16.7 mm) from Saur Kraut Cave (Claiborne County, Tennessee): e) telson. Paratype female (12 mm) from King's Saltpeter Cave (Claiborne County, Tennessee): f) telson.

Maxilla 2: Outer plate apically with 15-17 mostly naked setae. Inner plate with oblique row of 7 plumose setae and up to 20 plumose setae apically and subapically.

Maxilliped: Outer plate with 4 bladelike spines and 14-16 naked setae on inner margin. Inner plate apically with 4 naked, bladelike spines, 1 plumose apical spine, and 3-4 plumose setae on subapical inner margin (brush-type setae, Fig. 3c).

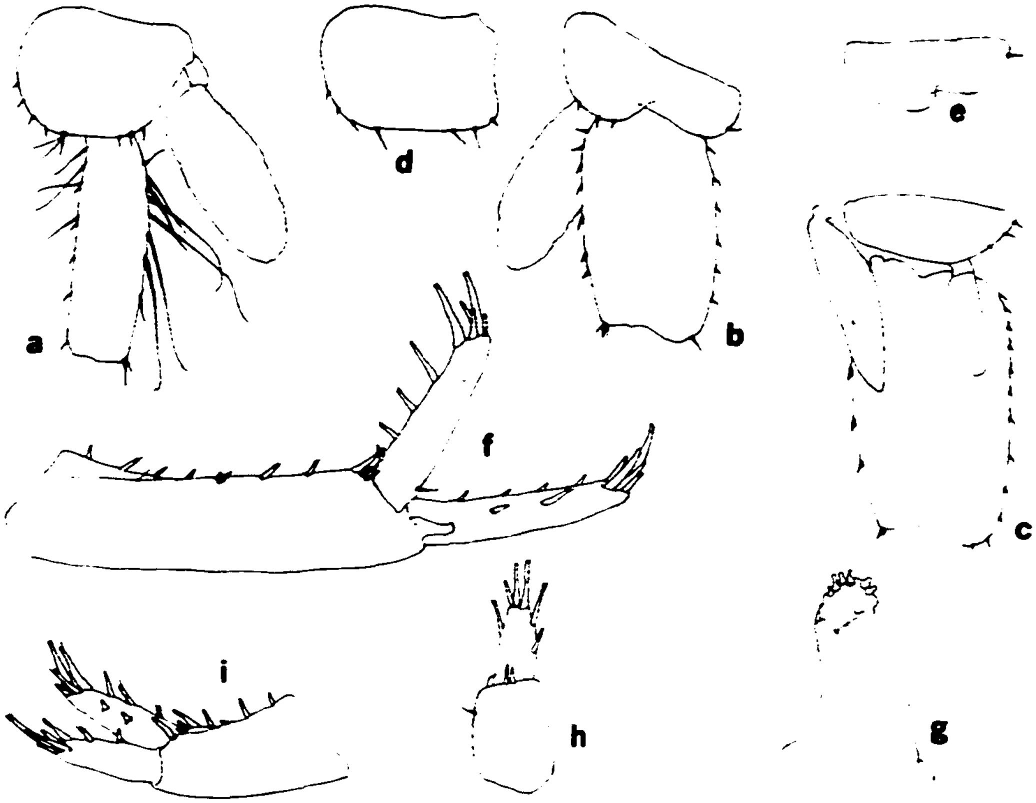


Fig. 5.22. *Bactrurus angulus* n. sp., holotype male (16.7 mm) from Saur Kraut Cave (Claiborne County, Tennessee): a-c) proximal parts of pereopods 3, 5 and 7, d, e) coxal plates of pereopods 4 and 6, f) uropod 1, g) peduncular process of uropod 1, h) uropod 3. Allotype female (11.2 mm) from Cumberland Gap Saltpeter Cave (Lee County, Virginia): i) uropod 2.

Gnathopod 1 (Fig. 5.21a): Basis with 5-7 sets of long, naked setae on posterior margin (with about 22 setae in total), 3-5 plumose setae on distoposterior corner, 2 naked setae on proximoanterior margin, 3 naked setae on distoanterior margin and 3 naked setae on medial surface. Carpus with 3 rastellate spines on distoposterior margin (see enlargement of Fig. 5.17b) and about 10 plumose setae on distal margin. Propod noticeably wider and slightly shorter and than propod of gnathopod 2; palmar margin almost even, surface crenulated, bearing ca. 8 spines on lateral margin and 9-10 spines on medial margin; defining angle distinct (ca. 120°), with 7 slender spines medially and row of 5-6 spines on lateral corner; posterior (subangular) margin 18% length of propod, with 2 sets of plumose setae; anteromedial surface bearing 4 rows of plumose setae. Dactyl ca. 95% length of propod. Coxal plate with 4 setae.

Gnathopod 2 (Fig. 5.21b): Basis with 8 sets of long setae on posterior margin (with ca. 14 setae in total), 3-5 plumose setae on distoposterior corner, 3 long, naked setae on proximoanterior margin, 3-5 naked setae on distoanterior margin and 2 short setae on medial surface. Carpus with 4-5 sets of plumose setae on posterior margin, 7-9 plumose setae on distal margin and 4-5 rastellate spines on distoposterior corner (see enlargement of Fig. 5.17b). Palm of propod crenulated, with 10-11 spines on lateral margin and 10-12 spines on medial margin; defining angle 135-140°, with 2-4 spines on lateral corner and row of 4-5 smaller spines on medial corner; proximal (subangular) margin ca. 31% length of propod, bearing 4-5 sets of plumose setae; anteromedial surface bearing 6 rows of plumose setae. Dactyl 81 % length of propod. Coxal plate slightly wider than long, bearing 6-7 setae.

Pereopod 3 (Fig. 5.22a): Basis with. 5-6 long setae and 1-2 short setae on posterior margin, 5-6 short setae on anterior margin and 4-6 long setae on proximoanterior margin and surface. Coxal plate with to 7-8 setae.

Pereopod 4: subequal to pereopod 3. Coxal plate with 6-9 setae (Fig. 5.22b).

Pereopod 5 (Fig. 5.22c): Anterior margin of basis with 5-7 short spines; posterior margin with 9-11 setae. Coxal plate with 3 setae on distoanterior lobe and 1 seta on distoposterior corner.

Pereopods 6 and 7 subequal to those of *B. wilsoni.* (Figs. 5.22d, e).

Coxal gills on pereopods 2-7, distinctly reduced on pereopod 7 (less than half the size of coxal gill on pereopods 5 and 6; Fig. 5.22e). Lateral sternal processes present on pereonites 6 and 7 (Fig. 5.22e).

Pleopods: Width of peduncles gradually increasing from pleopod 1-3. Number of unfused rami segments: pleopod 1 with 12 segments, pleopod 2 with 9-10 segments and pleopod 3 with 8 segments. Retinaculae asymmetrical, with unilateral row of small hooks (Fig. 5.20e).

Epimeral plates: Distoposterior margins with 4-5 short setae each; plate 1 without spines, plate 2 bearing 2 spines and plate3 with 3 spines.

Uropod 1 (Fig. 5.22f): Peduncle with 8-9 spines on dorsolateral margin, 3-4 spines on dorsomedial margin and 2-3 spines on distomedial corner. Rami about 51% length of peduncle; inner ramus with 4-5 apical spines and about 6 spines on dorsal surface (some of which relatively long). Outer ramus with 5-6 apical spines and ca. 6 spines on dorsal surface. Peduncle of male with distoventral, serrate process (Figs. 5.22f, g).

Uropod 2 (Fig. 5.22h): Peduncle with 3-4 spines on dorsolateral margin, 2 spines on dorsomedial margin and 3-4 spines on distomedial corner. Inner ramus longer than outer ramus, about 71% of length peduncle, bearing 4 apical spines and 5-6 spines on dorsal surface. Outer ramus with 5 apical spines and about 3 spines on dorsal surface.

Uropod 3 (Fig. 5.22i): Peduncle armed with 2 spines on distal margin. Inner ramus vestigial, only about 27% length of outer ramus. Outer ramus reduced, about 83% length of peduncle, with 3-4 apical spines, 1-3 subapical spines on lateral margin and 1-2 subapical spines on medial margin.

Telson (Figs. 5.21c-f): Apical margin with shallow notch or V-shaped excavation (5-19% cleft), bearing 11-14 apical spines. Width of male telson 73-87% of length, with relatively short apical spines;

width of female telson 85-106% of length, apical spines distinctly longer than spines of male telson.

Sexual dimorphism and variation: One of the two examined males had a telson which was distinctly longer than wide (holotype male), whereas the telsonic width/length ratio of the smaller male did not differ from those of the females. With the exception of 1 female telson with distinct, V-shaped excavation (19% cleft), the majority of the specimens examined had a telson with a shallow distal notch (5-7% cleft). Secondary sexual dimorphism was found in uropod 1 and telson.

Remarks: *Bactrurus angulus* is presently known only from two caves in northeastern Tennessee and one cave in southwestern Virginia. This species is very rare and several attempts to collect additional specimens have been unsuccessful (1996 and 1997). The type locality, located south of Powell River and northeast of Tazewell, contains about 100 m of passages and is developed in Middle Ordovician limestone. Despite two visits to the cave, only a single male (holotype) has been found to date. The specimen was collected from a shallow, mud-bottom drip (seep-fed) pool in a short dead end passage.

Like *B. wilsoni*, *B. angulus* differs from most other *Bactrurus* species by a serrate peduncular process on male uropod 1. It can be distinguished from *B. wilsoni* by the following characters: absence of large denticles on lacinia mobilis of right mandible; distinct defining angle of propod on gnathopod 1; dactyl of gnathopod 1 ca. 95% length of propod (86% in *B. wilsoni*); distal medial margin of carpus on gnathopod 2 with ca. 7 plumose setae (3 plumose setae in *B. wilsoni*); spines on anterior margin of basis of pereopod 5 short (relatively long in *B. wilsoni*); coxal gill on pereopod 7 distinctly reduced (less than half the size of coxal gill on pereopods 5 and 6); dorsomedial margin of peduncle on uropod 1 with 3-4 spines (1 in *B. wilsoni*); length and armature of outer ramus on uropod 3 showing less reduction than that of *B. wilsoni*.

The stygobiont amphipod *Crangonyx antennatus* was associated with *B. angulus* in small stream pools in Cumberland Gap Saltpeter and Kings Saltpeter caves.

Etymology: The epithet *angulus* (Latin for corner, angle) refers to the distinct defining angle of the propod of gnathopod 1.

Bactrurus cellulanus **new species**

Figs. 5.23-25

Bactrurus sp.-- Holsinger, 1986a: 95.

Type locality: small spring- or seep-fed stream in unfinished basement of Jordan Hall on campus of Indiana University, Bloomington (Monroe County, Indiana).

Material examined: INDIANA: Monroe Co.: Seep-fed stream in basement of Jordan Hall, Indiana University, Bloomington, 1 female paratype (JRH), F. Young, Dec. 1962; **HOLOTYPE** male (15.8 mm, on 2 slide mounts; USNM), and 1 male paratype (JRH), 8 Jan. 1963 (specimens collected by Young donated to study by J. J. Lewis); **allotype** female (15.5 mm; USNM acc. nr. 395927), N. Hynes, Jan. 1963.

Diagnosis: A medium-sized species morphologically very similar to *B. wilsoni* and *B. angulus*.

Bactrurus cellulanus can be distinguished by the following characters: Distal margin of right lacinia mobilis with large, irregular denticles. Propods of gnathopod 1 and 2 subequal in length, propod of gnathopod 1 noticeably wider than propod of gnathopod 2; defining angle of gnathopod 1 distinct (ca. 140°). Pereopod 5 about as long as pereopod 4. Coxal gills on pereopods 2-7, distinctly reduced on pereopod 7 (less than half the size of coxal gill on pereopod 6). Two pairs of lateral sternal processes on pereonites 6 and 7; median sternal processes absent. Male uropod 1 with serrate peduncular process. Telson of adult male sometimes slightly longer than telson of female, apical spines shorter than those of female; telson with shallow notch or V-shaped excavation (5-19% cleft). Largest male 15.8 mm, largest female 15.5 mm.

Description: Based on holotype male (15.8 mm) and allotype female (15.5 mm).

Antenna 1 subequal to that of *B. mucronatus*, 60-79% length of body. Primary flagellum with up to 47 segments.

Antenna 2 about 45-49% length of antenna 1 (Fig. 5.23a). Flagellum with up to 16 segments.

Lower lip subequal to that of *B. wilsoni.*

Mandibles (Fig. 5.23b): Row of 7 plumose spines between molar and lacinia mobilis; distal margin of right lacinia mobilis subequal to that of *B. wilsoni* (with large, irregular denticles; see enlargement Fig. 5.23b). Palp segment 2 with 13-14 setae on lateral and medial margins; palp segment 3 with 28-30 short, plumose D-setae, 3-5 long, plumose B-setae and 2-4 long, plumose A-seta.

Maxilla 1 subequal to that of *B. wilsoni*.

Maxilla 2 (Fig. 5.23c): Outer plate apically with up to 19 setae. Inner plate with oblique row of 10 strong, plumose setae and up to 19 plumose setae apically and subapically.

Maxilliped: Outer plate with 4-5 bladelike spines and ca. 12 naked setae on inner margin (Fig. 5.23d). Inner plate apically with 4 bladelike spines and 3-4 plumose setae (Fig. 5.23e).

Gnathopod 1 (Fig. 5.24a): Basis with approximately 18 long, naked setae on posterior margin, 5 plumose setae on distoposterior corner, 7-8 naked setae on anterior margin and 5 naked setae on medial surface. Carpus with 2 rastellate spines on distoposterior margin (see enlargement of Fig. 5.17b) and ca. 6 plumose setae on distal margin. Propod noticeably wider than propod of gnathopod 2 (but about equally long); palmar margin almost even, crenulated, armed with 15-17 spines on lateral margin and about 21 spines on medial margin; defining angle distinct (ca. 140°), bearing a row of 5 spines medially and 4 spines on lateral corner; posterior (subangular) margin 23% length of propod, with 4 sets of plumose setae (distal set naked); anteromedial surface bearing 5 rows of plumose setae. Dactyl ca. 82% length of propod. Coxal plate with 6 setae.

Gnathopod 2 (Fig. 5.24b): Basis with 7-9 sets of long setae on posterior margin (with about 11 setae in total), 4 plumose setae on distoposterior corner, 3 long, naked setae on proximoanterior margin, 5-6 naked setae on distoanterior margin and 6 setae on medial surface. Carpus with 6 sets of plumose setae on posterior margin, 5-6 plumose setae on distal margin and 4 rastellate spines on distoposterior corner (see enlargement of Fig. 5.17b). Palm of propod almost even, crenulated, with 12-13 spines on lateral margin and about 14 spines on medial margin; defining angle rounded, with row of 4 spines on lateral corner; medial corner with row of 4 spines, accompanied by 2 long spines and 1 short spine; proximal (subangular) margin ca. 31% length of propod, bearing 5 sets of plumose setae; anteromedial surface bearing 6 rows of plumose setae. Dactyl 76 % length of propod. Coxal plate wider than long, bearing 5-7 setae.

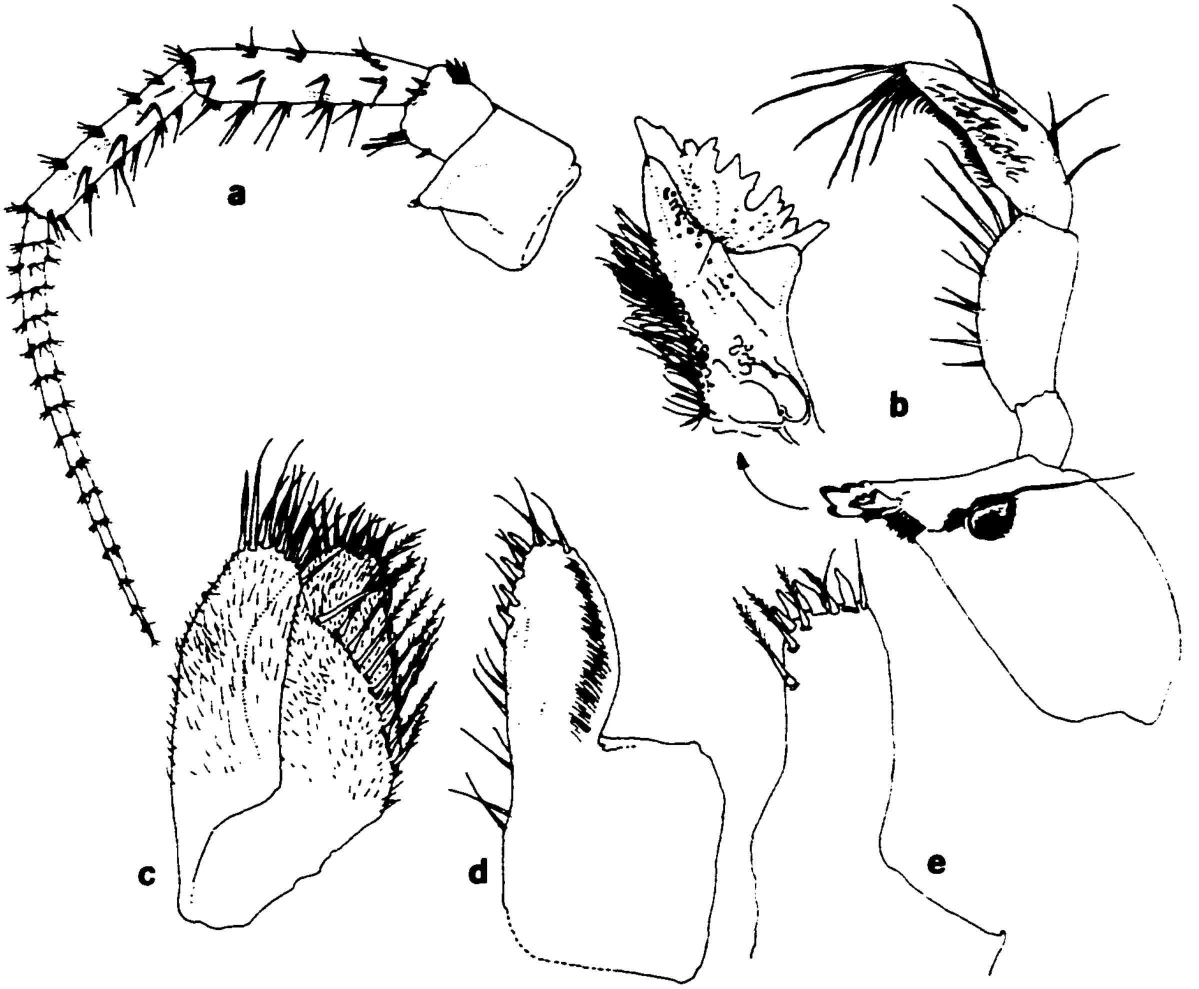


Fig. 5.23. *Bactrurus cellulanus* n. sp., holotype male (15.8 mm) from seep (Monroe County, Indiana): a) antenna 2. b) right mandible (lacinia mobilis enlarged). c) maxilla 2. d. e) outer and inner plates of maxilliped.

Pereopod 3 (Fig. 5.25a): Basis with 6 long setae and 2 short setae on posterior margin, 5-7 short setae on anterior margin and 4-7 long setae on proximoanterior margin and surface. Coxal plate with to 10-11 marginal setae.

Pereopod 4: subequal to pereopod 3. Coxal plate with 11marginal setae (Fig. 5.25b).

Pereopod 5 (Fig. 5.25c): Anterior margin of basis with 9 short spines; posterior margin with 15 short setae. Coxal plate with 4-5 setae on distoanterior lobe and 2 setae on distoposterior corner.

Pereopods 6 and 7 subequal. Anterior margins of bases with 6-8 short spines; posterior margins bearing up to 16 setae (including 2-3 slender spines). Coxal plate of pereopod 6 with 2 setae on distoposterior lobe (Fig. 5.25d). Coxal plate of pereopod 7 with 4 setae on posterior margin (Fig. 5.25e).

Coxal gills on pereopods 2-7, distinctly reduced on pereopod 7 (less than half the size of coxal gill on pereopod 6; Fig. 5.25e). Lateral sternal processes present on pereonites 6 and 7 (Fig. 5.25d, e).

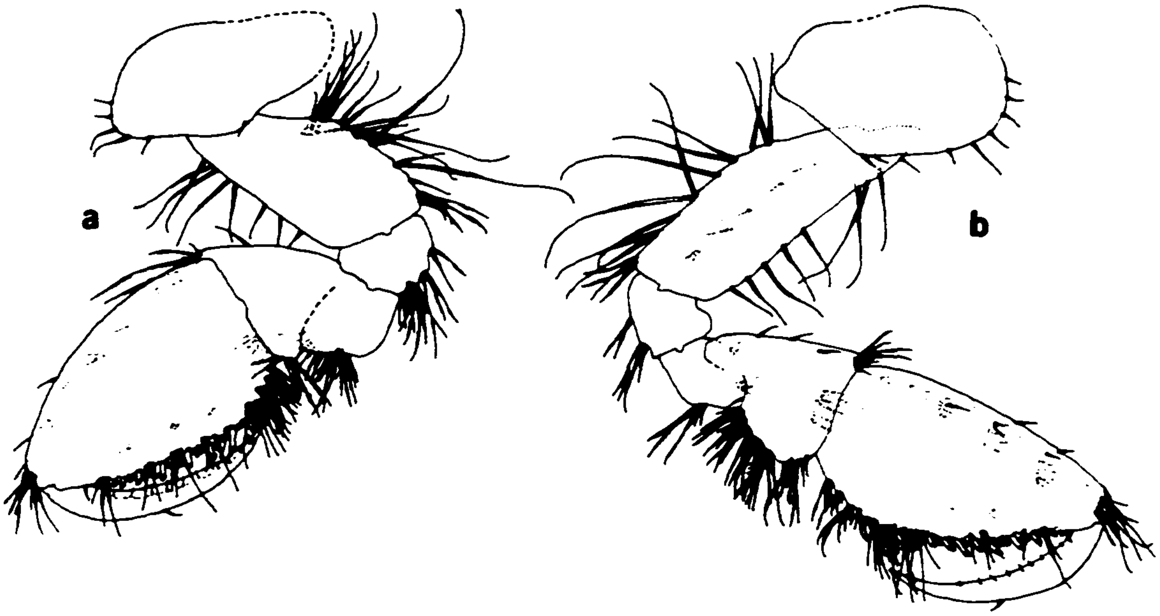


Fig. 5.24. *Bactrurus cellulanus* n. sp., holotype male (15.8 mm) from seep (Monroe County, Indiana): a) gnathopod 1 (lateral view), b) gnathopod 2 (lateral view).

Pleopods: Width of peduncles gradually increasing from pleopod 1-3. Number of unfused rami segments: pleopod 1 with 9-15 segments, pleopod 2 with 12-14 segments and pleopod 3 with 8-12 segments. Retinaculae reduced, asymmetrical (Fig. 5.25f).

Epimeral plates: Distoposterior margins with 5-7 short setae each; plate 1 without spines, plates 2 and 3 bearing 3 spines, respectively.

Uropod 1 (Fig. 5.25g): Peduncle with 12 spines on dorsolateral margin, 4-5 spines on dorsomedial margin and 4 spines on distomedial corner. Rami about 62% length of peduncle; inner ramus with 5 apical spines and about 8-9 spines on dorsal surface. Outer ramus with 5 apical spines and 8-10 spines on dorsal surface. Peduncle of male with distoventral, serrate process.

Uropod 2 (Fig. 5.25h): Peduncle with 3-5 spines on dorsolateral margin, 1 spine on dorsomedial margin (sometimes absent) and 2-4 spines on distomedial corner. Inner ramus longer than outer ramus, about 71% of length peduncle, bearing 4 apical spines and 7-11 spines on dorsal surface (some of which relatively long). Outer ramus with 5 apical spines and 5-7 spines on dorsal surface.

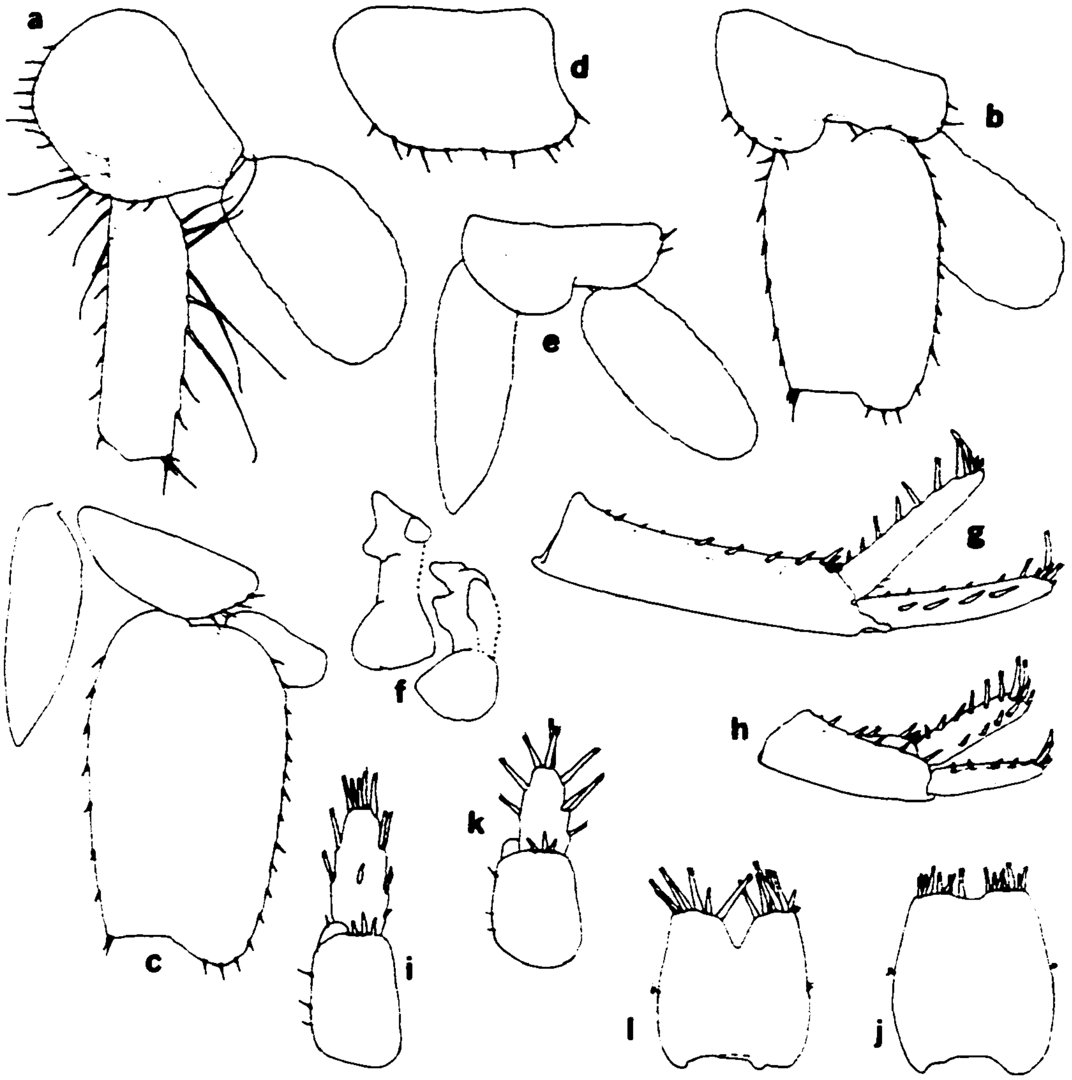


Fig. 5.25. *Bactrurus cellulanus* n. sp., holotype male (15.8 mm) from seep (Monroe County, Indiana): a-c) proximal parts of pereopods 3, 5 and 7, d) coxal plate of pereopod 4, e) coxal plate, gill and lateral sternal process of pereopod 6, f) retinaculae of pleopods, g-i) uropods 1-3, j) telson. Allotype female (15.5 mm) from same locality: k) uropod 3, l) telson.

Uropod 3 (Fig. 5.25i, j): Peduncle armed with 3 spines on distal margin. Inner ramus vestigial, 24-25% length of outer ramus (sometimes with 1 tiny apical seta). Outer ramus reduced (but relatively long), 80-101% length of peduncle, bearing 3-5 apical spines, 2-5 subapical spines on lateral margin and 2-

3 subapical spines on medial margin (sometimes 1 spine on dorsal surface).

Telson (Figs. 5.25l, k): Width 90% of length. Apical margin entire or with V-shaped excavation (15-23% cleft), bearing 13-15 apical spines. Male telson with relatively short apical spines; spines distinctly longer in female telson.

Sexual dimorphism and variation: Similar to *B. angulus*, variation could be found in the excavation of the distal telsonic margin (1 male with entire margin, other specimens with V-shaped excavation (15-23% cleft)). Secondary sexual dimorphism occurred as described in uropod 1 and telson.

Remarks: The type-locality is a small spring- or seep-fed stream in an unfinished basement beneath Jordan Hall on the campus of Indiana University. The terrain surrounding the seep is karst developed on Mississippian-aged limestone and is believed to have been a sinkhole complex prior to construction of Jordan Hall. Four specimens of the new species were collected in December 1962 and January 1963, but *Bactrurus* was not seen on a visit to the seep by JRH in June 1965. More recently, the diversion of the small stream and other work in the basement has apparently destroyed the type-locality (J. J. Lewis, pers. com.).

In addition to *B. cellulanus*, stygobiont amphipods (*Crangonyx packardi*) and isopods (*Caecidotea jordani*) and an epigean crayfish (*Cambarus*) have been collected from the type locality.

Bactrurus cellulanus is morphologically closely allied with *B. wilsoni* and *B. angulus*. Like the latter 2 species, *B. cellulanus* has a serrate peduncular process on the male uropod 1. It can be further distinguished as follows: inner plate of maxilla 2 with oblique row of 10 strong, plumose setae (6-7 in most other species); outer ramus of uropod 3 relatively long, bearing several subapical spines on both margins; peduncle of uropod 3 armed with 3 distal spines (usually 1-2 spines in other species).

Bactrurus cellulanus differs from *B. angulus* by the following characters: 21 medial and 15-17 lateral spines on palmar margin of gnathopod 1 (9-10 medial and 8 lateral spines in *B. angulus*); defining angle of propod on gnathopod 1 less distinct; rami of uropod 1 with 8-10 spines on dorsal surface (6 spines in *B. angulus*); dorsal margins of inner ramus on uropod with 7-11 spines, some of which relatively long (5 spines in *B. angulus*).

Bactrurus cellulanus can be distinguished from *B. wilsoni* as follows: outer plate of maxilliped with 4-5 bladelike spines (2-3 in *B. wilsoni*); posterior margin of carpus on gnathopod 2 with 6 sets of plumose setae (3 sets in *B. wilsoni*); proximal, subangular margin of propod on gnathopod 1 with 4 rows of plumose setae (less setose in *B. wilsoni*); anterior margin of basis on pereopod 5 with ca. 9 short spines (6 relatively long, slender spines in *B. wilsoni*); coxal gill of pereopod 7 less than half the size of coxal gill on pereopod 6 (only slightly reduced in *B. wilsoni*); dorsomedial margin of peduncle on uropod 1 with 4-5 spines (1 spine or absent in *B. wilsoni*); dorsal margins of inner ramus on uropod with 7-11 spines, some of which relatively long (4-5 spines in *B. wilsoni*).

Etymology: The epithet cellulanus (Latin for "hermit" or "recluse") refers to the "reclusive" habitat in the basement of Jordan Hall.

Phylogenetic analysis

Cladistic methods

The phylogenetic analysis was performed using PAUP, version 3.0s. Initially, all characters were left unordered and unweighted. Subsequently, some characters were ordered and weighted through outgroup comparison (see Character assessment and Appendix C). In the following text, these alternative runs will be referred to as 'unordered analysis' and 'partially ordered analysis'. During a Heuristic Search, the ancestral condition was left 'unknown' and the following search options were in effect: only minimal trees were kept, collapsing zero-length branches; TBR branch swapping was performed on minimal trees only (steepest descent by random stepwise addition). The trees obtained with these settings were used for subsequent runs, keeping all minimal trees (MULPARS option) that were as short as or shorter than those loaded into memory. These steps were repeated until no shorter trees could be found. A strict consensus tree was calculated from the trees generated by the Heuristic Search.

Character assessment

Except for the species of *Bactrurus* that were examined during the study, the assessment of character states is based on descriptions and drawings from the literature (see Appendix C). During the partially ordered analysis, more complex characters were ordered and weighted, assuming that their evolution had to be more directed and less likely than the development of more simple structures. For example, the different stages of reduction of coxal gills in several crangonyctid and bogidiellid taxa suggest a stepwise, gradual evolution over a long period of time, whereas the occurrence or loss of spines and setae on appendages may have evolved much faster (Koenemann & Holsinger, 1999a).

Terminal taxa

The strength of phylogenetic analyses is strongly dependent on the quality and quantity of the data employed. For example, to investigate the relationship of *Bactrurus* to its potential sister-genus *Stygobromus*, the morphological similarity of both genera has to be taken into consideration. In this case, the preferable approach was to analyze a selection of taxa that represent different species groups of *Stygobromus*. But we also wanted to investigate the phylogenetic relationship of *Bactrurus* and *Stygobromus* to other genera of the family Crangonyctidae. Therefore, in addition to the seven *Bactrurus* species, the following taxa were included to ensure that the analysis was as comprehensive as possible for the scope of this study (Table V.1):

- *Stygobromus mackini* Hubricht, 1943, and *Stygobromus hoffmani* Holsinger, 1978, occur primarily in limestone caves at the eastern margin of the *Bactrurus* distribution range (Fig. 5.26). Each represents a group of morphologically closely allied species, respectively: the mackini and emarginatus groups. Stygobromus grahami Holsinger, 1974, is a species of the *hubbsi* group found in the western United States. The 14 species of the *tenuis* group, represented in the analysis by *Stygobromus t. tenuis* (Smith, 1874), have the widest distribution range of all *Stygobromus* species groups. They are recorded from several states in eastern, southern, and central parts of the United States. *Stygobromus phreaticus* Holsinger, 1978, and *Stygobromus araeus* (Holsinger, 1969), are morphologically unique species that occur in shallow groundwater habitats in eastern Virginia.

- *Crangonyx forbesi* (Hubricht & Mackin, 1940), a stygophile inhabitant of caves and related groundwater habitats, with reduced eyes and pigmentation, frequently co-occurs with *B. brachycaudus*. A large part of the range of *C. forbesi* overlaps with that of *Bactrurus*. *Crangonyx subterraneus* Bate, 1859, was chosen as a European representative of the genus *Crangonyx*.
- *Synurella dentata* Hubricht, 1943, is an epigean crangonyctid amphipod from the east-central United States. Similar to *C. forbesi*, its range partly overlaps that of *Bactrurus*. *Synurella a. ambulans* (Müller, 1846) was included as a European species of the genus.
- The monotypic crangonyctid genus *Lyurella* Derzhavin, 1939, is endemic to the Caspian Sea region and shares several diagnostic characters with *Synurella* (e.g. coxal plates 1 and 2 longer than wide; presence of reduced eyes; telson longer than wide).
- *Stygonyx courtneyi* Bousfield & Holsinger, 1989, is a monotypic crangonyctid genus known only from a single hypogean freshwater locality in the state of Oregon. It is morphologically very similar to *Stygobromus*.
- *Gammarus pulex* Linnaeus, 1758, and *G. troglophilus* Hubricht & Mackin, 1940, were chosen as outgroup taxa. *Gammarus pulex* is a common epigean freshwater species in many parts of Europe and has been recorded as far east as Siberia, including Lake Baikal. *Gammarus troglophilus* is a stygophile that inhabits both epigean and hypogean habitats in the central United States; its range overlaps that of *Bactrurus*.
- The following stygobiont taxa were included as additional outgroups: *Niphargus fontanus* Bate, 1859 (widely distributed in Europe); *Spelaeogammarus spinilacertus* Koenemann & Holsinger, 2000, and *Megagidiella azul* Koenemann & Holsinger, 1999b, two genera of the family Bogidiellidae endemic to eastern and south- central Brazil, respectively.

Results

Both unordered and partially ordered analyses produced strict consensus trees with well-resolved, very similar topologies (Fig. 5.27). Both trees have a single stem, with the species of *Gammarus* as basal sister-group to all stygobiont and stygophile taxa. *Niphargus fontanus* appears as a sister-group to both Bogidiellidae and Crangonyctidae. The small, monophyletic clade of the bogidiellids is a sister-group to the Crangonyctidae. A somewhat unexpected result is that *Bactrurus* is not monophyletic. The genus is subdivided into two smaller clades: a trichotomy with *B. wilsoni B. angulus* and *B. cellulanus* (hereafter referred to as *wilsoni* group) and *B. brachycaudus* on a clade with the morphologically closely related *B. mucronatus* and *B. pseudomucronatus* (hereafter referred to as *brachycaudus* group). Interestingly, the analyzed data set renders *B. hubrichti* as a sister-group of both *Bactrurus* and *Stygobromus*.

TABLE V.1

Character matrix. See Appendix C for a description of characters (characters are numbered according to their listing in Appendix C). Outgroup taxa of the partially ordered analysis are capitalized.

GAMMARUS PULEX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GAMMARUS TROGLOPHILUS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NIPHARGUS FONTANUS	1	0	1	2	0	0	0	0	0	1	0	1	0	0	0	2
MEGAGIDIELLA AZUL	1	0	2	2	0	0	1	0	1	0	0	0	1	0	1	3
SPELAEOGAMMARUS SPINILACERTUS	1	0	3	2	0	0	1	0	1	0	0	1	1	0	1	1
Bactrurus brachycaudus	1	1	0	1	0	1	1	0	1	1	0	1	2	1	1	2
Bactrurus hubrichti	1	1	0	0	0	2	1	0	1	1	0	0	1	1	1	2
Bactrurus mucronatus	1	1	1	2	0	2	1	0	1	1	2	1	2	1	1	2
Bactrurus pseudomucronatus	1	1	0	1	1	1	1	0	1	1	2	1	2	1	1	2
Bactrurus wilsoni	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	2
Bactrurus angulus	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	2
Bactrurus cellulanus	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	2
Crangonyx forbesi	0	0	0	1	1	2	1	0	1	1	0	0	1	1	0	2
Crangonyx subterraneus	1	0	1	2	0	2	1	0	2	1	0	0	1	1	0	2
Lyurella	0	0	0	?	1	1	1	0	5	2	0	1	1	1	0	2
Stygobromus araeus	1	0	1	2	1	1	1	0	4	2	1	1	2	1	1	2
Stygobromus grahami	1	0	1	2	0	0	1	1	2	2	0	1	2	1	1	2
Stygobromus hoffmani	1	0	0	0	1	1	1	1	2	2	0	1	2	1	1	2
Stygobromus mackini	1	1	0	1	1	1	1	1	2	2	0	1	1	1	1	2
Stygobromus phreaticus	1	1	1	2	0	1	1	1	4	2	0	0	1	1	1	2
Stygobromus t. tenuis	1	1	0	1	1	2	1	1	3	2	1	1	2	1	1	2
Stygonyx courtneyi	1	0	0	1	0	1	1	1	2	2	0	0	1	1	0	2
Synurella a. ambulans	0	0	0	?	1	2	1	0	3	2	1	1	1	1	0	2
Synurella dentata	0	0	0	1	1	2	1	0	2	2	0	1	0	1	0	2

Although the crangonyctid taxa form a large, monophyletic clade in both consensus trees, the topology within the crangonyctids shows some interesting differences. The first point to note is that *Stygobromus* is not monophyletic in the consensus tree of the unordered analysis: a small clade with *Stygobromus phreaticus*, joined by Stygonyx courtneyi, appears as a sister-group to the remaining *Stygobromus* species. In the partially ordered analysis, *Stygonyx courtneyi* has become the sister-group to a monophyletic *Stygobromus* clade. The second difference between unordered and partially ordered analyses is a small clade with both *Synurella* species and *Lyurella*. This clade remains unresolved in the unordered analysis, whereas *Synurella dentata* is a sister-group to *Lyurella* and *Synurella a. ambulans* in the partially ordered analysis.

Discussion

Phylogenetic analysis

The congruent sequence of outgroup taxa in both analyses suggest monophyly for the Crangonyctidae, with Niphargidae and Bogidiellidae as sister-groups (Fig. 5.27). *Crangonyx* is paraphyletic but both species included occur at basal positions of a large, monotypic crangonyctid clade. The phylogenetic relationships of the genera *Crangonyx*, *Lyurella*, *Synurella*, *Bactrurus* and *Stygobromus* is in agreement with Holsinger's cladogram of the Crangonyctidae (1986a). Both unordered and partially ordered analyses maintain *Lyurella* and *Synurella* on a single clade, suggesting a close relationship of *Lyurella* to Synurella, as postulated by Holsinger (1977, 1986a). However, the different topology within this clade in both trees leaves the relationship of *Synurella* and *Lyurella* unresolved. The partially ordered analysis places *Lyurella* closer to the European *Synurella a. ambulans*. However, more data are needed to clarify the relationship of these genera.

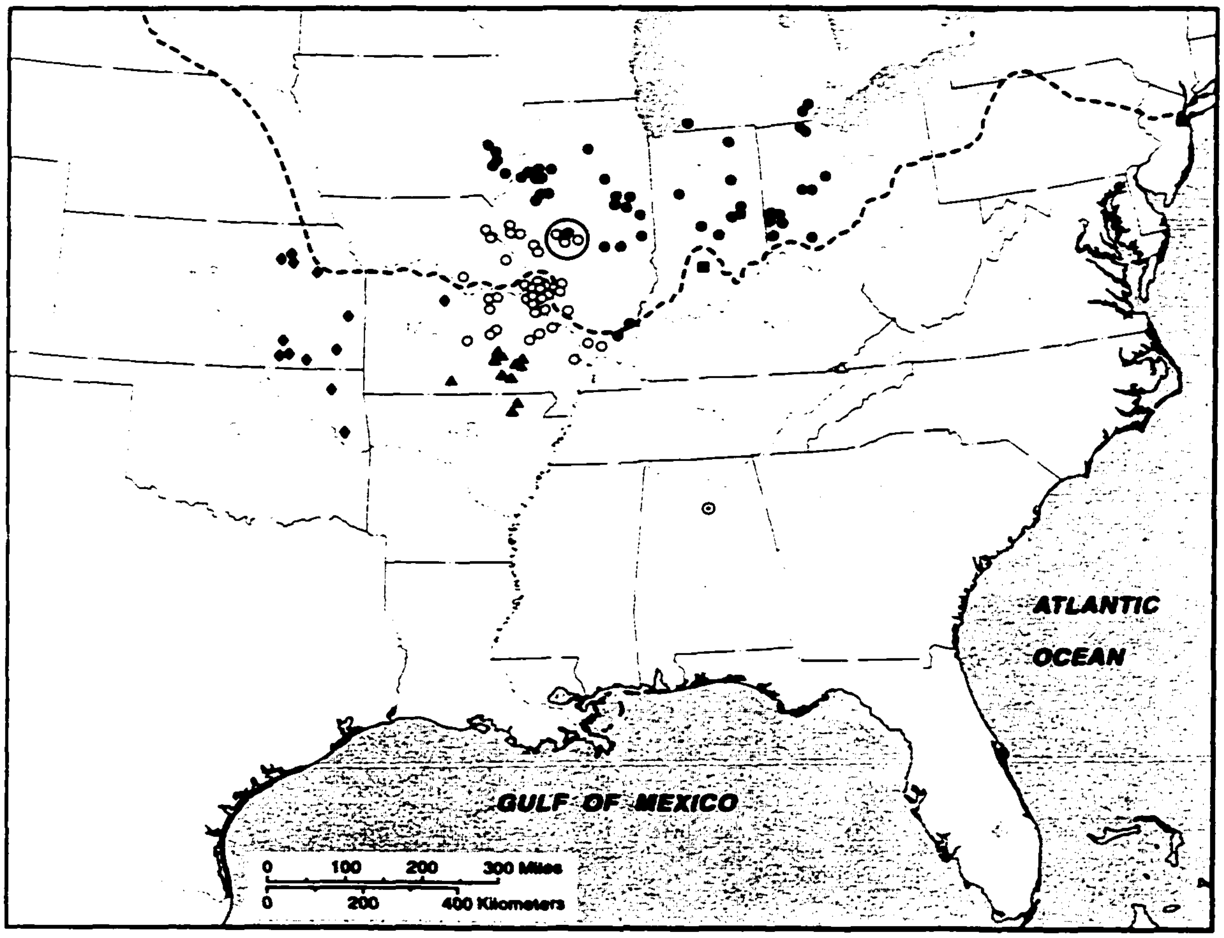


Fig. 5.26. Geographic distribution of *Bactrurus* in eastern and central North America. Each symbol represents approximately one locality record (the actual number of records may be slightly higher in some of the densely clustered areas). Open circles: *B. brachycaudus*; filled diamonds: *B. hubrichti*; filled circles: *B. mucronatus*; filled triangles: *B. pseudomucronatus*; dot surrounded by circle: *B. wilsoni*; filled square: *B. cellulanus*; open triangle: *B. angulus*. The large open circle in the glacial drift area (Illinois) surrounds several localities: three are the first records for *B. brachycaudus* from glacial drift habitats; one is a locality occupied by both *B. brachycaudus* and *B. mucronatus* (see text). The maximum southern extent of Pleistocene glaciation is indicated by the black dashed line. The dotted line delineates the maximum extent of a continental marine embayment in the middle to late Cretaceous (adapted from Holsinger, 1993).

Another difference of the unordered and partially ordered analysis affects the relationship of *Stygonyx courtneyi* with the *Stygobromus* taxa. Interestingly, the diagnostic distinction between both genera is mainly based on the comparatively longer outer ramus of uropod 3 in *Stygonyx*. Apart from this character, *Stygonyx* and *Stygobromus* are morphologically indistinguishable. Considering the comparatively greater variation of outer rami length on third uropods in *Bactrurus* (with *B. mucronatus* and *B. cellulanus* as the most extreme forms), the given topology probably correctly reflects the close phylogenetic relationship of *Stygonyx* and *Stygobromus*. A separate generic status for *Stygonyx courtneyi* is questionable and its assignment to the genus *Stygobromus* is perhaps justified (In this case, the analyses would imply monophyly for *Stygobromus*). But as in the case of *Lyurella/Synurella*, more data and certainly also additional records are needed to resolve the relationships of these genera.

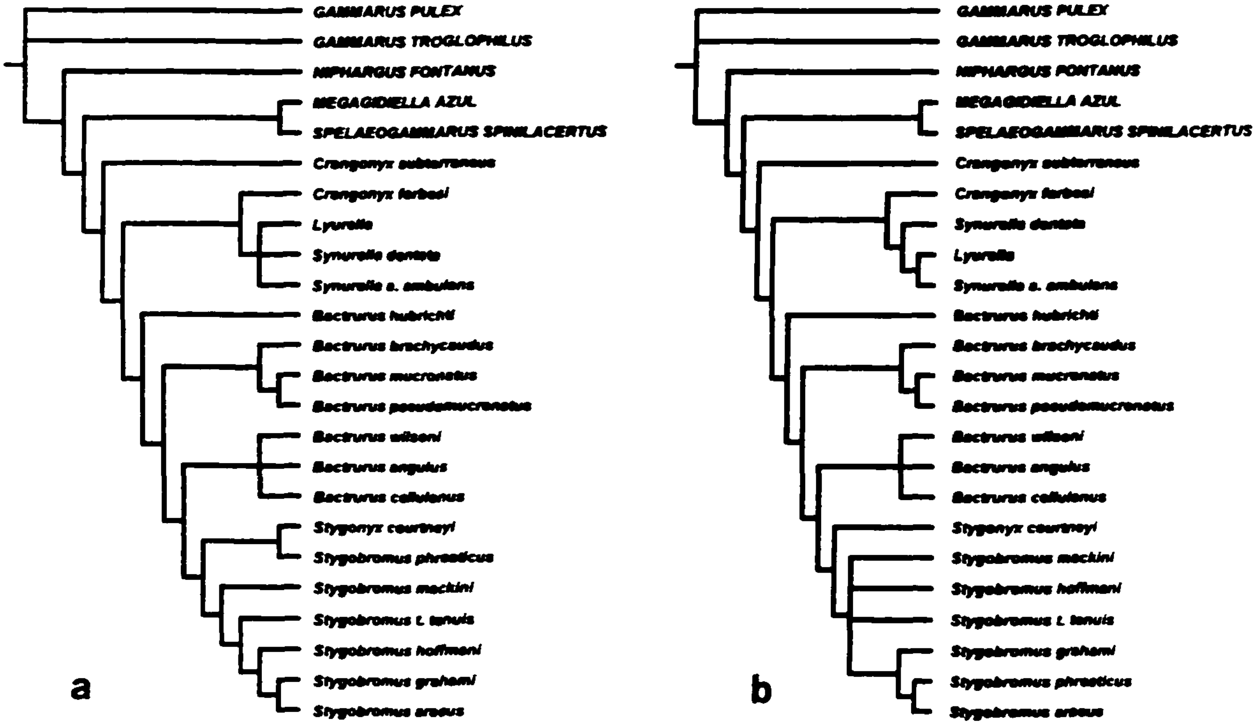


Fig. 5.27. Phylogenetic analysis of seven *Bactrurus* species, including selected crangonyctid genera and several outgroup taxa (capitalized). a) PAUP Strict Consensus tree: characters unordered and equally weighted (CI: 0.44; RI: 0.68; RC: 0.30; length: 65; min. possible length: 29; max. possible length: 143). b) PAUP Strict Consensus tree: characters partially ordered and weighted (CI: 0.43; RI: 0.70; RC: 0.30; length: 145; min. possible length: 63; max. possible length: 341).

In both trees, *Bactrurus* and *Stygobromus* are nested in a large clade, distinguished from the other crangonyctid taxa by two synapomorphies: character 2 (presence of rastellate spines on the carpus of gnathopod 2) and character 15 (coxal plates 1 and 2 wider than long). The paraphyly of *Bactrurus* is largely determined by two factors:

(1) The *wilsoni* group has become the sister-group to the *Stygobromus/Stygonyx* clade because it shares a distinct synapomorphy with these genera (character 8: peduncle of male uropod 1 with serrate process).
(2) The *brachycaudus* group is separated by a homoplasious character 13 (telson not cleft), which is also found in a clade with four *Stygobromus* species in the tree of the unordered analysis (*S. t. tenuis*, *S. hoffmani*, *S. grahami* and *S. araeus*).

The fact that *Bactrurus* appears as a paraphyletic genus is not too surprising when we compare it morphologically with *Stygobromus*. The taxonomic discrimination between both genera is based primarily on two diagnostic characters: (1) *Stygobromus* is distinguished from *Bactrurus* by the absence of an inner ramus and a more reduced outer ramus of uropod 3; (2) blade-like spines on the outer plate of the maxilliped are either absent or much reduced in number in most *Stygobromus* species. However, of these differences only the absence of the inner ramus on uropod 3 is a truly robust diagnostic character. The other two characters (length of outer ramus of uropod 3 and armature of outer plate of maxilliped) show a considerable amount of variation in both genera, so that a clear separation of two distinctly different degrees of reduction becomes nearly impossible. It appears that the length of the outer ramus of uropod 3 shows an almost continuous reduction from *Crangonyx* to *Bactrurus* to *Stygobromus*. Similarly, there are additional diagnostic characters that cannot be unambiguously used to morphologically distinguish *Bactrurus* from *Stygobromus*. For example, pereopod 7 varies from little to distinctly longer than pereopod 6 in *Bactrurus*, whereas in *Stygobromus* it is shorter than, equal to, or a little longer than pereopod 6.

The employment of different or additional characters may produce trees with different topologies for *Bactrurus* and *Stygobromus*. However, it is the relatively large amount of morphological variation, mainly within the genus *Stygobromus*, that is likely to complicate a character analysis and cloud its outcome. In the long run, the utilization of molecular analyses may be the best way to gain a more complete picture of the phylogeny within the Crangonyctidae.

The data used in the phylogenetic analysis support an ancient freshwater origin for the crangonyctids. Several taxa with distinct Holarctic distributions (*Crangonyx* and *Synurella*) appear at basal positions of a monophyletic Crangonyctidae. However, more data are needed to investigate the origin of crangonyctid amphipods in greater detail. A conclusive answer to this question will depend on whether or not we can establish monophyly for the Crangonyctidae.

The results of the phylogenetic analysis do not support *Bactrurus* as a monophyletic group. Furthermore, *Bactrurus* and *Stygobromus* do not appear as sister-groups. However, both genera appear on a separate clade together with *Stygonyx courtneyi*. Although the cladograms rendered a stable topological separation of both genera, we cannot unambiguously infer if a close relationship is based on homoplasious similarity or common ancestry.

The Biogeography of *Bactrurus*

The cladograms show an interesting branching pattern for *Bactrurus* that can be related to the geographic distribution of the individual species. *Bactrurus hubrichti* appears on a single branch as the sister-group of the other two clades with three species each.

- The first clade is composed of the three species of the *brachycaudus* group, each of which is relatively abundant and two of which have wide ranges. Together, they form a more or less continuous cluster of records, which embraces most of the range of *Bactrurus* (Fig. 5.26).
- A second, polytomous clade is formed by the *wilsoni* group, which is composed of three highly endemic species that share several apomorphic characters. Typically, the localities of the *wilsoni* group species are disjunct and few in number.
- The distribution of *B. hubrichti* is characterized by a pattern that differs from both the *brachycaudus* and *wilsoni* groups: It has a relatively wide range, but many of the localities are disjunct. With the exception of a single record from central Missouri, the range of *B. hubrichti* is separated from the *brachycaudus* group by a distinct gap (Fig. 5.26).

1. The *wilsoni* group

The three species of the *wilsoni* group are endemic to isolated areas. Two species occur in the Appalachians and thus are far removed from all other species in the genus. *Bactrurus cellulanus* and *B. wilsoni* are recorded from single localities, whereas *B. angulus* is found in three caves within a small area (Fig. 5.26). The distances between the range of *B. angulus* and *B. wilsoni* and the main cluster of species in the Central Lowland and Ozark Plateaus Physiographic provinces are remarkably large, with no recorded species in between. The greatly delimited range of *B. cellulanus* appears to be an exception to this pattern, inasmuch as it occurs in relatively close proximity to the extensive range of *B. mucronatus* in central and northern Indiana. However, all records of *B. mucronatus* in Indiana are from glaciated areas, whereas *B. cellulanus* was found in an unglaciated karst region south of the glacial drift areas. Hence, we may conclude that a dispersal barrier exists between the range of *B. cellulanus* and the groundwater aquifers of the adjacent drift region to the north. Repeated efforts to find *B. cellulanus* in caves and related groundwater habitats in the greater Bloomington area surrounding the type locality have been unsuccessful to date, leading to the conclusion that this species represents an isolated relict on the periphery of the range of *B. mucronatus*.

In addition to their isolated distributions, species of the *wilsoni* group also share several unique morphological characters: the males of all three species have serrate peduncular processes on uropod 1, which are absent in the other species of *Bactrurus*, but very common in *Stygobromus* (see also Phylogenetic analysis). Moreover, the species of the *wilsoni* group have an almost identical form of sexual dimorphism of the telson (differing width/length ratios and armature). The possession of several unique synapomorphies suggests that species of this group descended from a common ancestor with a continuous distribution. The occurrence of the *wilsoni* group in the Appalachians and Interior Low Plateau, at great distances from the extensive marine embayments of the Cretaceous (see Fig. 5.26), suggests that these species are probably remnants of a very old freshwater group, with a long since fragmented distribution pattern. Similarly, the other four species of *Bactrurus*, despite having comparatively much wider ranges, also occur in a part of the continent that were not exposed to marine waters during the Cretaceous. The evolutionary history of *Bactrurus* seems to be in accordance with that of other genera of the Crangonyctidae. The family is believed to be an "ancient" freshwater group that was already established on Laurasia prior to the separation of North America and Eurasia in the early Mesozoic (see Holsinger, 1986a, 1986b, 1994b).

2. The *brachycaudus* group

2.1. *Bactrurus mucronatus*

In the *brachycaudus* group, two species also share a unique, conspicuous apomorphy: males of *B. mucronatus* and *B. pseudomucronatus* have an extremely elongate telson. However, the distributions of the two species are disjunct. *Bactrurus pseudomucronatus* occurs exclusively in karst groundwater habitats in parts of the Ozark Plateaus of southern Missouri and northern Arkansas, whereas *B. mucronatus* inhabits almost exclusively glacial drift areas of the Central Lowland Physiographic Province. The only exceptions for *B. mucronatus* appear to be a single karst locality in southern Illinois (Equality Cave), where the species has been regularly collected from a cave stream over the last 60 years, and Bear Cave near Lake Michigan (Berrier County, Michigan). Prior to this study, the occurrence of the population in Equality Cave was believed to be isolated by a considerable distance from the closest recorded drift population in central Illinois (Holsinger, 1986a). However, new locality records obtained in May 1999 helped to fill in this gap. They included a locality in the drift area approximately 16 km north of Equality Cave. The new records indicate a more continuous distribution of *B. mucronatus* in the Central Lowland, as well as a possible hydraulic connection of karst and drift aquifers in southern Illinois.

On the distribution map of *Bactrurus*, *B. mucronatus* is the only species of the genus that occurs almost exclusively north the line that delineates the maximum extent of the Pleistocene glaciation (Fig. 5.26). Two alternative theories have been proposed to explain the occurrence of stygobiont crustaceans in glaciated areas:

- A few stygobionts survived periods of glaciation in groundwater refugia under the ice.
- Some stygobionts followed the receding ice northward at the end of the last Pleistocene glaciation and subsequently invaded and colonized newly available habitats in coarse sediments deposited by glaciers.

Because it was widely assumed that the Pleistocene ice sheets destroyed the hypogean biota they covered, most workers seemed to favor the second explanation (Holsinger, 1978; Lewis & Bowman, 1981). However, based primarily on the distribution of a number of *Stygobromus* species that occur north of the glacial boundaries, and which appear to be closely related to those in unglaciated areas, Holsinger (1978, 1981, 1986a) made a strong case for a subglacial refugia model. Nevertheless, survival in subglacial refugia was considered improbable for *B. mucronatus* (Holsinger, 1986a). This assumption was based primarily on the occurrence of the morphologically close populations south of the glacial boundary, described herein as

B. pseudomucronatus. *Bactrurus mucronatus* was believed to have split from an ancestral species (*B. pseudomucronatus*) by northward dispersal and subsequent geographic isolation. In this scenario, *B. mucronatus* originated from a region south of the maximum extent of the Pleistocene glaciations (e.g. southern Missouri and possibly southern Illinois) and followed the receding glaciers north to its present range. However, there are several biogeographic features of *B. mucronatus* that weaken the likelihood of lateral dispersal and invasion of glacial drift areas subsequent to glacial recession:

- The distribution range of *B. mucronatus* extends over a distance of approximately 650 km, from west to east. Given what are generally regarded as weak dispersal abilities of hypogean amphipods, a colonization of these dimensions is not very probable within the given time-frame (i.e., dispersal would have started at the end of last glaciation, perhaps 10000 to 16000 BP).
- A lateral (horizontal) invasion and colonization of drift is assumed to have started from an area south of the glaciated regions, presumably originating in proximity to the present range of *B. pseudomucronatus* (by splitting into two lineages). In this scenario, we should expect to find remnant populations between the center of dispersal and present range. However, *B. mucronatus* and *B. pseudomucronatus* are separated by a gap, which is occupied by *B. brachycaudus* and there are no records of *B. mucronatus* in this area.
- The dispersal from a hypothetical center should also result in a typical, branch-like pattern, following major drainage systems with decreasing densities from center to outer margins. It is obvious from the distribution map that the present range of *B. mucronatus* does not show this kind of pattern.
- *Bactrurus brachycaudus* inhabits karst groundwater habitats much closer to the drift areas than *B. pseudomucronatus*. Yet, several new localities for the former species in glacial drift discovered during the recent fieldwork appear to be exceptional occurrences. It is therefore likely that dispersal from karst into interstitial groundwater systems in western Illinois is restricted or obstructed by physiographic barriers.

Rather than pointing towards a dispersal center south of its present range, the distribution of *B. mucronatus* extends in a belt-like fashion from east to west, with the most densely populated areas along a center axis, also roughly following an east-west stretch (Fig. 5.26). In glaciated areas, specimens are often common at the outlets of drainage systems in farm fields (Fig. 5.28). During wet periods, usually in late winter and spring, many outlet pipes discharge groundwater without interruption, continually flushing out stygobiont invertebrates (mostly *B. mucronatus* accompanied by the stygobiont asellid isopod *Caecidotea kendeighi*). At several outlet catchment basins, one of us (SK) counted up to 200 flushed-out specimens of *B. mucronatus* and *C. kendeighi*. Large sample sizes (up to 100 specimens) from drain outlets were recorded in the early 1940s by Leslie Hubricht. This abundance is in sharp contrast to cave habitats that are typically sparsely populated. These findings suggest that the glaciated areas of the Central Lowland might have sheltered comparatively large and stable populations of *B. mucronatus* for a long period of time, stretching well back into the Pleistocene. How can this be explained?

Fig. 5.28. Two photographs showing outlets of drain pipes in central Illinois. Stygobiont amphipods and isopods are often found in these outlets, presumably having been flushed out of deeper, interstitial habitats and into drainage systems when groundwater tables are elevated during wet periods. Above, water is passing from a pipe into a ditch or small creek. Below, one can see the outlet pipe of a drainage system in a large, flat field. These systems are very common in glacial drift areas of the mid-west and in unconsolidated sediments on the eastern coastal plain. They consist of a series of perforated pipes, which are buried approximately 2 meters beneath the surface of poorly drained farm fields. In late winter and early spring, excess water from those fields is drained off in preparation for seasonal plowing and planting.

Over the last 1 to 2 million years, much of the Central Lowland was profoundly affected by periods of continental glaciation. Glaciers repeatedly advanced and retreated across the surface, disrupting preglacial drainage patterns and eroding both bedrock and older, unconsolidated sediments. The moving ice sheets carried vast amounts of debris, which was mostly deposited as unsorted sediments (till). In some regions, however, the melting glaciers formed well-sorted layers of sands and gravels. Many of these deposits were subsequently covered by till. Quaternary deposits in the Central Lowlands are between 10 to 400 feet thick. Geologic investigations in Illinois have revealed a complex, 3-dimensional network of groundwater aquifers in Quaternary drift (Larson et al., 1995, 1997). The distribution of subterranean aquifer systems depends on various hydrogeological factors, for example, stratigraphy and consistency of deposits, transmissivity and storage capacity of sediments, and also properties of the underlying bedrock (Larson et al., 1997).

The distribution of *B. mucronatus* in the Central Lowlands is apparently correlated with thicker Quaternary deposits (60-120 m) and/or deposits near major surface streams as shown in Fig. 5.29. Considering the complex distribution of shallow sand and gravel aquifers and deeper bedrock aquifers in the Central Lowland, the invasion of drift by *B. mucronatus* may have been an upward (vertical) rather than a lateral (horizontal) dispersal process. Since the permafrost during glaciation periods did not penetrate the ground deeper than approximately 5 m, the colonization of glacial deposits could have taken place much earlier than postulated by a lateral, post-glaciation dispersal model (i.e. between 10,000 and 16,000 BP). In a vertical dispersal scenario, the invasion of drift may have occurred during glacial and interglacial periods and date back as early as the onset of Pleistocene glaciation.

Bactrurus mucronatus is the most widespread and abundant species of the genus. Its distribution pattern suggests that the colonization of an extensive network of (newly) available interstitial habitats is the result of adaptive radiation. Moreover, groundwater aquifers in drift areas might have a considerably higher nutrient input in comparison with those in karst. The leaching of soluble constituents by precipitation is likely to play a more crucial role in the flat drift regions with extensive farm fields, where a relatively low run-off results in increased and more diffuse percolation and infiltration.

It is difficult to say whether *B. mucronatus* was already widespread in the Central Lowland before glacial deposits accumulated or whether it originated from ancestral relicts that were isolated somewhere within its present range in subglacial refugia and repeatedly re-invaded newly available aquifers. Larson et al. (1995 & 1997) point out that some deep bedrock aquifers yield groundwater to overlying glacial drift. The hydraulic connection is maintained through fractured bedrock (carbonates) or permeable units (sandstone). Vertical invasions from deeper bedrock aquifers into shallow sand and gravel habitats cannot be excluded as an important factor in the distribution of *B. mucronatus*.

2.2 *Bactrurus brachycaudus*

Bactrurus brachycaudus may be able to invade newly available habitats in much the same way as we have postulated for *B. mucronatus*. An illustrative example of the rapid colonization of new habitat space was observed by one of us (SK) in May, 1999. In a karst area at Tyson Research Center (Missouri), a former U.S. Army bunker had been modified into an artificial cave habitat. Mr. Earl Biffle, who designed and maintains the 'cave bunker', diverted part of the outflow of a natural spring, located on a slope just

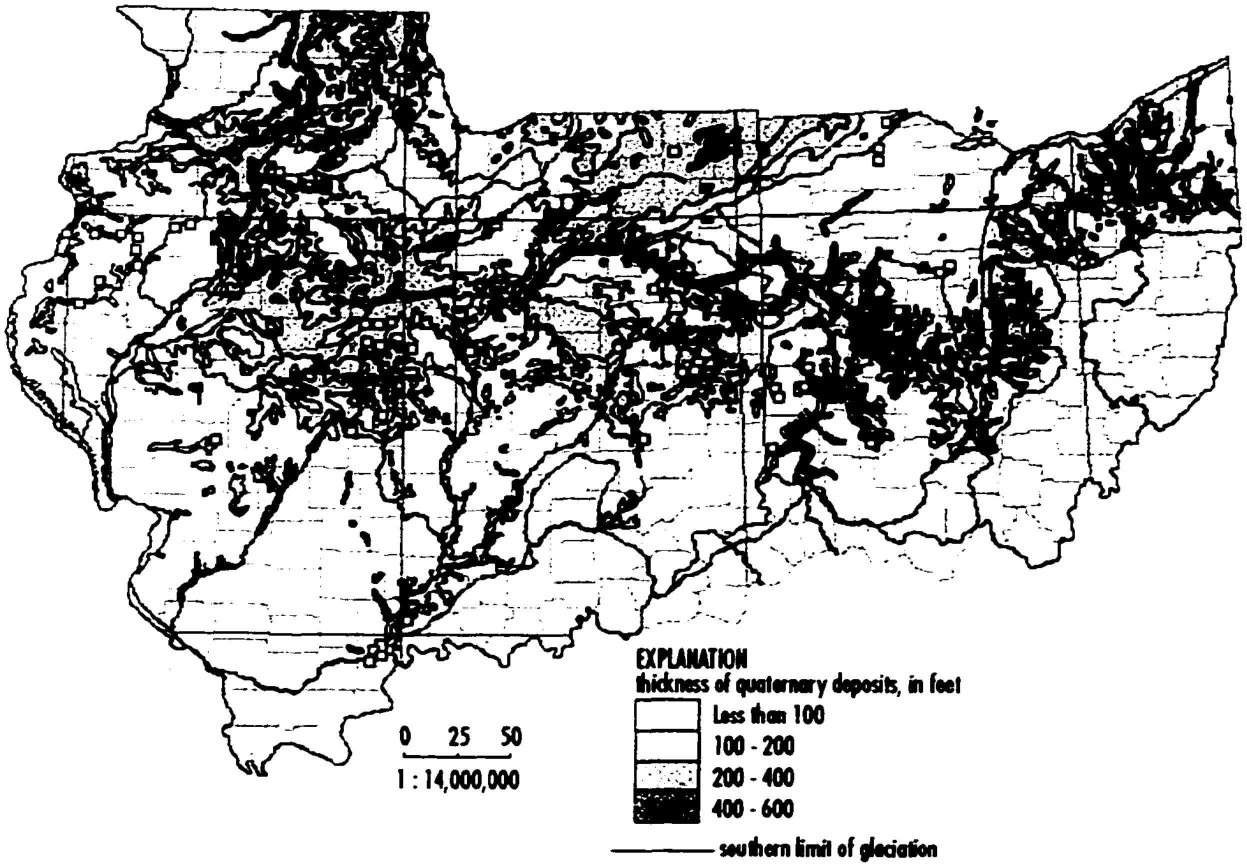


Fig. 5.29. Distribution of *B. mucronatus* in a part of the Central Lowland Physiographic Province that covers the states of Ohio, Indiana and Illinois. Each symbol (open squares) represents a collection record for this species. Quaternary deposits within pre-glacial bedrock valleys or along streams and rivers generally contain shallow sand and gravel aquifer systems. The unglaciated areas south of the glaciation line are rendered in the lightest shade of grey. (Map modified, with kind permission of U.S. Geological Survey)

above the bunker. He let the spring water flow through a simple pipe system, which entered the bunker at the back side. The interior of the bunker (ca. 5 x 20 m) was parted by a little, low wall that allowed the formation of a shallow pool (ca. 15 cm deep) and the discharge of excess water at the front of the bunker. After a long and thorough search at the mouth of the spring only one juvenile specimen of *B. brachycaudus* could be found, but the bunker itself was populated by several hundred individuals of that species. Interestingly, only a small number of specimens actually stayed in the artificial pool (which was inhabited by epigean gammarids and a few cave salamanders). Instead, swarms of juveniles and adults, many of which reached up to 30 mm in length, were crawling under aluminum foil covers outside the pool (!) in shallow puddles of water. Mr. Biffle assured us that he did not provide any supplemental nutrients. However, he introduced the salamanders as natural predators to the artificial habitat.

Another significant discovery during the 1999 field trip was a drain pipe sample that contained specimens of both *B. brachycaudus* and *B. mucronatus*. Not only is this the first record of *B. brachycaudus* from a non-karst habitat, it is also the first recorded co-occurrence of two *Bactrurus* species from the same locality. The sample was taken at the outer, northeastern margin of the range of *B. brachycaudus* rage in Montgomery County, Illinois. The specimens from this locality showed minor morphological variation in several characters when compared with *B. brachycaudus* specimens from karst habitats (see *B. brachycaudus*, Remarks). However, a conspicuous degree of sexual dimorphism was found in the telson, with male telsons up to 20% longer than those of females. It cannot be completely ruled out that these specimens are hybrids of a cross between *B. mucronatus* and *B. brachycaudus*.

The majority, if not all, of the localities of *B. brachycaudus* occur within the Mississippi River drainage system. This species is found in springs and caves in karst areas on both sides of the river, and it is possible that individuals of *B. brachycaudus* are occasionally carried into drift areas during extensive flooding of the Mississippi River.

A second case of a locally isolated population was found at the western margin of the range of *B. brachycaudus*. Similar to the specimens from the drift area in Illinois, a cave population from Texas Co. (Missouri) exhibited minor degrees of morphological variation, indicating possibly genetic isolation from the main population (see: *B. brachycaudus*, Sexual dimorphism and variation).

The range of *B. pseudomucronatus* is adjacent to that of *B. brachycaudus* and includes a few seeps and several caves in karst areas of southern Missouri and northern Arkansas (see Remarks under *B. pseudomucronatus*). *Bactrurus pseudomucronatus* is less widespread and abundant than *B. mucronatus* and *B. brachycaudus*. Yet, like the other two species, its localities are probably interconnected hydrologically and population sizes may be much larger than the records suggest.

Comparison of molecular sequence data

The pairwise sequence differences of the 18s rDNA (small subunit) gene for *B. brachycaudus*, *B. mucronatus* and the new species *B. pseudomucronatus* shows differences between 1.0 to 1.3%, respectively (Table V.2). Since intraspecific differences around 1% were found for different populations of *Gammarus pulex* (U. Englisch, pers. com.), the low interspecific differences among species of the *brachycaudus* groups may indicate a relatively recent splitting of this lineage.

The comparison of sequence differences reveals several noteworthy observations:

- The difference between *B. mucronatus* and *B. pseudomucronatus* (1.3%) is slightly larger than the difference between *B. brachycaudus* and *B. pseudomucronatus* (1.0%).
- The same tendency was found for the glacial drift population (Montgomery County, Illinois) of *B. brachycaudus*, which was found together with *B. mucronatus* in one locality.
- The slight difference of 0.3% between the glacial drift population and a specimen from the main range of *B. brachycaudus* seems to support the view that both specimens analyzed belong to the same species.

TABLE V.2

Pairwise sequence differences of 18S rDNA (small subunit) gene, calculated in Paup 4.0. *Bactrurus brachycaudus* GD = specimens from glacial drift area. Table adapted from Englisch & Koenemann (in manuscript).

	Bactrurus mucronatus	*Bactrurus pseudomucronatus*	*Bactrurus brachycaudus* GD	*Crangonyx forbesi*	*Niphargus fontanus*	*Gammarus troglophilus*	*Gammarus pulex*
Bactrurus brachycaudus	1.2%	1.0%	0.3%	7.6%	7.3%	9.9%	9.1%
Bactrurus mucronatus	-	1.3%	1.2%	8.2%	7.8%	10.2%	9.4%
Bactrurus pseudomucronatus	-	-	1.1%	7.8%	7.6%	9.9%	9.2%
Bactrurus brachycaudus GD	-	-	-	7.8%	7.2%	10.0%	9.3%
Crangonyx forbesi	-	-	-	-	10.4%	13.3%	11.9%
Niphargus fontanus	-	-	-	-	-	9.4%	8.7%
Gammarus troglophilus	-	-	-	-	-	-	2.7%

Moreover, the molecular data do not convincingly support a possible hybridization of *B. brachycaudus* and *B. mucronatus* in Montgomery County.

Summary

Bactrurus Hay is a relatively small generic group that inhabits caves and related subterranean groundwater habitats in parts of eastern and central USA. Two field trips, conducted in the spring of 1999, yielded important new locality records that give us a better knowledge of the distribution of the genus. The examination of fresh samples as well as older collection material has resulted in descriptions of four new species. Based on these descriptions and redescriptions of three previously known species, the taxonomy of the genus is revised according to current standards. In addition, a phylogenetic analysis using morphological data is employed to examine the relationship of *Bactrurus* to 12 selected crangonyctid taxa, including its potential sister-genus *Stygobromus* Cope. Five epigean and hypogean amphipod taxa were chosen as outgroups for the analysis. For interspecific and intergeneric comparisons, pairwise sequence differences of the 18S (small subunit) rDNA gene are given for three *Bactrurus* species and three of the outgroup taxa. Both the biogeography and reconstructed phylogeny of *Bactrurus* are compared and discussed in detail. The evaluation of new data obtained by this study contributes to a more complete understanding of the evolutionary history of the genus.

The geographic distribution of *Bactrurus* is characterized by different patterns, which reflect both dynamic and static elements, are differentiated as follows:

- Three isolated, endemic relict species (*wilsoni* group);
- three abundant and widespread species (*brachycaudus* group), all of which have more or less contiguous populations in hydrologically connected groundwater habitats;

- one species (*B. hubrichti*) is less widespread than the *brachycaudus* group species and has at least one population that is isolated by a distinct gap from other localities (Miller Co., Missouri).

The analysis of the biogeography of the seven species of *Bactrurus* in eastern and central United States allows us to draw two final conclusions:

- The occurrence of all seven species in interior regions of the North American continent that were not covered by a shallow marine embayment during the Cretaceous suggests that the present distribution is composed of remnants of an ancient freshwater group, possibly dating back to the late Paleozoic.
- The present distribution of *B. brachycaudus* and *B. mucronatus* was probably profoundly affected by glaciation and major drainage changes throughout the Pleistocene. Whereas the distribution of *B. brachycaudus* is largely in karst drained by the Mississippi River, *B. mucronatus* is distributed throughout the interstitial habitats of glacial drift. It is likely that relatively high nutrient and energy input, combined with extensive habitat space has, resulted in *B. mucronatus* being the most abundant and widespread species of the genus.

CHAPTER VI

PHYLOGENETIC ANALYSIS OF SUBTERRANEAN AMPHIPOD CRUSTACEANS, USING SMALL SUBUNIT rDNA GENE SEQUENCES[9]

Introduction

Stygobiont amphipod crustaceans in the suborder Gammaridea occur in groundwater environments in many parts of the world. Their habitats include caves, wells, springs, and the interstitial spaces of freshwater and marine sediments. In some cases, the taxonomic structure within the suborder does not offer satisfactory solutions, be it at the species, genus or family level. Numerous discoveries of new stygobiont taxa, especially during the last two decades, call for modern taxonomic revisions.

In some recent revisions, cladistic analyses based on morphological data have been used to re-organize taxonomic groups and obtain information about their phylogenetic relationships. Examples include the families Bogidiellidae Hertzog, 1936 and Crangonyctidae Bousfield, 1973 (Holsinger, 1994; Koenemann, Vonk & Schram 1998; Koenemann & Holsinger, 1999). Another interesting case study for phylogenetic analyses is the crangonyctid genus *Bactrurus* Hay, 1902. *Bactrurus* is endemic to parts of eastern and central United States. The distribution of the genus is characterized by both isolated, highly endemic relicts and species with widespread ranges (see Fig. 5.26). *Bactrurus mucronatus* (Forbes, 1876) is the most abundant species with the widest range. It is also the only species of *Bactrurus* that almost exclusively inhabits non-karst environments in glaciated regions of the Central Lowland Physiographic Province. All other species of the genus occur in groundwater habitats related to karst terranes. A recent collection trip led to the discovery of what appeared to be a second species within the range of *B. mucronatus* in a glaciated area (Montgomery County, Illinois). In one instance, both species were even collected from the same locality. The species that co-occurred with *B. mucronatus* was morphologically very similar to *B. brachycaudus* Hubricht & Mackin, 1940. In contrast to *B. brachycaudus*, however, specimens from the glaciated area had sexually dimorphic telsons. Male telsons were about 20% longer than those of females. Sexually dimorphic telsons are common for *Bactrurus* and only two of the seven species of the genus, *B. brachycaudus* and *B. hubrichti* Shoemaker, 1945, have morphologically indistinguishable telsons in both sexes. Yet, despite sexually dimorphic telsons, specimens from the glacial drift area in Illinois appeared to share most diagnostic characters with *B. brachycaudus*. In a recent revision of *Bactrurus*, the specimens from Illinois were, therefore, regarded as isolated, geographic variants of *B. brachycaudus* rather than a new species (Koenemann & Holsinger, in ms.).

The objective of this project was to investigate the phylogenetic relationships of several closely similar species of *Bactrurus*, including *B. brachycaudus* and its geographic variant from glaciated parts of Illinois. Two other morphologically similar species, *B. mucronatus* and *B. pseudomucronatus* (Koenemann & Holsinger, in ms.), the ranges of which are adjacent to that of *B. brachycaudus*, were also included in the analyses. Because of the similarity of the species of *Bactrurus*, the limited availability of reliable diagnostic characters obstructs the application of morphological data to phylogenetic investigations. Therefore, we performed sequence analyses of the small subunit rDNA gene for several species of *Bactrurus* and selected stygobiont outgroup taxa. For the first time, the analysis of DNA data is not only applied to subterranean amphipods but to groundwater crustaceans in general.

Material and methods

Abbreviations: *B. brachycaudus* GD - specimens of *B. brachycaudus* that occur in non-karst environments of glacial drift areas; found together with *B. mucronatus* in one locality (Montgomery Co., Illinois).

Collection methods and selection of taxa: Specimens were collected from various subterranean habitats, including caves and springs in karst areas, and drainage systems of farm fields in glaciated areas. Moreover, a Bou-Rouch pump was employed to sample interstitial sediments of surface streams. All freshly collected material was immediately preserved in 95% ethanol.

The following species were collected during the field trips: *Bactrurus mucronatus*, *B. brachycaudus*, *Bactrurus pseudomucronatus*, *Crangonyx forbesi* (Hubricht & Mackin, 1940), and *Gammarus troglophilus* Hubricht & Mackin, 1940 (Table VI.1).

Sequence data from the European subterranean genus *Niphargus* represented by *N. fontanus* Bate, 1859, and the epigean freshwater species *G. pulex* Linnaeus, 1758 were included in the phylogenetic analyses. *Gammarus pulex* and the stygophile freshwater species *G. troglophilus* were used as outgroup taxa.

DNA Extraction: DNA was obtained using the QIAamp Tissue Kit (Qiagen). Instructions of the Mouse Tail protocol were exactly followed, with the exception of the last step: the DNA was eluted with 2 x 100 µl H_2O instead of 2 x 200 µl.

PCR Amplification: PCR was performed following a standard protocol: a total volume of 50 µl consisting of 1 x PCR buffer, 1x Q-Solution (Qiagen), 125 pM dNTPs, 25 pM of primer small subunit F and 50 pM of primer small subunit R, 1.25 U Taq DNA polymerase (Qiagen), and 1 µl DNA extract. The PCR cycle was programmed as follows: 1 x 5 min at 94° C; 35 x 30 sec at 94° C, 50 sec at 52.5° C and 3 min 20 sec at 72° C; 1 x 7 min at 70° C. The amplified PCR product was purified using the QIAquick PCR Purification Kit (Qiagen). Primer sequences are given in Table VI.2.

DNA Cloning and Sequencing: The purified PCR products were ligated into the pCR®-TOPO vector (TOPO TA Cloning Kit, Invitrogen) and cloned in heat shock competent Top 10 F' One Shot™ cells (Invitrogen). Plasmids were purified with the S.N.A.P. ™ MiniPrep Kit (Invitrogen).

Cycle Sequencing was conducted in a LI-COR 4200 automated sequencer, using the Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham).

TABLE VI.1

Taxa used for sequence analyses. Stygobionts are obligatory groundwater organisms, whereas stygophiles can be defined as facultative (e.g. temporarily) subterranean species.

Hypogean Species		**Epigean Species**
Stygobiont Species	**Stygophile Species**	
Bactrurus mucronatus (Crangonyctidae)	*Crangonyx forbesi* (Crangonyctidae)	
Bactrurus pseudomucronatus (Crangonyctidae)		
Bactrurus brachycaudus (Crangonyctidae)	*Gammarus troglophilus* (Gammaridae)	*Gammarus pulex* (Gammaridae)
Bactrurus brachycaudus GD (Crangonyctidae)		
Niphargus fontanus (Niphargidae)		

Sequence Analyses: Sequences were aligned with the software package ClustalW and corrected by eye according to a secondary structure presented by Crease & Colbourne (1998) in Genetic Data Environment (GDE). The Chi-square test and the pairwise sequence differences were calculated in Paup 4.0 (Swofford 1998).

Phylogenetic Analyses: Eight taxa were included in the phylogenetic analyses. *Gammarus pulex* and *G. troglophilus* were chosen as outgroups for three methods of phylogenetic inference, each of which was computed with Paup 4.0. The following settings were chosen for the individual methods:

Parsimony Analyses: Branch-and-Bound search with 1000 bootstrap replicates; both MulTrees and Sequence Addition option 'simple' were activated and a 50% Majority Rule consensus tree was generated.

Distance Analyses: Neighbour Joining (NJ), using Kimura 2-parameter and the Tajima & Nei model for nucleotide substitution. The data set was resampled with 1000 bootstrap replicates.

Maximum Likelihood Analyses: Branch-and-Bound search (1000 bootstrap replicates), with MulTrees and the default Sequence Addition in effect. A 50% Majority Rule consensus tree was calculated.

Results

Sequencing and Alignment: All of the 8 complete ssu rDNA sequences differed greatly in length from the approximately 1,800 nucleotides (nt) regarded as typical in crustaceans and most other animals (Table VI.3). Insertions in regions V4, V7 and V9 (Crease & Colbourne 1998) increased the length to a range between 2237 nt and 2329 nt.

TABLE VI.2

Oligonucleotides used for PCR and sequencing.

PRIMER	SEQUENCE	ORIENTATION
PCR		
Small subunitF	CCTA(CT)CTGGTTGATCCTGCCAGT	Forward
Small subunitR	TAATGATCCTTCCGCAGGTT	Reverse
CYCLE SEQUENCING		
M13universal CS(-43)	CGCCAGGGTTTTCCCAGTCACGAC	Forward
M13reverse(-29)	CAGGAAACAGCTATGAC	Reverse
400F	ACGGGTAACGGGGAATCAGGG	Forward
400R	CCCTGATTCCCCGTTACCCGT	Reverse
700F	GTCTGGTGCCAGCAGCCGCG	Forward
700R	CGCGGCTGCTGGCACCAGAC	Reverse
1000F	CGATCAGATACCGCCCTAGTTC	Forward
1000R	GAACTAGGGCGGTATCTGATCG	Reverse
1155F	CTGAAACTTAAAGGAATTGACGG	Forward
1155R	CCGTCAATTCCTTTAAGTTTCAG	Reverse
1250F	CCGTTCTTAGTTGGTGGAGCG	Forward
1250R	CGCTCCACCAACTAAGAACGGCC	Reverse
1500R	CATCTAGGGCATCACAGACC	Reverse
1600F	CGTCCCTGCCCTTTGTACACACC	Forward

In Table VI.4, the pairwise sequence differences are shown. Within the genus *Bactrurus* sequence differences range between 1-1.3 %. Only the difference between *B. brachycaudus* and *B. brachycaudus* GD shows a value much lower than 1 %. A Chi-square test of the homogeneity of base frequencies across the included taxa results in a P-value of 1. Thus, there is no significant correlation between the base distribution and the pairing of single sequences.

The ClustalW multiple alignment was manually corrected due to secondary structure features and resulted in 2464 alignment positions. 2004 of these positions are constant. 203 of the remaining 460 positions are parsimony informative positions.

Phylogenetic Analyses: The trees obtained for each of the three methods of phylogenetic inference (maximum likelihood, distance, and parsimony) had the same basic topological structure, with *N. fontanus* as a sister-group to the crangonyctid taxa, and *C. forbesi* as a sister-group to a clade that comprised the three species (and single specimen) of *Bactrurus* (Fig. 6.1). Because the resulting trees of maximum likelihood and distance analyses were identical, one tree (maximum likelihood) was chosen to

TABLE VI.3

Sequence length, GC-content and Genebank Accession numbers for the taxa included in the analyses.

Species	Sequence Length	GC Content	GB Accession No.
Bactrurus brachycaudus	2322 bp	55%	AF202979
Bactrurus mucronatus	2329 bp	55%	AF202978
Bactrurus pseudomucronatus	2319 bp	55.2%	AF202985
Bactrurus brachycaudus GD	2324 bp	55%	AF202984
Crangonyx forbesi	2331 bp	53.7%	AF202980
Niphargus fontanus	2237 bp	54.9%	AF202981
Gammarus troglophilus	2307 bp	54.7%	AF202983
Gammarus pulex	2250 bp	54.3%	AF202982

represent both methods (Fig. 6.1a). As opposed to the maximum likelihood tree, the tree produced by the parsimony analysis failed to keep the morphologically closely related species *B. mucronatus* and *B. pseudomucronatus* in a separate clade. Instead, *B. mucronatus* formed a sub-clade together with *B. brachycaudus* and *B. brachycaudus* GD (Fig. 6.1b).

TABLE VI.4

Pairwise sequence differences for *Bactrurus* and selected outgroup taxa, calculated in Paup 4.0. A Chi-square test was performed to test the homogeneity of base frequencies across the included taxa.

	B. mucronatus	*B. pseudomucronatus*	*B. brachycaudus* GD	*C. forbesi*	*N. fontanus*	*G. troglophilus*	**G. pulex**
B. brachycaudus	1.2%	1%	0.3%	7.6%	7.3%	9.9%	9.1%
B. mucronatus	-	1.3%	1.2%	8.2%	7.8%	10.2%	9.4%
B. pseudomucronatus	-	-	1.1%	7.8%	7.6%	9.9%	9.2%
B. brachycaudus GD	-	-	-	7.8%	7.2%	10%	9.3%
C. forbesi	-	-	-	-	10.4%	13.3%	11.9%
N. fontanus	-	-	-	-	-	9.4%	8.7%
G. troglophilus	-	-	-	-	-	-	2.7%

Discussion

The consensus tree obtained by the maximum likelihood (and distance) method is in general accordance with both morphological and geographic characteristics of the species of *Bactrurus* included in the analyses (Fig. 6.1a):

- *Bactrurus brachycaudus* and *B. brachycaudus* GD appear together on a sub-clade. The decision to treat the specimens of the glacial drift area as a geographic variant rather than a new species is also supported by the comparatively low sequence difference between both populations (Table VI.4).

- Similarly, *B. mucronatus* and *B. pseudomucronatus* are rendered on a second sub-clade. Both species are morphologically distinguished from the other species of the genus by an extreme case of sexually dimorphic telsons: in males, the telson is conspicuously elongate, reaching up to 1/3 of the length of the body. Because of this distinct similarity, *B. pseudomucronatus* has been frequently misidentified as *B. mucronatus* (hence, the epithet *pseudomucronatus*, meaning 'false *mucronatus*'). Yet, both species are distinguished by several less obvious diagnostic characters. Moreover, their distribution is disjunct (see Fig. 5.26). *Bactrurus mucronatus* occurs almost exclusively in glacial drift areas, whereas *B. pseudomucronatus* inhabits karst regions of the Ozark Plateaus. The results of the analyses support the assumption that some time before the Pleistocene an ancestral species with elongate male telsons split into two populations, one of which subsequently invaded and colonized the coarse grained sediments of aquifers in the glaciated parts of the Central Lowlands.

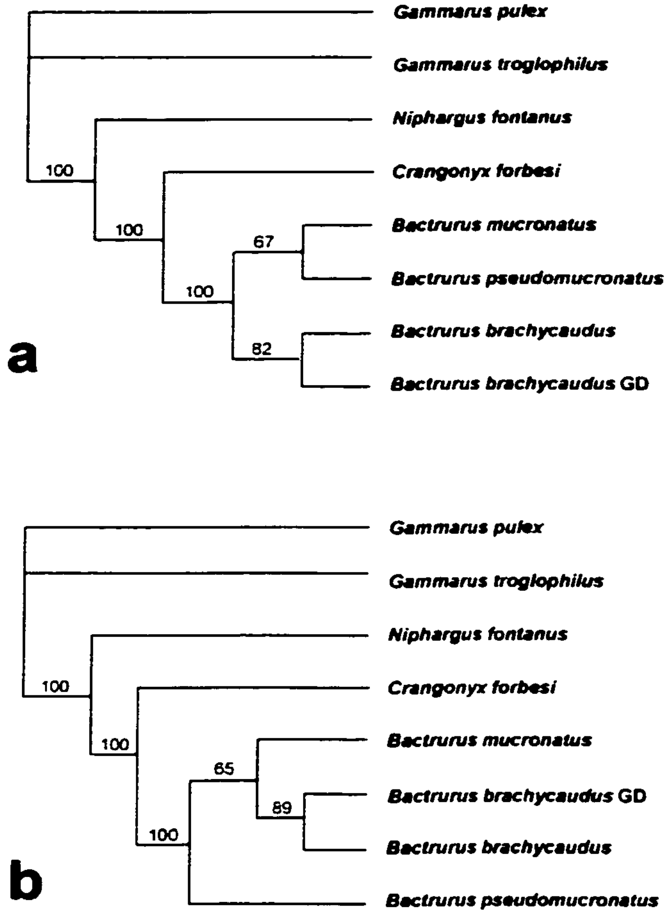


Fig. 6.1. 50% Majority Rule consensus trees obtained by maximum likelihood (a) and parsimony methods (b). Both trees were calculated using the Branch and Bound algorithm with 1000 bootstrap replicates (bootstrap values are shown for internal nodes). The trees were rooted using *Gammarus pulex* and *G. troglophilus* as outgroups.

The parsimony analysis rendered a different phylogeny for *Bactrurus* (Fig. 6.1b). The consensus tree shows *B. pseudomucronatus* as a sister-group to *B. mucronatus* and the *brachycaudus* specimens. This branching pattern seems to contradict the morphological data. For example, it is highly unlikely that a distinct synapomorphy (extremely elongate, sexually dimorphic telson) is reduced completely back into the primitive state (normal telson in *B. brachycaudus*). This inconsistency of the tree in Figure 6.1b may reflect the tendency of parsimony to underestimate the amount of changes on long branches (Sullivan et al., 1997; Maley & Marshall, 1998).

Each method of phylogenetic inference resulted in identical topologies of basal branches. The selection of crangonyctid taxa appears as monophyletic group. Interestingly, the pairwise sequence differences render the North American species *C. forbesi* closer to the European *G. pulex* than to *G. troglophilus* (North America), the range of which even partly overlaps with that of *C. forbesi*.

This study demonstrates that the use of the small subunit gene produces reliable results for groundwater amphipods at the species level and above. In a future project, it would be interesting to further investigate relationships of additional stygobiont and/or stygophile taxa. A subsequent approach should, for example, include several selected stygobiont taxa of all major global distribution areas, combined with co-ocurring epigean amphipods of marine or freshwater habitats.

Summary

The exclusively subterranean amphipod genus *Bactrurus* (Crangonyctidae) occurs in central and eastern parts of the United States. *Bactrurus* is characterized by morphologically similar species. In at least one instance, the distinction between separate species and geographic variants appears to be blurred. A sequence analysis of the small subunit (18S) rDNA gene was conducted for eight amphipod taxa. Both maximum likelihood and distance methods resulted in phylogenies for several species of *Bactrurus* that are in accordance with morphological data. These results could not be validated by a parsimony analysis. All three methods of phylogenetic inference, however, produced identical basal branching patterns. The molecular analyses do not support the recognition of what initially appeared to be a new species of *Bactrurus* from glaciated areas in Montgomery County, Illinois.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Phylogenetic reconstructions

Morphological data

The increasing availability of computers since the early eighties opened a new domain for reconstructing phylogenies. Hennig86 was one of the first software programs that provided a powerful tool for comprehensive cladistic character analyses. Although the analyzable characters of a taxon cover a wide range of qualities and attributes, including ethological and physiological properties, the vast majority of early computerized phylogenetic investigations were based on external morphological characters. Yet, the use of morphological characters for phylogenetic analyses is subjected to several limitations. Most importantly to note, morphological analyses are based on assumptions and, hence, involve a certain level of bias that can never entirely be avoided. The assumptions an investigator has to make comprise the selection of independently evolved characters, the coding of character states, as well as character type and weighting assessments. Minor changes to a data matrix, e.g. the single change of a character state or weight, can already produce significantly different cladograms.

An analysis that aims to investigate phylogenetic relationships within a taxon relies primarily on the principle of homology. One of the basic principles of cladistics states that only homologous characters, which were derived from a common ancestor and are shared among its descendants, can produce reliable phylogenetic reconstructions. Unfortunately, it is often very difficult, if not impossible, to distinguish between homologous and homoplasious traits in subterranean amphipods. The adaptation to life in cold and dark aquatic environments seems to favor the development of similar structures in unrelated groups (which are, thus, not derived from a common ancestor; see also Chapter IV: Character assessment and choice of outgroup). It may, therefore, often be necessary to carry out preliminary character analyses to determine which characters are true homologies.

The availability of phylogenetically reliable characters varies greatly among different groups of stygobiont amphipods. For example, 27 characters were employed for the analyses of the Bogidiellidae opposed to 16 characters used for the Crangonyctidae (Chapters 4 and 5). The fact that only 11 of these 43 characters were shared by both families reflects a typical dilemma: many gammaridean amphipod taxa are morphologically very similar, which limits the choice of reliable characters. Consequently, the choice of characters is often tailored to the taxon under investigation.

Of course, this does not mean that the utilization of morphological characters should be avoided for cladistic analyses. Some workers have produced excellent results with morphological data. The limitations merely show how important it is to 'know your group' and develop a good understanding of the evolution of individual characters. This can be achieved, for example, by identifying homologous traits through preliminary studies.

Molecular data

The analyses of molecular sequences opened a completely new category of data for systematists. This approach was developed in the mid fifties when amino acid sequences of proteins were investigated. In the sixties and seventies, semi-automated sequencing of universal proteins, e.g. cytochrome b or c and hemoglobin, allowed the comparison of a new class of data among taxa. During the last 15 years, DNA sequencing has become an increasingly important instrument for systematic studies.

Molecular data have several advantageous properties when compared with morphological characters. They usually provide much larger amounts of equivalently comparable evidence, which can be recognized as independent units. For example, the 18S (small subunit) rDNA gene has approximately 2,500 nucleotides that can be treated as 2,500 pieces of phylogenetic evidence. The occurrence of mutations in different fragments is very likely independent. In contrast, 2,500 characters would be an impracticably large number for morphological analyses. Moreover, it is often impossible to decide whether particular structures evolved independently or whether a structural change in character A is equivalent to a modified character B. We cannot be sure whether the reduction of rami segments of the pleopods is not developmentally linked with a modification of rami spines, or if the modification of rami spines is equivalent to the loss of gills on the seventh pereopod.

Although DNA data do not exhibit the shortcomings typical for morphological analyses, they are characterized by limitations of their own. In certain cases, it may be extremely difficult, if not impossible, to determine which nucleotides or sequences of nucleotides are homologous. This problem particularly arises when a gene under investigation differs significantly among the analyzed taxa. The probability that different bases at same positions have evolved independently is very high (e.g. insertions or deletions). Another important requirement for comparative molecular analyses is the assumption that a particular gene evolved at the same evolutionary rate in different organisms. Recent publications have implied that some genes tend to produce conflicting phylogenies when compared with well-established morphological or fossil data (Maley & Marshall, 1998).

Suggestions for future studies

Obviously, the weaknesses of individual analytical tools can be minimized by combining different methods. The ideal phylogenetic reconstruction, especially at higher taxonomic levels, compiles information from several, independent sources, for example, morphological, physiological, ethological and molecular data, and combines them to a single, comprehensive analysis ('total evidence' approach).

However, choosing a comprehensive approach may not always be feasible due to temporal or financial restrictions. In this case, a 'simple' phylogenetic analysis would be preferable. Phylogenetic reconstructions that employ information from a single source, e.g. only morphological or molecular data, can definitely produce excellent results (which do not necessarily have to differ from those obtained by more comprehensive analyses). Yet, these analyses depend much more on the reliability of the analyzed data set and are, therefore, more easily prone to failure. With regard to the difficulties encountered during this research project, I would like to address two factors that significantly contribute to the success of phylogenetic studies:

- **Taxonomic descriptions**: The assessment of morphological characters often relies on the literature. Although the quality of taxonomic descriptions of hypogean amphipods has considerably improved during the past 50 years, the identification of useful characters still is often very difficult if not impossible. The main problems to be noted are qualitative and quantitative differences among descriptions. The occurrence of potentially useful characters is only vaguely mentioned or not described at all in some publications, for example, broodplates, retinaculae of the pleopods, or even the armature of mouthparts and appendages. As a result, unidentifiable structures have to be coded as 'unknown' ('?'), which may increase polytomies or produce less reliable phylogenies. In the worst case, a character has to be excluded from the analysis because too many taxa with unknown conditions are involved. From a cladistic point of view, it would be highly recommendable to introduce a binding protocol for taxonomic descriptions that guarantees a consistent standard for both describing text and accompanying drawings.
- **Collection and preservation techniques**: Unfortunately, for the molecular analyses (Chapter VI), all older specimens from research collections failed to yield DNA products whereas 80% of the freshly collected specimens successfully yielded DNA. The specimens collected during the 1999 field trip were preserved in 90-95% ethanol immediately after the samples were taken. In comparison, research collection material is usually preserved in 70-75% alcohol (some museums still preserve their specimens in formalin, which makes DNA extraction almost impossible). In order to prevent post-mortem breakdown of DNA by nucleases, the following steps should be considered: (1) preservation in purified alcohol (90-95%); (2) preservation immediately after collection; (3) storage at -20 to 4° C until extraction. These simple procedures are easy to carry out and can save considerable amounts of time and money (not to mention the frustration of unsuccessful analyses).

Biogeography and evolutionary history

The geographic distribution of crangonyctid and bogidiellid taxa is rather unusual. It can best be analogized with an incomplete mosaic, the remaining pieces of which are scattered over several continents and islands. A comparison of the distributions of both families reveals several interesting differences (Table VII.1).

TABLE VII.1

Comparison of biogeographic and taxonomic features of the families Bogidiellidae and Crangonyctidae. See Table VII.2 for details on habitat types and the geographic distribution of crangonyctid taxa.

[1] Not including approximately 78 additional, unpublished species, the descriptions of which are in preparation.

	Bogidiellidae	**Crangonyctidae**
Distribution	worldwide (predominantly Mediterranean and Caribbean regions, and Central and South America)	Holarctic (predominantly continental USA and eastern Europe)
Habitat types	exclusively hypogean	hypogean and epigean
	mostly freshwater (some brackish and marine taxa)	exclusively freshwater
Taxonomic structure	110 species in 33 genera	ca. 150 species in 6 extant genera[1]

The Bogidiellidae

All species of the Bogidiellidae occur in subterranean environments. They have almost a worldwide distribution, except for boreal, arctic and Antarctic regions. The geographic distribution of the family is characterized by four major concentrations of taxa in Central and South America, the West Indian region, and especially in the larger Mediterranean region. If we compare the species/genera ratios of certain regions, we find that generic diversity is highest in the western hemisphere, whereas species richness is greater in the Mediterranean region (Chapter IV, Discussion).

The characteristics of this unusual distribution pattern allows several conclusions to be drawn:

- The bogidiellids have a scattered distribution over several continents. In addition, some taxa occur in isolated, continental freshwater habitats, for example, in Brazil, Afghanistan, China and Europe. Some of these habitats probably have been separated from marine waters since the Paleozoic. Stock (1981) suggested that the Bogidiellidae were already a well-established freshwater group prior to the break-up of Pangaea, about 180-200 MY BP.
- There are three clusters of taxa in the western hemisphere and one in the Mediterranean region. Each of these regions has an individual, complex geological history, completely different from that of other regions. What they may have in common, however, is that most of these coastal regions and islands emerged from the sea far more recently than the middle to late Paleozoic. The geologically youngest regions are probably the present Mediterranean coastal areas. Most of these regions have evolved during the Tertiary, some as early as the Miocene (Vonk et al., 1999; Karaman & Karaman, 2000). Although the remarkable abundance of bogidiellids in these regions is certainly influenced by a variety of ecological factors (Sket, 1999), the relatively recent geological evolution of the Mediterranean is likely to be the key element regarding species richness and distribution in this part of the world. The present taxonomic structure and distribution pattern suggest that invasions of newly available habitats,

even re-invasions of marine environments, and a subsequent diversification or large-scale adaptive radiation in these areas, took place more recently than, for example, in the western hemisphere.

The Crangonyctidae

The Crangonyctidae are one of the largest freshwater families of the Amphipoda, with ca. 153 species in seven genera. The species/genera ratio of the crangonyctids is significantly higher than the highest ratio found for the bogidiellids in the Mediterranean region (33 species in 4 genera). Unlike the Bogidiellidae, crangonyctids occur exclusively in freshwater habitats of the Holarctic region (Holsinger, 1977). Approximately 80% of the crangonyctid species are recorded from hypogean habitats (Table VII.2). Most of the epigean species live in surface freshwater environments that may be hydraulically connected with underlying groundwater aquifers, for example, seeps, bogs, small streams, etc.

Holsinger (1986a) suggested an ancient freshwater origin for the Crangonyctidae, dating back to the late Mesozoic. He pointed out that crangonyctids probably were already a well established freshwater group on the Laurasian landmass prior to the break-up of North America and Europe and supported his assumptions with four lines of evidence as follows: (1) Crangonyctid taxa appear to have no close phylogenetic relationship to marine groups; (2) the larger genera occur in both North America and Eurasia (see Fig. 7.1); (3) their high specific and generic diversity and distribution over immense geographic areas may be the result of a long period of colonization and adaptation to freshwater environments; and (4) the "crangonyctid-like" genus *Palaeogammarus*, fossilized in Baltic amber from the Eocene or Oligocene, implies that the family was already adapted to freshwater habitats at least by the early Tertiary.

TABLE VII.2

Geographic distribution and habitat types of genera and species of the Crangonyctidae. Numbers in parentheses denote unpublished species (descriptions in manuscript or preparation). Table adapted from Holsinger, 1977.

	Habitat				**Distribution**	
Genus	**Described species**	**Hypogean**	**Epigean**	**Hypogean/epigean**[2]	**North America**	**Eurasia**
Bactrurus	3 (4)	3 (4)	-	-	3 (4)	-
Crangonyx	23 (24)	9 (6)	11 (15)	3 (3)	19 (24)	4
Lyurella	1	-	1	-	-	1
Palaeogammarus[1]	3	-	3	-	-	3
Stygobromus	101 (50)	101 (50)	-	-	98 (50)	3
Stygonyx	1	1	-	-	1	-
Synurella	18	5	12	1	4	14

[1] Extinct genus, known only from Baltic amber fossils.

[2] Species found in both subterranean and epigean habitats.

The geographic distribution of the genus *Bactrurus* provides excellent exemplary data that allows us to draw conclusions about the age of the Crangonyctidae. Interestingly, the distribution of *Bactrurus* shows similar patterns when compared with the distribution of the Bogidiellidae: It is composed of relict species as well as more abundant taxa with widespread ranges (Chapter V, Discussion).

- Three highly endemic new species occur in isolated habitats, and are known from single or few localities in Alabama, the Cumberland Gap area (Virginia and Tennessee) and in an unglaciated part of southern Indiana. The remarkable morphological similarity of these three species in addition to several distinct synapomorphies strongly suggests their descent from a widespread common ancestor.
- In contrast, the occurrence of two more abundant species with wider ranges seem to point towards the colonization of newly available habitat space some time during the Pleistocene, when the glaciers repeatedly advanced and retreated, and deposited thick layers of sediments in the Central Lowlands.

The fact that all species of *Bactrurus* occur in regions that were apparently not inundated by a shallow marine embayment during the middle to late Cretaceous points towards a freshwater origin at least in the early Mesozoic, probably even in the middle to late Paleozoic. This pattern is similar for *Crangonyx* and *Stygobromus* (there are a few exceptional occurrences in formerly inundated areas that are very likely recolonizations). All three genera have what may be relicts in karst regions at relatively high elevations, for example, in the Appalachians and also in western North America.

Summary

The comparison of the biogeography and taxonomy of the Bogidiellidae and Crangonyctidae reveals significant differences:

- Both families have distinctly different geographic distributions, taxonomic structures and ecological preferences (Table VII.1).
- Given the significantly higher species/genera ratio of the Crangonyctidae, we might expect the family to be a relatively young group, based on the assumption "the higher the generic diversity, the older the group". Yet, both bogidiellids and crangonyctids are characterized by ancient freshwater relicts, possibly dating back to the middle to late Paleozoic, as well as regions with high diversification and abundance that are very likely the result of much younger vicariant events.

The conclusions discussed in this chapter are based primarily on geographic and/or geological data. A final answer to the origin of freshwater amphipods can probably not be expected before different data sources, e.g. comprehensive morphological and molecular analyses, are investigated. As we may have reached the limitations of morphological character analyses, it becomes evident that the sequencing of a single gene cannot resolve the remaining grey areas of the evolutionary history of the Amphipoda. At present, numerous projects are being carried out or being planned that have the potential to unravel high-level, large-scale phylogenies for the Amphipoda, including the investigation of different genes, allozymes, amino acids, and even karyotypes. The compilation and evaluation of large data sets from numerous sources remains the most promising option for a reliable reconstruction of amphipod phylogeny.

REFERENCES

ADAMS, C. G., 1981. An outline of Tertiary palaeogeography. In: L. R. M. COCKS (ed.), The evolving earth: 221-235. (British Museum (Natural History); Cambridge University Press, Cambridge).

BARNARD, J. L., 1958. Index to the families, genera and species of the gammaridean Amphipoda (Crustacea). Occasional papers of the Allan Hancock Foundation **19**: 145 pp.

BARNARD, J. L. & C. M. BARNARD, 1983. Freshwater Amphipoda of the world. I. Evolutionary patterns.: i-xvii, 1-358. (Hayfield Associates, Mt. Vernon, Virginia).

BATE, C. S., 1859. On the genus *Niphargus* (Schiödte). Proceedings of the Dublin University Zoological and Botanical Association **1**: 237-244.

BIRSTEIN, J. A. & S. I. LJOVUSCHKIN, 1968. A representative of the family Bogidiellidae (Crustacea, Amphipoda), new for the USSR, in subterranean waters of Central Asia. Zoological Journal, **47**: 676-683. [In Russian].

BOTOSANEANU, L. & J. NOTENBOOM, 1988. Un amphipode stygobie de la Thaïlande (Crustacea: Amphipoda). Annales de Limnologie, **24**: 51-59.

BOTOSANEANU, L. & J. H. STOCK, 1989. A remarkable genus of cavernicolous Bogidiellidae (Crustacea, Amphipoda) from Thailand. In: L. J. VAN DER STEEN (ed.), Studies in honour of Dr. Pieter Wagenaar Hummelinck. Uitgaven "Natuurwetenscappelijke Studiekring voor Suriname en de Nederlandse Antillen, **123**: 171-184.

BOU, C. & S. RUFFO, 1979. Contributo alla conoscenza delle *Bogidiella* di Grecia (Crustacea Amphipoda Gammaridae). Natura, Società Italiana di Scienze Naturali, Museo Civico di Storia Naturale di Milano, **70**: 295-309.

BOUSFIELD, E. L., 1973. Shallow-water gammaridean Amphipoda of New England. (Cornell Univ. Press; Ithaca, New York).

-- --, 1977. A new look at the systematics of gammaroidean amphipods of the world. Crustaceana, **4**: 282-316.

BOUSFIELD, E. L. & J. H. HOLSINGER, 1981. A second new subterranean amphipod crustacean of the genus *Stygobromus* (Crangonyctidae) from Alberta, Canada. Can. J. Zool., **59**: 1827-1830.

BRADBURY, J. H. & W. D. WILLIAMS, 1996. Freshwater amphipods from Barrow Island, Western Australia. Records of the Australian Museum, **48**: 33-74.

CHILTON, C., 1882. On some subterranean Crustacea. Transactions Proc. New Zealand Institute, **14**: 174-180.

-- --, 1894. II. The subterranean Crustacea of New Zealand: with some general remarks on the fauna of caves and wells. Trans. Linn. Soc. London, 2nd series, vol. **6**: 163-284.

COINEAU, N. & G. CHANDRASEKHARA RAO, 1972. Isopodes et Amphipodes des sables intertidaux des Iles Andaman et Nicobar (Golfe du Bengale). Vie et Milieu (Ser. A), **23**: 65-100.

COINEAU, N., 1969. Contribution à l'étude de la faune interstitielle - Isopodes et Amphipodes. Mémoires du Muséum National d'Histoire Naturelle (Série A), **55**: 145-216.

-- --, 1977. Le genre *Pseudoingolfiella* aux Kerguelen. Extrait de: CNFRA, **42**: 287-293.

COPE, E. D., 1872. On the Wyandotte Cave and its fauna. American Naturalist **6**: 406-422.

CREASE, T. J. & J. K. COLBOURNE, 1998. The unusually long small-subunit ribosomal RNA of the crustacean, *Daphnia pulex*: Sequence and predicted secondary structure. J. Mol. Evol. **46**: 307-313.

CULVER, D. C. & J. R. HOLSINGER, 1969. Preliminary observations on sex ratios in the subterranean amphipod genus *Stygonectes* (Gammaridae). The American Midland Naturalist (Notes and Discussion) **82** (2): 631-633.

DA SILVA BRUM, I. N., 1975. *Spelaeogammarus bahiensis* g.n. sp.n. de Anfipodo Cavernicola do Brasil. (Amphipoda-Bogidiellidae). Atas da Sociedade de Biologia di Rio de Janeiro, **17**: 125-128.

DANCAU, D., 1973. Observations sur les Amphipodes souterrains de l'île de Majorque. Genre *Bogidiella* Hertzog. Travaux de l'Institut de Spéologie 'Emile Racovitza', Bucharest, **12**: 113-119.

DERZHAVIN, A. N., 1939. Presnovodnye perakaridy talysha. Akademiia Nauk Azerbaidzhanskii SSR Filial, Trudy Zoologicheskogo Instituta, **10**: 43-58.

DIVIACCO, G. & S. RUFFO, 1985. Nuovi bogidiellidi delle acque sotterranee Africane (Crustacea Amphipoda). Italian Journal of Zoology, **7** (Suppl. XX): 135-148.

ENGLISCH, U. & S. KOENEMANN, in manuscript. Phylogenetic analysis of subterranean amphipod crustaceans, using small subunit rDNA gene sequences.

FITZPATRICK, J. F., 1983. How to know the freshwater Crustacea. The Pictured Key Nature Series: 227 p. (Wm. C. Brown Company Publishers, Dubuque, Iowa).

FORBES, S. A., 1876. List of Illinois Crustacea, with descriptions of new species. Bulletin of the Illinois Museum of Natural History, **1**: 3-25.

GARDNER, J. E., 1986. Invertebrate fauna from Missouri caves and springs. Missouri Department of Conservation, Natural History series No. **3**: 72 p.

GORDON, I., 1957. On *Spelaeogriphus*, a new cavernicolous crustacean from South Africa. Bulletin of the British Museum of Natural History (Zoology), **5**: 31-47.

GRIFFITHS, C. L., 1989. The Ingolfiellidae (Crustacea: Amphipoda) of southern Africa, with descriptions of two new species. Cimbebasia, **11**: 59-70.

GROSSO, L. E. & G. L. CLAPS, 1984. Tercer Bogidiellidae (Crustacea Amphipoda) de la cuenca del Rio Grande (Jujuy, Argentina). Neotropica, **30**: 223-231.

-- -- & -- --, 1985. Distribución geográfica de la familia Bogidiellidae (Crustacea, Amphipoda) en la República Argentina, con la descripción de un nuevo subgénero y una nueva especie. Physis (Buenos Aires), Secc. B, **43**: 49-55.

GROSSO, L. E. & H. R. FERNÁNDEZ, 1985. Una nueva *Bogidiella* (Amphipoda Bogidiellidae) hiporreica de la provincia de Tucumán (Argentina). Neotropica, **31**: 201-209.

-- -- & -- --, 1988. Un caso de simpatria de tres especies del género *Bogidiella* (Crustacea, Amphipoda) en el noroeste Argentino, con la description de dos nuevoas especies. Stygologia, **4**: 64-78.

-- -- & -- --, 1993. Nuevo género cavernicola austral de Bogidiellidae; *Patagongidiella* n.gen. del noroeste Patagónico (Neuquén, Argentina). Bolletino del Museo Civico di Storia Naturale, Verona, **17**: 357-372.

GROSSO, L. E. & R. A. RINGUELET, 1979. Fauna subterránea de las aguas dulces de la República Argentina. I. Dos nuevas especies de Amphipodos del género *Bogidiella*. Limnobios, **1**: 381-394.

HAY, O. P., 1882. Notes on some fresh-water Crustacea, together with descriptions of two new species. Am. Nat., **16**: 241-243.

HAY, W. P. 1902. Observations on the crustacean fauna of Nickajack Cave, Tennessee and vicinity. Proc. U. S. nat. Mus., **25**: 417-439.

HERTZOG, L. 1933. *Bogidiella albertimagni* sp. nov. ein neuer Grundwasseramphipode aus der Rheinebene bei Strassburg. Zoologischer Anzeiger, **102** (9/10): 225-227.

-- --, 1936. Crustacés de biotopes hypogés de la vallée du Rhin d'Alsace, II. Bull. Soc. zool. France, **61**: 356-372.

HOLSINGER, J. R., 1967. Systematics, speciation, and distribution of the subterranean amphipod genus *Stygonectes*

(Gammaridae). U.S. Nat. Mus. Bull., **259**: 176 p.

-- --, 1969. The systematics of the North American genus *Apocrangonyx* (Gammaridae), with remarks on ecology and zoogeography. The American Midland Naturalist, **81**/1: 1-28.

-- --, 1972. The freshwater amphipod crustaceans (Gammaridae) of North America. Biota of Freshwater Ecosystems, Identification Manual U.S. No. **5** (for the Environmental Protection Agency): 1-89.

-- --, 1974. Systematics of the subterranean amphipod genus *Stygobromus* (Gammaridae), part I: species of the western United States. Smithsonian Contributions to Zoology, **160**: 63 p.

-- --, 1977. A review of the systematics of the Holarctic amphipod family Crangonyctidae. Crustaceana 4: 244-281.

-- --, 1978. Systematics of the subterranean amphipod genus *Stygobromus* (Crangonyctidae), part II: species of the eastern United States. Smithsonian Contributions to Zoology, **266**: 144 p.

-- --, 1980. *Stygobromus canadensis*, a new subterranean amphipod crustacean (Crangonyctidae) from Canada, with remarks on Wisconsin refugia. Can. J. Zool., **58**: 290-297.

-- --, 1981. *Stygobromus canadensis*, a troglobitic amphipod crustacean from Castleguard Cave, with remarks on the concept of cave glacial refugia. Proceedings of the 8th International Congress of Speleology, Bowling Green, Kentucky. Vol. **1**: 93-95.

-- --, 1986a. Zoogeographic patterns of North American subterranean amphipod crustaceans. In: R. H. GORE & K. L. HECK (eds.). Crustacean Biogeography in Crustacean Issues: 85-106. (Balkema, Rotterdam).

-- --, 1986b. Amphipoda: Holarctic crangonyctid amphipods. In: BOTOSANEANU, L. (ed.), Stygofauna Mundi: 548-556. (E. J. Brill, Leiden).

-- --, 1993. Biodiversity of subterranean amphipod crustaceans: global patterns and zoogeographic implications. Journal of Natural History, **27**: 821-835.

-- --, 1994a. Amphipoda. In: C. JUBERTHIE & V. DECU (eds), Encyclopaedia Biospeologica, Tome I. Société de Biospéologie.

-- --, 1994b. Pattern and process in the biogeography of subterranean amphipods. Hydrobiologia, **287**: 131-145.

HOLSINGER, J. R. & D. C. CULVER, 1988. The invertebrate cave fauna of Virginia and a part of eastern Tennessee: zoogeography and ecology. Brimleyana, **14**: 164 p.

HOLSINGER, J. R. & G. LONGLEY, 1980. The subterranean amphipod crustacean fauna of an artesian well in Texas. Smithson. ContriBog. Zool., **308**: 1-62.

HOLSINGER, J. R., J. S. MORT & A. RECKLIES, 1983. The subterranean crustacean fauna of Castleguard Cave, Columbia Icefields, Alberta, Canada, and its zoogeographic significance. Arctic and Alpine Research, **15**/4: 543-549.

HOVENKAMP, F., W. HOVENKAMP & J. J. VAN DER HEIDE, 1983. Two new hyporheic amphipods, *Bogidiella* (*Bogidiella*) *cyrnensis* n. sp. and *Bogidiella* (*Medigidiella*?) *paolii* n. sp. from Corsica. Bijdragen tot de Dierkunde, **53**: 82-92.

HUBRICHT, L., 1943. Studies on the Nearctic freshwater Amphipoda, III. Notes on the freshwater Amphipoda of eastern United States, with description of ten new species. The American Midland Naturalist, **29**/3: 683-712.

-- --, 1959. Malacostraca (Amphipoda). In: W. T. EDMONDSON (ed.); Freshwater Biology, 2nd edition: 876-878. (Wiley & Sons, Inc. New York).

HUBRICHT, L. & J. G. MACKIN, 1940. Description of nine new species of fresh-water amphipod crustaceans with notes and new localities for other species. The American Midland Naturalist, **23**: 187-218.

KARAMAN, G. S., 1974a. 58. Contribution to the knowledge of Amphipoda. Genus *Synurella* Wrzes. in Yugoslavia with remarks on its all World known species, their synonymy, bibliography and distribution (fam. Gammaridae). Poljoprivreda I Šumarstvo Titograd, **35**: 49-60.

-- --, 1974b. 59. Contribution to the knowledge of Amphipoda. Revision of the genus *Stygobromus* Cope 1872 (Fam. Gammaridae) from North America. Glasnik Republickog zavoda za zaštitu prirode, I Prirodnjackog muzeja u Titogradu, **7**: 97-125.

-- --, 1979. Contribution to the knowledge of Amphipoda 106. Two new *Bogidiella* species (fam. Gammaridae) from Italy. Glasnik Republickog zavoda za zastitu prirode, I Prirodnjackog muzeja u Titogradu, **12**: 101-105.

-- --, 1981. Revision of bogidiella-group of genera with description of some new taxa (fam. Gammaridae). Contribution to the knowledge of Amphipoda 121. Poljoprivreda I Sumarstvo Titograd, **27**: 23-44.

-- --, 1982. Critical remarks to the recent revisions of bogidiella-group of genera with study of some taxa (fam. Gammaridae). Contribution to the knowledge of Amphipoda 126. Poljoprivreda I Sumarstvo Titograd, **28**: 31-57.

-- --, 1987. New species of the family Bogidiellidae (Gammaridea) from Yugoslavia, *Bogidiella serbica*, n. sp. Glasnik Prirodnjckog muzeja u Beogradu (ser. B), **42**: 37-50.

-- --, 1988a. New genera and species of the subterranean family Bogidiellidae from the Near East. Contribution to the knowledge of Amphipoda 179. Studia Marina, Kotor, **19**: 31-51.

-- --, 1988b. Two new species of genus *Bogidiella* Hert. from Sardinia and France, with remarks to *Bog. vandeli* Coineau 1968 (Gammaridea, fam. Bogidiellidae). Contribution to the knowledge of Amphipoda 180. Poljoprivreda I Sumarstvo Titograd, **34**: 25-41.

-- --, 1988c. The new genus of the family Bogidiellidae (Gammaridea) from coastal seawater (mesopsammon) of Italy, *Aurobogidiella*, n. gen. Contribution to the knowledge of Amphipoda 182. Poljoprivreda I Sumarstvo Titograd, **34**: 93-105.

-- --, 1989a. *Bogidiella sketi*, new freshwater species of the family Bogidiellidae from Dalmatia (Yugoslavia) with remarks to some other *Bogidiella* species. Contribution to the knowledge of Amphipoda 188. Poljoprivreda I Sumarstvo Titograd, **35**: 49-60.

-- --, 1989b. *Bogidiella cypria*, new species of the family Bogidiellidae from Cyprus Island in the Mediterranean Sea. Contribution to the knowledge of Amphipoda 190. The Montenegrin Academy of Sciences and Arts, Glasnik of the Section of Natural Sciences, **7**: 7-23.

-- --, 1990a. One new species of the family Bogidiellidae from Creta Island, Greece, *Bogidiella (Medigidiella) aquatica* n. sp. Contribution to the knowledge of Amphipoda 192. Glasnik Prirodnjckog muzeja u Beogradu (ser. B), **43**: 27-39.

-- --, 1990b. *Bogidiella stocki*, a new species from the Sinai Peninsula (Amphipoda, Bogidiellidae). Contribution to the knowledge of Amphipoda 193. Beaufortia, **41**: 141-149.

-- --, 1990c. *Bogidiella barbaria*, new species from the Iberian Peninsula (fam. Bogidiellidae). Contribution to the knowledge of Amphipoda 203. Glasnik Republickog zavoda za zastitu prirode, I Prirodnjackog muzeja u Titogradu, **23**: 7-23.

KARAMAN, G. S. & J. L. BARNARD, 1979. Classificatory revisions in gammaridean Amphipoda (Crustacea), part 1. Proceedings of the Biological Society of Washington, **92**: 106-165.

KARAMAN, G. S. & B. Sket, 1990. *Bogidiella sinica* sp. n. (Crustacea: Amphipoda) from southern China. Bioloski Vêstnik, Lubljana, **38**: 35-48.

KARAMAN, S. L., 1933. Über zwei neue Amphipoden, *Balcanella* und *Jugocrangonyx* aus dem Grundwasser von Skopje. Zoologischer Anzeiger, **103** (1-2): 41-47.

-- --, 1953. Über subterrane Amphipoden und Isopoden des Karstes von Dubrovnik und seines Hinterlandes. Acta Musei Macedonici Scientarium Naturalium, Skopje, **1** (7):137-167.

-- --, 1959. Über eine neue Art und Unterart der Gattung *Bogidiella* (Crust. Amphipoda) aus Jugoslavien. Acta Zool. Acad. Sc.Hungaricae, **4** (3-4): 339-348.

KIMURA, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

KOENEMANN, S., 1997. Biological studies on groundwater crustaceans in southwest Anatolia, Turkey. List of sampling stations June-July 1996. Verslagen en Technische Gegevens (Universiteit van Amsterdam), **70**: 1-14.

KOENEMANN, S. & J. R. HOLSINGER, 1999a. Phylogenetic analysis of the amphipod crustacean family Bogidiellidae, s. lat. and revision of taxa above the species level. Crustaceana, **72/8**: 781-816.

-- -- & -- --, 1999b. *Megagidiella azul* a new genus and species of a cavernicolous amphipod crustacean (Bogidiellidae) from Brazil, with remarks on its biogeographic and phylogenetic relationships. Proceedings of the Biological Society of Washington 112 (3): 572-580.

-- -- & -- --, 2000. Revision of the subterranean amphipod genus *Spelaeogammarus* (Bogidiellidae) from Brazil, including descriptions of three new species and considerations of their phylogeny and biogeography. Proceedings of the Biological Society of Washington 113 (1): 104-123.

-- -- & -- --, in manuscript. Systematics of the North American subterranean amphipod genus *Bactrurus* (Crangonyctidae). Submitted to Beaufortia/Bulletin of the Zoological Museum Amsterdam (June 2000).

KOENEMANN, S., R. VONK & F. SCHRAM, 1998. Cladistic analysis of 37 Mediterranean Bogidiellidae (Amphipoda), including *Bogidiella arista*, new species, from Turkey. J. Crust. Biol., **18/2**: 383-404.

KREBS, J. R. & N. B. DAVIES, 1993. An Introduction to Behavioural Ecology. 3rd Edition. Blackwell Scientific Publications, Oxford.

LARSON, D. R., B. L. HERZOG, R. C. VAIDEN, C. A. CHENOWETH, Y. XU & R. C. ANDERSON, 1995. Hydrogeology of the Green River Lowland and associated bedrock valleys in northwestern Illinois. Environmental Geology (Department of Natural Resources; Illinois State Geological Survey), **149**: 19 p.

LARSON, D. R., J. R. KEMPTON & S. MEYER, 1997. Geologic, geophysical, and hydrologic investigations for a supplemental municipal groundwater supply, Danville, Illinois. Cooperative Groundwater Report (Department of Natural Resources), **18**: 63 p.

LEWIS, J. J., 1983. The obligatory subterranean invertebrates of glaciated southeastern Indiana. NSS Bulletin, **45**: 34-40.

LEWIS, J. J. & T. E. BOWMAN, 1981. The subterranean asellids (*Caecidotea*) of Illinois (Crustacea: Isopoda: Asellidae). Smithsonian Contributions to Zoology, **335**: 66 p.

LINNAEUS, C., 1758. *Systema Naturae*. Editio Decima, Tomus I. Holmiae (Stockholm): Laurentii Salvii.

MALEY, L. E. & C. MARSHALL, 1998. The coming of age of molecular systematics. Science, **279**: 505-506.

MATEUS, A. & M. MACIEL DE LOURDES, 1967. Description d'une espéce de *Bogidiella* (Crustacea, Amphipoda) du psammon du Portugal et quelques notes sur son genre. Publicações do Instituto de Zoologia 'Dr. Augusto Nobre', Faculdade de Ciências do Porto, **100**: 11-47.

MEŠTROV, M., 1961. Über neue *Bogidiella*-Arten (Crustacea, Amphipoda) aus unterirdischen Gewässern Kroatiens und Sloweniens. Zoologischer Anzeiger, **167**: 74-80.

MÜLLER, F., 1846. Über *Gammarus ambulans*. Archiv f. Naturg., Jahrg. **12**: pp.

NICHOLAS, B. G., 1960. Checklist of macroscopic organisms of the United States. American Midland Naturalist, **64**: 123-160.

NOODT, W., 1959. Estudios sobre crustaceos Chilenos de aguas subterráneas. I. *Ingolfiella chilensis* n. sp. de la playa marina de Chile Central (Crustacea, Amphipoda). Investigaciones Zoológicas Chilenas, **5**: 199-209.

-- --, 1965. Interstitielle Amphipoden der konvergenten Gattungen *Ingolfiella* Hansen und *Pseudingolfiella* n. gen. aus Suedamerika. Crustaceana, **9**: 17-30.

NOTENBOOM, J. 1991. Marine regressions and the evolution of groundwater dwelling amphipods (Crustacea). Journ. of Biogeography, **18**: 437-454.

PECK, S. B. & J. J. LEWIS, 1977. Zoogeography and evolution of the subterranean invertebrate faunas of Illinois and southeastern Missouri. NSS Bulletin, **40**: 39-63.

PESCE, G. L., 1980. *Bogidiella aprutina* n. sp. a new subterranean amphipod from phreatic waters of central Italy. Crustaceana, **38**: 139-144.

-- --, 1981. A new phreatic *Bogidiella* from subterranean waters of Sardinia (Crustacea Amphipoda, Gammaridae). Revue Suisse de Zoologie, Genève, **88**: 157-162.

PINTO-DA-ROCHA, R., 1995. Sinopse da fauna cavernicola do Brasil (1907-1994). Papéis Avulsos de Zoologia (S. Paulo) 39: 61-173.

PIRES, A. M. S., 1987. *Potiicoara brasiliensis*: a new genus and species of Spelaeogriphacea (Crustacea: Peracarida) from Brazil with a phylogenetic analysis of the Peracarida. Journal of Natural History, **21**: 225-238.

PRETUS, J. L. & J. H. STOCK, 1990. A new hyporheic *Bogidiella* (Crustacea, Amphipoda) from Mallorca. Endins, **16**: 47-51.

RUFFO, S., 1952. *Bogidiella neotropica* n. sp. nuovo Anfipodo dell' Amazonia. Rivista Svizzera di Idrologia, **14/1**: 129-134.

-- --, 1958. Due nuove specie di Anfipodi delle acque sotteranee dell' Afghanistan. Studi sui Crostacei Anfipodi LIII. Memorie Museo Civico di Storia Naturale, Verona, **6**: 389-403.

-- --, 1963. Una nuova specie di *Bogidiella* (Crust. Amphipoda) della depressione del Mar Morto. Studi sui Crostacei Anfipodi LVII. Bulletin of the Research Council of Israel, **11 B**: 188-195.

-- --, 1970a. *Bogidiella somala* n. sp. delle acque sotterranee della Somalia (Crustacea Amphipoda). Studi sui Crostacei Anfipodi LXVI. Monitore Zoologico Italiano, **3**: 159-171.

-- --, 1970b. Descrizione di *Kerguelenella macra* n. g n. sp. (Amphipoda Gammaridae). Studi sui Crostacei Anfipodi LXVIII. Boll. Mus. civ. di St. nat. Verona, **18**: 43-54.

-- --, 1973. Contributo alla revisione del genere *Bogidiella* Hertzog (Crustacea Amphipoda, Gammaridae). Studi sui Crostacei Anfipodi LXXIV. Bolletino dell' Istituto di Entomologia della Università degli Studi di Bologna, **31**: 49-77.

-- --, 1974a. Nuovi Anfipodi interstiziali delle coste del Sud Africa. Atti dell' Istituto Veneto di Scienze, Lettere ed Arti, **132**: 399-419.

-- --, 1974b. Note Brevi: Due cambiamenti di nome di generi di Crostacei Anfipodi. Boll. Mus. civ. di St. nat. Verona, **1**: 507.

-- --. 1979. Descrizione di due nuovi Anfipodi anoftalmi dell'Iran e del Madagascar (*Phreatomelita paceae* n. g. n. sp. *Dussartiella madegassa* n. g. n. sp.). Boll. Mus. civ. di St. nat. Verona, **6**: 419-440.

-- --. 1982. Nuovi Anfipodi di acque sotterranee della Somalia. Studi sui Crostacei Anfipodi 92. Monitore Zoologico Italiano 27: 97-113.Monitore Zoologico Italiano. **27**: 97-113.

-- --. 1984. *Bogidiella nubica* n. sp. from interstitial waters of the Sudan (Crustacea: Amphipoda). Studi sui Crostacei Anfipodi XCV. Hydrobiologia, **110**: 131-134.

-- --. 1985. Nouvi Anfipodi mesopsammici delle isole Andamane (Crust. Amphipoda). Studi sui Crostacei Anfipodi XCVIII. Boll. Mus. civ. di St. nat. Verona, **10**: 485-509.

-- --. 1994. New stygobiont amphipods (Crustacea Amphipoda) from the Philippine Islands. Studies on Crustacea Amphipoda CXXII. Tropical Zoology. **7**: 355-366.

RUFFO. S. & C. DELAMARE DEBOUTTEVILLE. 1952. Deux nouveaux Amphipodes souterrains de France. *Salentinella angelieri* n. sp. et *Bogidiella chappuisi* n. sp. C. R. Acad. Sci. Paris. **234**: 1636-1638.

RUFFO. S. & U. SCHIECKE. 1976. Una nouva *Bogidiella* di Creta (Amphipoda, Gammaridae). Bolletino Museo Civico di Storia Naturale. Verona, **3**: 147-155.

RUFFO. S. & A. VIGNA TAGLIANTI. 1973. Three new subterranean *Bogidiella* from Mexico and Guatemala (Crustacea, Amphipoda). Subterranean fauna of Mexico. II. Accademia Nazionale dei Lincei. **171**: 105-133.

-- -- & -- --. 1975. Una nouva *Bogidiella* della Sardegna. Fragmenta Entomologica, Roma, **11**: 73-82.

-- -- & -- --. 1977. Secondo contributo alla conoscenza del genere *Bogidiella* in Messico e Guatemala (Crustacea, Amphipoda, Gammaridae). Subterranean fauna of Mexico. III. Accademia Nazionale dei Lincei. **171**: 125-172.

SÀNCHEZ. E. L.. 1991. Stygofauna of the Canary Islands. 22. *Bogidiella* (*Stygogidiella*) *atlantica* n. sp. (Amphipoda) from interstitial waters on the western Canary Islands. Crustaceana, **61**:113-124.

SCHELLENBERG. A.. 1936. Die Amphipodengattungen um *Crangonyx*. ihre Verbreitung und ihre Arten. Mitteilungen aus dem Zoologischen Museum in Berlin, **22**: 31-43.

SCHIECKE. U.. 1978. Neue Amphipoda (Crustacea) vom Golf von Neapel (Italia). Bolletino Museo Civico di Storia Naturale. Verona, **5**: 355-368.

SHOEMAKER. C. R.. 1945. Notes on the amphipod genus *Bactrurus* Hay. with description of a new species. Journal of the Washington Academy of Sciences. **35**/1:24-27.

-- --. 1959. Three new amphipods from the West Indies. Journal of the Washington Academy of Sciences. **49**: 273-283.

SIEWING. R.. 1953. *Bogidiella brasiliensis*. ein neuer Amphipode aus dem Kuestengrundwasser Brasiliens. Kieler Meeresforschungen. Band 9. Heft 2: 243-247.

SKET. B.. 1985. *Bogidiella* (s. l.) *gammariformis* sp. n. (Amphipoda) from Equador. Biol. Vestn.. **33**: 81-88.

-- --. 1999. High biodiversity in hypogean waters and its endangerment - the situation in Slovenia, the Dinaric Karst, and Europe. Crustaceana, **72** (8): 767-780.

SKET. B. & T. M. ILIFFE. 1980. Cave fauna of Bermuda. Int. Rev. ges. Hydrobiol.. **65** (6): 871-882.

SMITH. S. I.. 1874. The Crustacea of the freshwaters of the United States. In part 2 of: Report of the United States Fish Commission (1872 and 1873): 637-665.

STEBBING. T. R.. 1899. Amphipoda from Copenhagen Museum and other sources. Part 2. Trans. Linn. Soc. London, 2. Zool.. 7: 395-432.

-- --. 1906. Amphipoda. I. Gammaridea. Das Tierreich. **21**: 1-806.

STOCK. J. H.. 1974. The systematics of certain Ponto-Caspian Gammaridae (Crustacea, Amphipoda). Mitt. Hamburg.

Zool. Mus. Inst., **70**: 75-95.

-- --. 1978. *Bogidiella martini*, un nouvel Amphipode souterrain de l'Île Saint-Martin (Antilles) et la zoogeógraphie des Bogidiellidae. International Journal of Speleology, **9**: 103-113.

-- --. 1981. The taxonomy and zoogeography of the family of Bogidiellidae (Crustacea, Amphipoda), with emphasis on the West Indian taxa. Bijdragen tot de Dierkunde (Amsterdam), **51**: 345-374.

-- --. 1983. Discovery of a bogidiellid amphipod crustacean in inland waters of the East Indian Archipelago: *Bogidiella (Medigidiella) sarawacensis* n. sp. Crustaceana, **44**: 198-204.

-- --. 1984. First record of Bogidiellidae (Crustacea, Amphipoda) from the Pacific: *Bogidiella* (*Xystriogidiella* n. subgen.) *capricornea* new species from the Great Barrier Reef. Bulletin of Marine Science, **34**: 380-385.

-- --. 1985. Bogidiellidae (Amphipoda) from Haiti and some general rules on the occurrence of Crustacea Malacostraca in inland groundwaters of the West Indies. Stygologia, **1**: 208-223.

-- --. 1988. Stygofauna of the Canary Islands, 8. Amphipoda (Crustacea) from inland groundwaters of Fuerteventura. Bulletin Zoölogisch Museum (Universiteit van Amsterdam), **11**: 105-112.

-- --. 1990. Discovery of the male and subgeneric status of *Bogidiella cypria* Karaman, 1989 (Crustacea, Amphipoda). Stygologia, **7**: 117-118.

-- --. 1992. *Bogidiella* (Amphipoda) in Japanese inland waters. Crustaceana, **62**: 273-282.

-- --. 1993. Some remarkable distribution patterns in stygobiont Amphipoda. Journal of Natural History, **27**: 807-819.

-- --. 1994. A new member of the family Bogidiellidae (Crustacea, Amphipoda) from the poikilohaline waters of Madeira. Bocagiana, **171**: 1-8.

STOCK, J. H. & L. BOTOSANEANU, 1988. Découverte du premier Bogidiellide (Amphipodes cavernicoles) á l'est de la ligne de Wallace en Indonésie. Stygologia, **4/4**: 371-377.

STOCK, J. H., B. SKET & T. M. ILIFFE, 1987. The status of *Bogidiella balearica* Dancau, 1973, a stygobiont amphipod from Mallorca. Endins, **13**: 39-46.

STOCK, J. H. & J. NOTENBOOM, 1988. Five new bogidiellid Amphipoda from Spain - the first freshwater records in the Iberian Peninsula. Hydrobiologia, **164**: 75-95.

STOCK, J. H. & L. M. RONDÉ-BROEKHUIZEN, 1987. Stygofauna of the Canary Islands, 3. The genus *Bogidiella* (Crustacea, Amphipoda). Revue de Zoologique Africaine, **101**: 439-461.

STOCK, J. H. & R. VONK, 1992. Marine interstitial Amphipoda and Isopoda (Crustacea) from Santiago, Cape Verde Islands. Bijdragen tot de Dierkunde, **62**: 21-36.

SULLIVAN, J., A. M. MARKERT & C. W. KILPATRICK, 1997. Phylogeography and molecular systematics of the *Peromyscus aztecus* species group (Rodentia: Muridae) inferred using parsimony and likelihood. Syst. Biol., **46** (3):426-440.

SWOFFORD, D. L., 1998. PAUP*. Phylogenetic Analysis using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

TAJIMA, F. & M. NEI, 1984. Estimation of evolutionary distance between nucleotide sequences. Mol. Biol. Evol., **18**: 387-404

TRAJANO, E. & L. E. SÁNCHEZ, 1994. Bresil. In: JUBERTHIE, C. & V. RECU (eds.), Encyclopaedia Biospeologica, Tome I: pp. 527-540.

VILLALOBOS, A. F., 1961. Un anfipodo cavernícola nuevo de México: *Bogidiella tabascensis* n. sp. Anales del Instituto de Biología, **31**:317-334.

VONK, R., S. SEVESO & J. NOTENBOOM, 1999. The groundwater amphipod *Bogidiella turcica* n. sp. and other bogidiellids (Malacostraca, Amphipoda) in southwest Turkey. Crustaceana, **27** (8): 817-836.

WECKEL, A. L., 1907. The fresh-water Amphipoda of North America. Proc. U.S. Nat. Mus., **32**: 25-58.

WIENS, J. J., 1995. Polymorphic characters in phylogenetic systematics. Systematic Biology, **44**: 482-500.

-- --, 1998. The accuracy of methods for coding and sampling higher-level taxa for phylogenetic analysis: a simulation study. Systematic Biology, **47** (3): 397-413.

YEATES, D. K., 1995. Groundplans and exemplars: paths to the tree of life. Cladistics, **11**: 343-357.

APPENDICES

A - List of all described genera and species of the revised family Bogidiellidae (Chapter IV)

- *Actogidiella* Stock, 1981
  - *A. cultrifera* Stock, 1981
- *Aequigidiella* Botosaneanu & Stock, 1989
  - *A. aquilifera* Botosaneanu & Stock, 1989
- *Afridiella* Karaman & Barnard, 1979
  - *A. messanai* Diviacco & Ruffo, 1985
  - *A. pectinicauda* Ruffo, 1982
  - *A. somala* Ruffo, 1970 (cf. Ruffo, 1970a)a
- *Antillogidiella* Stock, 1981
  - *A. martini* (Stock, 1978)
- *Arganogidiella* n. gen.
  - *A. arganoi* (Ruffo & Vigna Taglianti, 1973)
  - *A. arganoides* (Ruffo & Vigna Taglianti, 1977)
- *Argentinogidiella* n. gen.
  - *A. horcomollensis* (Grosso & Fernandez, 1988)
  - *A. lavillai* (Grosso & Claps, 1984)
- *Artesia* Holsinger, 1980
  - *A. subterranea* Holsinger, 1980 (in Holsinger & Longley, 1980)
  - *A. welbourni* Holsinger, 1992
- *Aurobogidiella* Karaman, 1988 (cf. Karaman, 1988c)
  - *A. italica* (Karaman, 1979)
- *Bermudagidiella* n.gen.
  - *bermudensis* (Stock, Sket & Iliffe, 1987)
- *Bogidiella* Hertzog, 1933 – ***albertimagni* group (A)** (inner rami of pleopods absent)
  - *B. albertimagni* Hertzog, 1933
  - *B. aprutina* Pesce, 1980
  - *B. balearica* Dancau, 1973
  - *B. broodbakkeri* Stock, 1992
  - *B. convexa* Stock & Notenboom, 1988
  - *B. cyrnensis* Hovenkamp, Hovenkamp & Van der Heide, 1983
  - *B. glabra* Stock & Notenboom, 1988
  - *B. glacialis* S. Karaman, 1959
  - *B. helenae* Mateus & Maciel, 1967
  - *B. hispanica* Stock & Notenboom, 1988
  - *B. ichnusae* Ruffo & Vigna Taglianti, 1975
  - *B. madeirae* Stock, 1994
  - *B. neotropica* Ruffo, 1952

B. semidenticulata Mestrov, 1961

B. torrenticola Pretus & Stock, 1990

Bogidiella – ***skopljensis* group (B)** (inner rami of pleopods 1-segmented, reduced)

B. calicali Karaman, 1988 (cf. Karaman, 1988b)

B. copia Karaman, 1988 (cf. Karaman, 1988a)

B. gammariformis Sket, 1985

B. longiflagellum S. Karaman, 1959

B. nicolae Karaman, 1988 (cf. Karaman, 1988b)

B. serbica Karaman, 1987

B. skopljensis S. Karaman, 1933

Bogidiella – ***niphargoides* group (C)** (one or more characters distinctly modified: inner rami of pleopods reduced, vestigial, or absent)

B. cooki Grosso & Ringuelet, 1979

B. niphargoides Ruffo & Vigna Taglianti, 1977

B. thai Botosaneanu & Notenboom, 1988

B. vomeroi Ruffo & Vigna Taglianti, 1977

Bogidiella – ***lindbergi* group (D)** (only one sex described)

B. barbaria Karaman, 1990 (cf. Karaman, 1990c)

B. cerberus Bou & Ruffo, 1979

B. deharvengi Stock & Botosaneanu, 1988

B. lindbergi Ruffo, 1958

B. michaelae Ruffo & Vigna Taglianti, 1977

B. paolii Hovenkamp et al., 1983

B. ruffoi Birstein & Ljovuschkin, 1968

B. sinica Karaman & Sket, 1990

B. silverii Pesce, 1981

B. sketi Karaman, 1989 (cf. Karaman, 1989a)

B. stocki Karaman, 1990 (cf. Karaman, 1990b)

B. vandeli Coineau, 1969

Bogidomma Bradbury & Williams, 1996

B. australis Bradbury & Williams, 1996

Cabogidiella Stock & Vonk, 1992

C. littoralis Stock & Vonk, 1992

Dycticogidiella Grosso & Claps, 1985

D. talampayensis Grosso & Claps, 1985

D. ringueleti Grosso & Fernandez, 1988

Eobogidiella Karaman, 1982

E. purmamarcensis (Grosso & Ringuelet, 1969)

Grossogidiella n. gen.

G. mauryi (Grosso & Fernandez, 1990)

Guagidiella Stock, 1981

  - *G. holsingeri* (Ruffo & Vigna, Taglianti 1973)
  - *G. pasquinii* (Ruffo & Vigna Taglianti, 1977)
- *Hagidiella* Stock, 1985
  - *H. prionura* Stock, 1985
- *Hebraegidiella* Karaman, 1988 (cf. Karaman, 1988a)
  - *H. bromleyana* Karaman, 1988 (cf. Karaman, 1988a)
- *Indogidiella* n. gen.
  - *I. sarawacensis* (Stock, 1983)
  - *I. daccordii* (Ruffo, 1994)
- *Maghrebidiella* Diviacco & Ruffo, 1985
  - *M. maroccana* Diviacco & Ruffo, 1985
- *Marigidiella* Stock, 1981
  - *M. brasiliensis* (Siewing, 1953)
  - *M. crassipes* Stock, 1981
- *Marinobogidiella* Karaman, 1982
  - *M. thyrrenica* (Schiecke, 1978)
- *Medigidiella* Stock, 1981
  - *M. antennata* Stock & Notenboom, 1988
  - *M. aquatica* Karaman, 1990 (cf. Karaman, 1990a)
  - *M. arista* Koenemann, Vonk & Schram, 1998
  - *M. chappuisi* (Ruffo, 1952) (in Ruffo & Delamare Deboutteville, 1952)
  - *M. dalmatina* (S. Karaman, 1953)
  - *M. hebraea* (Ruffo, 1963)
  - *M. minotaurus* (Ruffo & Schiecke, 1976)
  - *M. paolii* Hovenkamp, Hovenkamp & Van der heide, 1983
  - *M. paraichnusae* (Karaman, 1979)
  - *M. uncinata* Stock & Notenboom, 1988
- *Megagidiella* Koenemann & Holsinger, 1999
  - *M. azul* Koenemann & Holsinger, 1999
- *Mesochthongidiella* Grosso & Fernandez, 1985
  - *M. tucumanensis* Grosso & Fernandez, 1985
- *Mexigidiella* Stock, 1981
  - *M. chitalensis* Karaman, 1982
  - *M. hamatula* Stock, 1985
  - *M. mexicana* Karaman, 1982
  - *M. sbordonii* (Ruffo & Vigna Taglianti, 1973)
  - *M. tabascensis* (Villalobos, 1961)
- *Nubigidiella* Karaman, 1988a
  - *N. nubica* (Ruffo, 1984)
- *Orchestigidiella* Stock, 1981
  - *O. orchestipes* (Ruffo & Vigna, Taglianti 1977)

Parabogidiella Holsinger, 1980
- *P. americana* Holsinger, 1980 (in Holsinger & Longley, 1980)

Patagongidiella Grosso & Fernandez, 1990
- *P. danieli* Grosso & Fernandez, 1990

Spelaeogammarus da Silva Brum, 1973
- *S. bahiensis* da Silva Brum, 1973
- *S. spinilacertus* Koenemann & Holsinger (in press)
- *S. trajanoae* Koenemann & Holsinger (in press)
- *S. santanensis* Koenemann & Holsinger (in press)

Stygogidiella Stock, 1981
- *S. atlantica* Sànchez, 1991
- *S. bredini* (Shoemaker, 1959)
- *S. cypria* Stock, 1990
- *S. perla* Stock, 1981
- *S. purpuriae* Stock, 1988
- *S. uniramosa* Stock & Rondé-Broekhuizen, 1987
- *S. virginalis* Stock, 1981

Xystriogidiella Stock, 1984
- *X. capricornea* Stock, 1984
- *X. spathulata* Stock & Rondé-Broekhuizen, 1987

B - List of characters and character states employed in the cladistic analysis of the Bogidiellidae (Chapter IV)

References to taxa are based on the unrevised family Bogidiellidae s. lat. (see Chapter IV, Taxa)

(1) Modifications of the outer ramus in male pleopod 1

State 0 = absent

State 1 = present

Alternative run: weight factor: 6: ordered. Modified outer rami on pleopod 1 occur in three taxa only. *Mesochtongidiella* bears 1 modified spine on segment 2, whereas *Mexigidiella* has 1 modified spine on segment 2 and 3, respectively. The modifications of *Stygogidiella* B are less pronounced but still distinguishable (see Chapter IV, New taxa).

(2) Modifications of the outer ramus in male pleopod 2

State 0 = absent

State 1 = present

Alternative run: weight factor: 6: ordered. Modifications in the male pleopod 2 occurred more frequently than in pleopod 1. The majority of affected taxa had 1 modified spine on segment 2. A few taxa showed additionally slightly modified segments or differently reduced segments (see Chapter IV, Characters and Incompatible taxa).

(3) Number of outer ramus segments in pleopods 1-3

State 0 = 3 or more segments

State 1 = 3 segments

State 2 = 2 or 3 segments

State 3 = 2 or less segments

Alternative run: weight factor: 6: ordered. State 0 comprises genera with more than 3 outer rami segments in pleopods 1-3 (*Artesia* and *Dussartiella*), genera with 3 segments in the outer rami of 1 pleopod and more than 3 segments in the outer rami of the other 2 pleopods (*Aurobogidiella* and *Paracrangonyx*), and genera with a varying number of outer rami segments (*Aequigidiella* with 3 or 4 segments and *Patagongidiella* with 3-5 segments. In *Marigidiella*, the specimens had 3-segmented outer rami in pleopod 1+2 and a 2-segmented outer ramus in pleopod 3 (state 2). State 3 refers to a differently varying number of segments in both sexes (*Bollegidia*: 0-2 segments) and also to a 1-segmented outer ramus (*Bollegidia* and *Pseudingolfiella*) (see also Chapter IV, Characters).

(4) Inner rami of pleopods 1-3

State 0 = multiarticulate, aequiramous

State 1 = uniarticulate, aequiramous

State 2 = uniarticulate, small

State 3 = uniarticulate, reduced

State 4 = vestigial

State 5 = absent

Alternative run: weight factor: 6; ordered. State 2 includes inner rami that are shorter than the outer ramus but distinctly longer than segment 1 of the outer ramus. A uniarticulate, reduced inner ramus that is shorter than segment 1 of the outer ramus is coded as state 3. Vestigial inner rami (state 4) are small, bud-like structures without setae. Majority Coding was applied to the following OTUs: *Bogidiella* C (1 species with absent inner rami, 1 species with reduced inner rami, 2 species with vestigial inner rami on pleopods 1+2 and absent inner ramus on pleopod 3: state 4); *Stygogidiella* A (3 species with absent inner rami, 2 species with reduced inner rami: state 5); *Antillogidiella martini* (male with reduced inner rami, female inner rami are lacking: state 3); *Guagidiella* (2 species with vestigial inner rami on pleopods 1+2 and absent inner ramus on pleopod 3: state 4).

(5) Modifications in male uropod 1

State 0 = absent
State 1 = spines modified
State 2 = rami/peduncle and spines modified

Alternative run: weight factor: 6; ordered. State 2 could be observed in two taxa. In *Patagongidiella danieli*, a process of the uropod 1 peduncle was apparently fused with a suspiciously modified spine. Similarly, *Guagidiella* had 1 strongly modified spine on a distinctly reduced inner ramus that was partly fused with the peduncle.

(6) Modifications in male uropod 2

State 0 = absent
State 1 = spines modified
State 2 = rami and spines modified

Alternative run: weight factor: 6; ordered. As with state 2 of character 5, a strongly reduced inner ramus, bearing 1 modified spine, appears to be in different states of fusion with the peduncle in *Mesochthongidiella* and *Dycticogidiella* (state 2). A reduced inner ramus plus modified spine without any signs of fusion occurs in *Aequigidiella* (state 2).

(7) Dagger-shaped rami in female uropod 1

State 0 = absent
State 1 = present

Alternative run: weight factor: 6; ordered. "Dagger-shaped rami" was the most commonly used term in the literature for advanced reductions in uropods 1+2 (characters 7-10). It covers a variety of different reduction states from distinctly pointed rami tips without apical spines to slightly pointed tips with 1 or 2 apical spines. In all cases, however, the term "dagger-shaped" seemed to be appropriate. The only exceptions were *Bollegidia* and *Pseudingolfiella*, showing pointed, dagger-shaped reductions as well as sack-like rami (in some instances, differently reduced on outer and inner ramus of the same uropod). Because dagger-shaped rami occurred randomly in either sex on both uropods 1+2, this reduction was split into 4 independent characters.

(8) Dagger-shaped rami in female uropod 2

State 0 = absent
State 1 = present

Alternative run: weight factor: 6; ordered.

(9) Dagger-shaped rami in male uropod 1

State 0 = absent

State 1 = present

Alternative run: weight factor: 6; ordered.

(10) Dagger-shaped rami in male uropod 2

State 0 = absent

State 1 = present

Alternative run: weight factor: 6; ordered.

(11) Rami of uropod 3

State 0 = biramous, aequiramous

State 1 = biramous with inner ramus greatly in size reduced

State 2 = uniramous

Alternative run: weight factor: 6; ordered. A greatly reduced, almost scale-like inner ramus on uropod 3 is exceptional for bogidiellids. It occurs in *Dussartiella* and *Paracrangonyx*. *Pseudingolfiella* is the only bogidiellid genus with a uniramous uropod 3 (see Chapter IV, Incompatible taxa).

(12) Outer ramus of uropod 3

State 0 = 1-segmented

State 1 = 2-segmented

Alternative run: weight factor: 6; ordered. A 2-segmented outer ramus occurs in *Dussartiella*, *Paracrangonyx*, and *Pseudingolfiella* only (see Chapter IV, Incompatible taxa). Because only some species in a few amphipod families have 2-segmented outer rami, a 1-segmented outer ramus was considered the plesiomorphic state.

(13) Gills

State 0 = on pereonites 2-6

State 1 = on pereonites 3-6

State 2 = on pereonites 4-6

Alternative run: weight factor: 3; ordered. Majority Coding has been applied to *Stygogidiella* A (state 2). According to Sànchez (1991), *Bogidiella (Stygogidiella) atlantica* has gills on pereonites 3-5, which makes it the only species in the family with this character.

(14) Oostegites

State 0 = on pereopods 2-5

State 1 = on pereopods 2-5 or 3-5

State 2 = on pereopods 3-5

State 3 = on pereopods 3+4

State 4 = "absent"

Alternative run: weight factor: 3; ordered. Oftentimes, this character is poorly or not at all described in the literature. Consequently, some states are adapted from drawings. State 1 occurred in the polytypic taxa *Artesia*, *Bogidiella* A, and *Mexigidiella*. State 3 and 4 are autopomorphic modifications of *Bollegidia* and *Pseudingolfiella*. The term "absent" is adopted from the literature. Apparently, brood plates were lacking in all females of both *Pseudingolfiella* sp.

(15) Number of segments in flagellum of antenna 2

State 0 = 5 or more segments

State 1 = 5 segments

State 2 = 5 or less segments

Alternative run: unweighted; ordered. The number of segments in the flagellum of antenna 2 is a relatively constant character. In some instances, a varying number of segments occurred intraspecific as well as interspecific. Examples for intraspecific variation are *Aequigidiella* (5-7 segments = state 0) and *Orchestigidiella* (4-5 segments = state 2). Interspecific variation could be observed in the polytypic taxa *Medigidiella* A (4-5 or 5 segments = state 2), *Medigidiella* C (5 or 6 segments = state 0), and *Guagidiella* (4 or 5 segments = state 2).

(16) Number of segments in accessory flagellum

State 0 = 3 or more segments

State 1 = 2 or 3 segments

State 2 = 2 segments

State 3 = 1 segment

Alternative run: unweighted; ordered. Corresponding to character (15), state 1 was assigned to the polytypic OTUs *Afridiella*, *Bogidiella* A+B, *Medigidiella* A, and *Stygogidiella* A.

(17) Number of palp segments in maxilla 1

State 0 = 2 segments

State 1 = 1-2 segments

State 2 = 1 segment

Alternative run: weight factor: 3, ordered. Three taxa showed an interesting case of variable numbers of palp segments in maxilla 1, coded as state 1: *Stygogidiella horcomollensis (Stygogidiella* B) obviously bears a 1-segmented palp. In some individuals, however, a weakly developed articulation, separating palp segments 1 and 2, could be perceived visually. *S. lavillai*, the other species of *Stygogidiella* B had a 2-segmented palp. Almost identical with the situation in *Stygogidiella* B, both species of *Dycticogidiella* had 2-segmented and 1-segmented, weakly articulated palp segments and state 1 was applied, too. The third OTU with state 1 was the only described specimen of *Dussartiella madegassa* which had a long 2-segmented palp on the right, and a 1-segmented, reduced palp on the left maxilla 1.

(18) Number of setae on inner lobe of maxilla 1

State 0 = 3 setae

State 1 = 2 or 3 setae

State 2 = 2 setae

State 3 = 1 or 2 setae

State 4 = 1 seta

State 5 = setae absent

Alternative run: unweighted; ordered. Characters 18 and 19 were left unweighted because their assumed minor phylogenetic significance. See Chapter IV. Characters for an explanation of transitional states in characters 18 and 19.

(19) Number of spines on outer lobe of maxilla 1

State 0 = 9 spines
State 1 = 7 or 8 spines
State 2 = 7 spines
State 3 = 6 or 7 spines
State 4 = 6 spines

Alternative run: unweighted; ordered.

(20) Maxilla 2

State 0 = normal
State 1 = reduced

Alternative run: unweighted; ordered. In the literature, the choice of descriptive terms for the inner plates on maxilla 2 varies considerably. State 1 comprises inner plates that are usually described as "small", "reduced", or "weak".

(21) Mandibular molar

State 0 = triturative
State 1 = non-triturative

Alternative run: unweighted; ordered. Like in character 20, state 1 refers to molars that are described as "small", "weak", etc.

(22) Coxal plates

State 0 = longer than wide
State 1 = wider than long

Alternative run: unweighted; ordered. In *Artesia*, coxal plates 1 and 2 were wider than long or as wide as long, whereas coxae 3-7 were distinctly longer than wide. We chose state 0 as apt coding for *Artesia*.

(23) Telson shape

State 0 = longer than wide
State 1 = approximately as long as wide
State 2 = wider than long

Alternative run: unweighted; ordered. The majority of bogidiellid amphipods have a telson that is wider than long. State 0 seems to be typical for non-European taxa. The Majority Coding method has been applied to *Bogidiella* A and C, and *Medigidiella* A (all state 2).

(24) Apical spines of telson

State 0 = 4 or more spines
State 1 = 2 or 3 spines
State 2 = 2 spines
State 3 = 1 or 2 spines
State 4 = 1 spine
State 5 = spines absent

Alternative run: unweighted; ordered. See Characters for an explanation of variable or transitional states 1 and 3 of characters 24 and 25. The Majority Coding method has been applied to *Stygogidiella* A (state 3).

(25) Subapical spines of telson

State 0 = 3-5 spines
State 1 = 2-4 spines
State 2 = 2 spines
State 3 = 1 or 2 spines
State 4 = 1 spine
State 5 = 0 or 1 spine
State 6 = spines absent

Alternative run: unweighted; ordered. State 0 and 1 occurred as highly variable, autapomorphic characters in *Megagidiella* and *Spelaeogammarus*, respectively. The 3 subapical spines in *Hebraegidiella* were coded as transitional condition of state 1. We applied the Majority Coding method to *Bogidiella* C (state 4).

(26) Mediosternal processes

State 0 = absent
State 1 = present

Alternative run: weight factor: 6; ordered. Mediosternal processes were present in *Patagongidiella mauryi* and *Patagongidiella danieli* only. We are of the opinion that these structures are both morphologically and physiologically different from the mediosternal gills in *Paracrangonyx*. Therefore, the gills in *Paracrangonyx* have been treated as true autapomorphies and excluded from the analysis.

(27) Eyes

State 0 = eyes present
State 1 = 2-3 unpigmented lens cells
State 2 = eyes absent

Alternative run: weight factor: 6; ordered. As mentioned above, we performed an phylogenetic analysis on the (unrevised) family Bogidiellidae s. lat., attempting to code all different states of homologue structures as independent characters. Since *Paracrangonyx* is removed from the family, however, the phylogenetic relationship of reduced eye structures in *Paracrangonyx* (state 1) and large, fully developed eyes observed in *Bogidomma* (state 2) becomes irrelevant. In the revised family, *Bogidomma australis* remains the only bogidiellid with eyes, a fact that certainly raises attention because of its uniqueness. The biology of this species from Barrow Island, Western Australia, may be worth further study. All the more, because it is reported from a cave (!) at the outer margin of the bogidiellid distribution range, with a possible connection to marine waters.

C - List of characters and character states used in the phylogenetic analysis of *Bactrurus* and selected taxa (Chapter V)

If not explicitly indicated otherwise, a character was coded as unordered and unweighted (i.e., equally weighted with weight factor 1) for both unordered and partially ordered analysis (see Chapter V, Cladistic methods)

(1) Eyes

State 0 = eyes present
State 1 = eyes absent

Partially ordered analysis: weight factor 3. For this character, state 0 was assumed as the ancestral condition of an epigean predecessor. Although different degrees of reduction could be observed in some of the stygophile taxa, a differentiation into more than two states (absent and present) seemed doubtful because of the discrete appearance of this trait.

(2) Rastellate spines on carpus of gnathopod 2

State 0 = absent
State 1 = present

The occurrence of 2-6 rastellate spines (sometimes reduced to strong brush-like setae) on the distoposterior margin of the carpus of gnathopod 2 was considered an apomorphic condition. It is present in all *Bactrurus* species and most terminal taxa of the genus *Stygobromus*. Rastellate spines and setae usually occur on both gnathopods. The only exception was *Stygobromus t. tenuis* which had no rastellate spines on the first gnathopod. To avoid overrating of this trait by scoring rastellate spines for both gnathopods as two independent characters, we decided to include the character 'rastellate spines' only once, for the second gnathopod.

(3) Coxal gills

State 0 = 6 pairs (present on pereopods 2-7)
State 1 = 5 pairs (present on pereopods 2-6)
State 2 = 4 pairs (present on pereopods 3-6)
State 3 = 3 pairs (present on pereopods 4-6)

Partially ordered analysis: ordered; weight factor 3. A progressive reduction of coxal gills was regarded as adaptation to subterranean environments and, therefore, state 0 was coded as plesiomorphic character state.

(4) Coxal gills on pereopod 7

State 0 = normal
State 1 = reduced
State 2 = absent

Partially ordered analysis: ordered. Similar as in character 3, a continuous reduction of this trait was hypothesized, with state 2 as apomorphic condition.

(5) Median sternal processes

State 0 = absent

State 1 = present

Median sternal processes occur in many crangonyctid taxa. More than 95% of *B. brachycaudus* specimens had no median sternal processes. According to the majority of occurrences, state 0 was chosen for this species (Majority Coding method; see Koenemann & Holsinger, 1999a).

(6) Lateral sternal processes

State 0 = absent

State 1 = on pereonites 6 and 7

State 2 = on pereonites 6 and 7 and pleonite 1

Partially ordered analysis: ordered; weight factor 3. The function of sternal processes is still unknown. We think that it may be an adaptation to life in cold subterranean freshwater environments and assume a linear, progressive evolution from state 0 to state 2. Approximately 86% of all *B. mucronatus* specimens examined had lateral sternal processes on pereopods 6 and 7 and on pleonite 1 (as opposed to sternal processes on pereopods 6 and 7 only). Therefore, state 2 was chosen for *B. mucronatus*.

(7) Uronites with dorsal setae

State 0 = present

State 1 = absent

The presence of dorsal setae on uronites 1-3 is common in *Gammarus* and considered the ancestral condition of an epigean predecessor.

(8) Peduncle of male uropod 1 with distal process

State 0 = absent

State 1 = present

Partially ordered analysis: weight factor 3. A sexually dimorphic uropod 1 with peduncular process occurs in some species of *Bactrurus* and almost all species of *Stygobromus*. Modified uropods and/or pleopods are relatively common in the stygobiont family, the Bogidiellidae. Sexually dimorphic modifications of posterior appendages might be functionally related to reproductive success in hypogean environments and, therefore, are treated as apomorphic adaptation.

(9) Outer ramus of uropod 3

State 0 = 2-segmented

State 1 = 1-segmented, not reduced, with apical and subapical spines

State 2 = 1-segmented, distinctly reduced, with few subapical spines

State 3 = 1-segmented, strongly reduced, with no subapical spines

State 4 = 1-segmented, scale-like, with only 1-2 apical spines

State 5 = absent

Partially ordered analysis: ordered; weight factor 3. Although a reduced outer ramus is common for both *Bactrurus* and *Stygobromus*, a distinction could be made between different degrees of reduction. The outer ramus is clearly more reduced in all *Stygobromus* taxa, bearing no subapical spines and with only 1-3 apical spines in the majority of species. In *Stygobromus*, the outer ramus reaches 10-42% of the length of the peduncle or it is sometimes absent. In *Bactrurus*, the most advanced reduction was observed in *B. mucronatus*, but even this species had an outer ramus that reached 54% of the length of the peduncle, with at least 1 or 2 subapical spines. The most developed outer ramus of all *Bactrurus* taxa was found in *B. cellulanus*. Because this outer ramus was very similar to those of both *Crangonyx* species, character state 2 was applied for *Bactrurus* and *Crangonyx*. In some specimens of *Stygobromus phreaticus*, the outer ramus of uropod 3 is absent. However, state 4 was applied since this reflects the condition in the majority of species.

(10) Inner ramus of uropod 3

State 0 = present, > 70% length of outer ramus

State 1 = present, < 35% length of outer ramus

State 2 = absent

Partially ordered analysis: ordered: weight factor 3. Similar to the reduction of the outer ramus, we hypothesized a progressive reduction for this character, with state 0 as ancestral condition.

(11) Sexual dimorphism of the telson

State 0 = absent

State 1 = present, but minor degree of dimorphism

State 2 = present, distinct degree of dimorphism

Partially ordered analysis: ordered: weight factor 3. State 1 refers to the short apical spines found in males of *B. wilsoni*, *B. angulus* and *B. cellulanus* (*wilsoni* group), opposed to distinctly longer spines in females. In the *wilsoni* group, the female telson apparently also tends to be wider and shorter than the male telson. The same dimorphism, albeit much more extremely developed, occurs in *B. mucronatus* and *B. pseudomucronatus*. The differences between the male and female telson can be observed without optical aids: in adult males the telson reaches up to 1/3 of the body length. Since both character states 1 and 2 are sexually dimorphic, they may be related to the reproductive success of males.

(12) Length/width ratio of telson

State 0 = as wide as long or wider than long

State 1 = distinctly longer than wide

A telson scored as distinctly longer than wide was approximately twice as long as wide (length 150-200% of width). The lowest length/width ratio was found in *Stygobromus mackini* (110%). This condition still was easily distinguishable from state 0.

(13) Cleft of telson

State 0 = cleft to base (90-100%)

State 1 = cleft 5-40% of length of telson

State 2 = telson entire (cleft 0-2% of length of telson)

Bactrurus wilsoni, *B. angulus* and *B. cellulanus* had dimorphic telsons: in some individuals, state 1 was observed while other specimens had state 2 (see Chapter V, Taxonomic part). Unfortunately, there were not enough specimens available to apply the Majority Coding method. In this case, the intermediate character condition (state 1) seemed to be the appropriate choice.

Although the difference between the extremes of character state 1 (5%) and state 2 (2%) is only marginal, a distinction of two different states was justifiable because both values could be measured in only some specimens of a taxon, respectively. For example, the apical notch in *Stygobromus phreaticus* was 5-9%, whereas most specimens of *B. brachycaudus* had a convex apical margin or a very small notch (0-2%).

The hypothesis that a deeply cleft telson is the plesiomorphic condition is speculative. For the analysis, we assumed a progressive reduction from a deeply cleft telson with subapical and apical spines to an elongated, entire telson with apical spines only. Therefore, the deeply to completely cleft telson was chosen as the ancestral condition

(14) Subapical spines of telson

State 0 = absent
State 1 = present

(15) Coxal plates 1 and 2

State 0 = longer than wide
State 1 = wider than long

Partially ordered analysis: ordered; weight factor 3. State 1 is a common condition for many stygobiont amphipods. The evolution of shorter coxal plates may be an adaptation to life in subterranean environments.

(16) Number of segments in accessory flagellum

State 0 = 4-6 segments
State 1 = 3 segments
State 2 = 2 segments
State 3 = 1 segment

State 0 occurred in the outgroup taxa only, all crangonyctid species analyzed had a 2-segmented accessory flagellum.

VITA

Work address:

Stefan Koenemann
Old Dominion University
Dept. of Biological Sciences
Norfolk, VA 235299-0266
USA

Education:

1991-1994

B.S. -- Carl-von-Ossietzky University (Oldenburg, Germany)

1994-1997

M.S. -- University of Amsterdam (Netherlands)

Meetings:

1997

- Partnership for Enhancing Expertise in Taxonomy (PEET); meeting and workshop, Woods Hole Oceanographic Institution and Marine Biological Laboratory (Massachusetts)

1998

- 4th International Crustacean Congress, Amsterdam; oral paper presentation, poster presentation (co-author)
- International Amphipod Meeting, Kronenburg (Germany)

2000

- Partnership for Enhancing Expertise in Taxonomy (PEET); conference, Smithsonian Institution, Washington D.C.; poster presentation
- 10th International Colloquium on Amphipoda, Heraklion (Crete, Greece); two oral presentations

Fellowships and grants:

1996

- STIR-netwerk (University of Amsterdam)

1997

- University of Amsterdam

1997-2000

- Partnership for Enhancing Expertise in Taxonomy (PEET/NSF, USA)

1999

- Cave Conservancy of the Virginias (USA)
- Cave Research Foundation (USA)