Volume 9 Number 1 June 1977 NE ISSN 0020-7691

International Journal of Speleology

Editor-in-Chief: R. Husson, Dijon
Book and News Editor: Brother G. Nicholas, Philadelphia

PUBLISHED BY

SWETS & ZEITLINGER B.V. - AMSTERDAM

INTERNATIONAL JOURNAL OF SPELEOLOGY

is issued in volumes of 4 numbers not more than one volume being published annually.

All correspondence regarding subscriptions should be sent to the publishers

SWETS & ZEITLINGER B.V.
Publishing Department
347B, Heereweg
2160 AH LISSE, The Netherlands

Manuscripts, illustrations and all communications relative to them should be addressed to the Editor-in-Chief, Professor Dr. R. Husson, Biologie Animale et Génerale, Faculté des Sciences, Université de Dijon, 6 Boulevard Gabriel, 21 Dijon, France. All matter relating to the section on Abstracts and News should be sent to: Brother G. Nicholas, F. S. C., Department of Biology, La Salle College, Philadelphia, Pa. 19141, USA. For instructions to authors with regard to the manuscript see below and inside of back cover.

Printed in The Netherlands by Offsetdrukkerij Kanters B.V., Alblasserdam

---

NOTICE TO CONTRIBUTORS

1 Two copies of the complete text of each article should be submitted. Submission of a paper will be taken to imply that it is unpublished and is not being considered for publication elsewhere. Papers published in the International Journal of Speleology may not be reprinted or published in translation without permission. Reproduction of illustrations requires similar permission.

2 Papers should be written in English, French, German, Italian or Spanisch. Authors using a language not their own are urgently requested ta have their manuscripts checked for linguistic correctness before submission. All papers should finish (in addition) with an English summary, giving a synopsis of the paper with sufficient detailed information concerning the methods used and the results obtained.

3 Papers should be typed in double spacing, on one side of the paper, quarto size, leaving top and left hand margin at least 2.5 cm (one inch) wide.

4 Papers should be headed by a title, the initials(s) and name(s) of author(s) and an exact description of the post held and business address of the author(s). Dates should be in the form "5 February 1959".

Continuation see inside of back cover.

Int. J. Speleol. 9 (1977), pp. 1-19.

Nitrobacter in Mammoth Cave

by

C. B. FLIERMANS* and E. L. SCHMIDT**

INTRODUCTION

Mammoth Cave, a large natural limestone cavern formed 20 to 30 million years ago in rocks laid down during the Mississippian Period, lies in west-central Kentucky and borders on the western coal basin and the Mississippian Plateau. Historically, over 1800 tons of nitrate sediments were mined from Mammoth Cave prior to and during the War of 1812, and were subsequently processed for gunpowder. The extensiveness of the operation is substantiated by the large number of mining archeological artifacts that remain in the cave (Faust, 1967).

Although the mechanism of saltpetre formation, $CaNO_3$, in cave ecosystems is unknown, various hypotheses have been suggested for saltpetre formation. Brown (1809) suggested that nitrates are leached into the cave sediments through drainage water since high concentrations of nitrates are sometimes found in cavernous sandstone rock. Priestley (1809) on the other hand suggested that weak nitrous acid produced in the atmosphere resulted in the deposition of saltpetre. Generally, it is thought that nitrate deposits in caves are formed by the degradation of bat guano (Clark, 1924); Hess (1900) reported, however, that deposits of nitrate extended over five miles into the cave, and such distances are not usually traversed by bats. Faust (1967) suggested that saltpetre formation was mediated by free-living (non-symbiotic) nitrogen fixing bacteria capable of fixing atmospheric nitrogen and using carbon dioxide as the sole energy source with the concomitant formation of $CaNO_3$. Yet, such an organism has never been reported nor isolated. Thus, the mode of formation of such large saltepetre deposits within Mammoth Cave and the role of bacteria in their formation remains unclear.

Cave ecosystems provide the microbial ecologist with a selective natural

---

* Savannah River Laboratory, E. I. du Pont de Nemours and Co., Inc., Aiken, S. C. 29801, USA.
** Department of Microbiology, University of Minnesota, Minneapolis, Minn. 55101, USA.

habitat in which to work. The environment for microbial growth is both extreme and constant in that the bacteria experience no light (in the non-tourist areas of the caves), constant temperatures, low nutrient levels, and a habitant whose pH is well buffered circumneutral. Such conditions provide selective pressures for the growth and proliferation of certain bacteria.

There are two basic approaches for studying bacteria in a natural ecosystem such as Mammoth Cave: the direct approach, which relies on viewing and recognizing bacteria in their natural habitat without enriching or culturing the bacterium on artificial media; secondly, the indirect approach, which removes the bacterium from its natural habitat and relies on the detection of the microbe or a microbial product in order to establish the presence of a given bacterium.

Isolated studies (Caumartin, 1963 and Gounot, 1967) have described indirect enrichment techniques for culturing organisms from cave sediments. Such techniques depend on observing the growth of the organisms after they are removed from their natural habitat and subjected to conditions dissimilar to those found *in situ.* Estimates of bacterial types and population densities by indirect procedures, i.e. plate counts, dilution plating, or most probable number analyses, may not reflect the bacteria present in the habitat (Wiebe, 1971). The indirect approach is limited by the fact that any single medium is not capable of supporting the growth of all bacterial types, thus certain bacteria will not be isolated, cultured and/or identified. On the other hand, the use of a wide variety of media and growth conditions is impractical and duplication of bacteria occurs. Additionally, separation of single bacterial colonies is often difficult, due to either the failure to separate single cells initially or the overgrowth of slower growing organisms. Since population estimates are based on visualization of colonies, the number of colonies on a given petri dish must be statistically numerous, yet not so large that crowding and overlapping occurs. Moreover, the development of colonies is a function in part of growth temperature, incubation time, and nutrient levels.

Direct procedures depend on the recognition of the bacterium of choice in its natural habitat without supplimental enrichment and growth. Such recognition is often very difficult, since most bacteria are not morphologically distinct. The development of the direct fluorescent antibody technique (Bohlool and Schmidt, 1968) and the implementation of the technique in natural ecosystems (Fliermans et al., 1974) has greatly expanded the field of microbial ecology and has made the direct approach to bacterial identification and quantification in various ecosystems possible. The fluorescent antibody technique has been described in detail elsewhere (Schmidt, 1973; Fliermans et al., 1974) and will only be outlined here.

The FA technique is derived from the high degree of specificity which occurs in an antigen-antibody reaction. A particular bacterium of interest (in this research, *Nitrobacter agilis* or *N. winogradskyi*) is isolated into pure cultures, cultivated, and used as the antigen for the preparation of specific antisera in rabbits. After a series of intravenous injections, specific antibodies against the injected antigen are produced. Antisera are then removed from the rabbit by cardiac puncture and the globulins containing the active antibodies are separated then purified by ammonium sulfate precipitation. These antibodies are conjugated to a fluorochrome dye, usually fluorescein isothiocyanate (FITC), to form the fluorescent antibody (FA), is then used as a stain for samples taken from the natural environment. The bacterium of interest, if present in the sample, forms a specific antigen-fluorescent antibody complex which can be visualized by fluorescent microscopy. Such a technique is specific for the homologous system and highly sensitive, since as little as 10^{-15} g of FITC on a bacterium can be detected (Goldman and Carver, 1961).

We chose to use this direct fluorescent antibody technique to study the presence, distribution and population densities of the chemautotrophic nitrifiers, *Nitrobacter agilis* and *Nitrobacter winogradskyi,* in Mammoth Cave and other saltpetre caves in the southeastern United States. Recent studies (Fliermans et al., 1974) demonstrated that fluorescent antibodies for *Nitrobacter* were species specific and could be used to evaluate the presence of these organisms in saltpetre caves.

MATERIALS AND METHODS

Cultures. All cultures were maintained as described by Fliermans et al., 1974. New isolates of nitrifying bacteria were obtained from cave sediments through a series of selective enrichments and final isolates were picked from streak plates (Schmidt, 1973). Since *Nitrobacter* spp. are considered to be strict chemoautotrophs, unable to grow on organic compounds, all cultures were routinely checked using five different heterotrophic media for purity. The absence of *Nitrobacter* growth in these five media (Clark and Schmidt, 1967) and uniformity of organisms observed under light microscopy were confirmation of cultural purity.

Sampling. Cave sediment samples were aseptically taken with either an alcohol flamed spatula or soil corer, immediately placed in sterile Whirl Pak bags (NASCO), and returned to the laboratory for processing. All samples were processed within 24 hours of sampling.

Chimical Analysis. Each sediment sample was measured for pH, % moisture, nitrite and nitrate concentrations. Sediment moisture was determined gravimetrically by placing cave sediment samples into tared 35 mm metal screw-capped film cans directly in the field. In the laboratory the samples were weighed and dried to a constant weight at 110°C with the lids loose. The samples were then placed in a dessicator for temperature equilibration and reweighed. The amount of water lost was expressed as a percentage of the sediment sample. Sediment pH values were measured on a 1:1 w/v slurry with distilled water using an Orion portable pH meter with a combination electrode.

Qualitative spot tests for nitrate and/or nitrite were taken extensively throughout the cave ecosystem, using diphenylamine in concentrated sulfuric acid (Pramer and Schmidt, 1964). Sediment samples were extracted with distilled water and filtered in the field using a filter holder (Swinnex-25, Millipore Corp.) and a 0.45μ membrane filter. Three drops of the filtrate were placed in white porcelain plates and an equal amount of reagent added. A complex between the diphenylamine and the nitrate or nitrite resulted in a deep blue color, indicating the presence of NO_3^- or NO_2^-.

Nitrites were measured quantitatively using the colorimetric procedure of Shinn (1941). Nitrates and nitrites from 50 g of cave sediment were extracted with 250 ml of 0.015M $CaSO_4$. The supernatant was filtered through a Whatman No. 42 filter and nitrite levels determined. Nitrate analyses were performed by passing the filtrate through a cadmium reduction column, measuring the nitrite concentration colorimetrically, and calculating the nitrate concentration by difference (Strickland and Parsons, 1968). The efficiency of nitrate reduction was 93-97%.

Leaching Studies. Composite samples each containing 300 g of Mammoth Cave sediments from thirty sites were placed in two chromatographic columns (40 x 600 mm) and leached free of detectable nitrates and nitrites with 400 ml filter sterilized distilled water. Leachate was collected aseptically in 50 ml aliquots and measured qualitatively for the removal of nitrates and nitrites. Total bacterial and *Nitrobacter* population densities in the soil column and in the leachate were measured by direct microscopy (Fliermans and Schmidt, 1975) and immunofluorescence (Fliermans et al., 1974), respectively.

RESULTS

Samples were taken from areas indicated by an "x" on the surveyed passages shown in Figure 1. Although the Mammoth Cave system contains more than 248 km of passageways, samples were taken from 55 km of passages, of which less than 10% were accessible to tourist. Samples were collected from areas within the passages where public influence was deemed negligible, i.e., ceilings, walls, crevasses, etc. Sampling was concentrated in the Rotunda and Booth's Amphitheater areas since archeological evidence indicates that extensive saltpetre mining took place in these areas. A more specific description of some of the sampling sites within Mammoth Cave along with chemical data of pH, NO_3^-, NO_2^- and percent moisture, are shown in Table I. Values for pH ranged from 5.95 to 8.99 with a mean of 7.94. This is as expected since the cave is formed in a limestone region where the buffering capacity of the parent material is high. Nitrite levels were generally less than 0.2 ppm NO_2^--N but did occur as high as 19.5 ppm. On the other hand nitrate levels were high, ranging from 1 to 660 ppm NO_3^--N with a mean of 223 ppm. Samples of water coming into the cave were always low in nitrates having less than 5 ppm, while soil samples above the cave were always less than 25 ppm NO_3^--N. Moisture content of the sediment samples was low, except for samples taken where water was actively moving into the cave such as at Side Saddle Pit and Richardson's Spring. Sediment moisture levels ranged from 1.1 to 28.6% with a mean of 8.2%. The highest moisture levels occurred in the deepest part of the cave nearest the ground water, while lower moisture levels were generally observed in the upper passages.

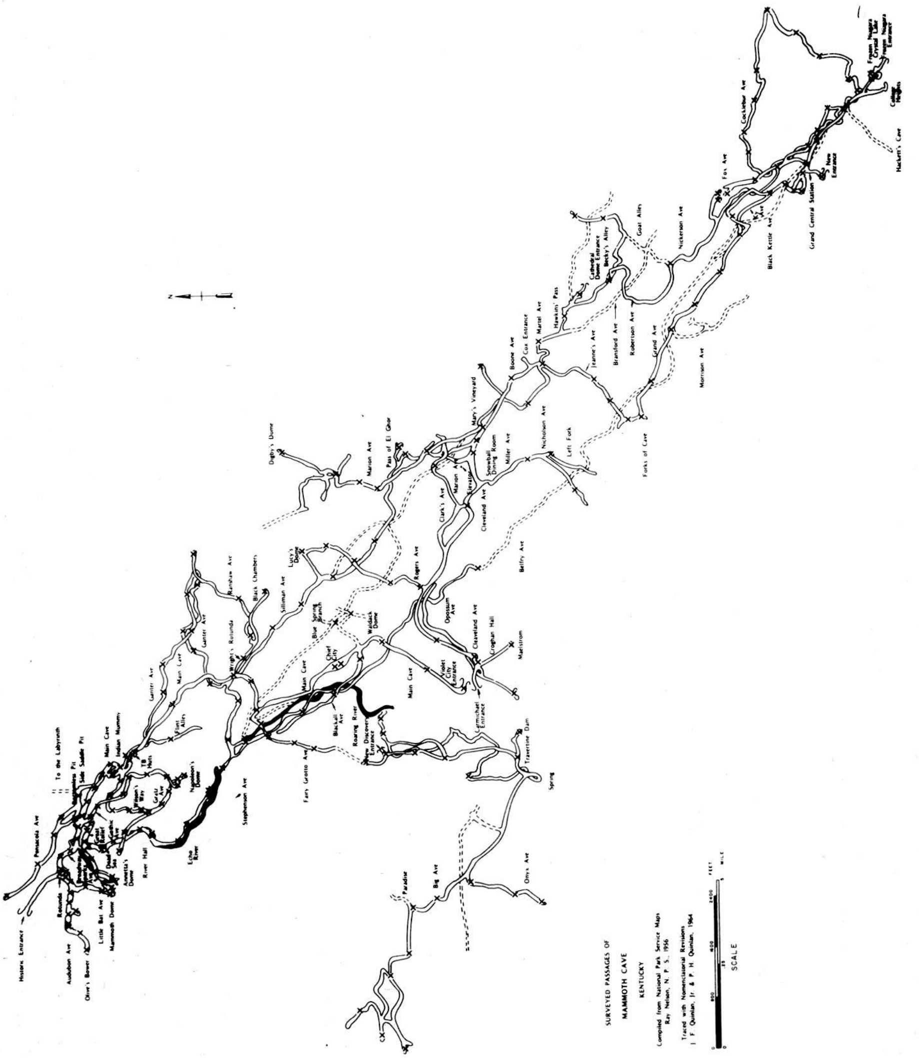


Fig. 1. Samples taken from areas designated by an "x" within passageways of Mammoth Cave. Only general sample locations are marked.

Table 1. Specific chemical and physical parameters of samples taken from a variety of habitats in Mammoth Cave.

Sample	Description	pH	% Moisture	NO_2^-	NO_3^-
114-1	Surface sample from 1st saltpetre vats in Rotunda	7.40	7.64	19.5	80
114-2	Subsurface sample (5 cm); 1st saltpetre vats in Rotunda	7.43	7.73	3.5	260
114-3	Subsurface sample (20 cm); 1st saltpetre bed gray powder material	7.89	5.51	<0.2	1
114-4	Adjacent 1st saltpetre vat in Rotunda; surface sample	7.08	N.D.	<0.2	190
114-5	Fine silt from 2nd saltpetre vat in Rotunda; from leaching trough	7.52	5.26	<0.2	390
115-1	Scrapings from collecting trough 2nd saltpetre bed; Rotunda	7.99	N.D.	17.5	115
115-2	Silt from final holding tank; Rotunda	7.60	N.D.	<0.2	275
115-3	Base of wall beyond Rotunda	7.14	N.D.	<0.2	275
115-4	Scrapings off wall 2M above 115-3	6.88	6.90	<0.2	420
115-5	East of Rotunda; surface sample 3M off trail near “Old Trail”	5.95	6.65	<0.2	410
115-6	East of Rotunda; base of wall near “Old Trail”	7.86	7.73	<0.2	470
115-7	Scrapings from wall above “Methodist Church”	7.42	9.05	<0.2	490
116-1	Silt from top of “Pulpit Rock”	7.86	N.D.	<0.2	440
116-2	Sample across from 2nd set of leaching vats	7.37	10.3	<0.2	455
116-3	Sample from reddish bank across from 2nd set of saltpetre vats	7.52	8.31	<0.2	660
116-4	Surface sample between 2nd set of leaching vats	7.42	N.D.	<0.2	140
116-5	Final leaching troughs; second set of leaching vats	7.63	N.D.	15.5	320
116-6	Scrapings from wall at “Boones Rock”	7.14	N.D.	<0.2	500
116-7	Sample below vats at “Booth’s Amphitheatre”	7.46	5.31	4	245
117-1	Sample behind last leaching vat at “Booth’s Amphitheatre”	6.54	N.D.	<0.2	455
117-2	Scrapings from side of ledge across from bleachers at “Gothic Avenue”	7.45	12.4	<0.2	510

117-3	Sample behind bleachers at "Gothic Avenue"	7.19	4.45	<0.2	140
117-4	Beneath ledge close to opening into main part of the cave at "Gothic Avenue"	7.28	N.D.	<0.2	142
117-5	Sample from floor of "Gothic Avenue"	7.52	7.61	<0.2	490
117-7	"Standing Rock," often used for excretory purposes	7.53	7.83	<0.2	455
117-8	Sample adjacent to old cart near "Standing Rock"	6.90	2.85	<0.2	120
117-9	Sample from under ledge at "Acute Angle"	6.79	7.65	<0.2	450
118-2	Sample from ledge inside "Acute Angle"	8.05	N.D.	<0.2	150
118-3	Sample near ceiling 1 m from gate at "Acute Angle"	8.15	N.D.	<0.2	290
118-4	Sample from floor at base of gate at "Acute Angle"	7.98	N.D.	<0.2	215
118-6	Sample from baseboard around the 1st "T. B. Hut"	7.67	7.81	<0.2	455
118-8	Sample from ledge at "Star Chamber"	8.12	11.6	<0.2	450
118-9	Sample between first two "T. B. Huts"	8.27	N.D.	2	425
119-2	Sample near "hoe marks" in "Cyclops Avenue"	7.27	4.18	<0.2	50
119-3	Sample from ledge in "Cyclops Avenue"	7.78	7.91	<0.2	160
119-4	Sample 1 m above 119-3	7.23	6.00	<0.2	200
119-5	Ceiling scrapings from "Backslider"	8.35	N.D.	<0.2	14
119-6	Sample from sediment wall in "Backslider" (0-2 mm)	7.85	15.9	<0.2	38
119-7	Same as 119-6 (2 mm-50 mm)	7.09	12.0	<0.2	14
120-1	Sample from floor of "Backslider" near 119-6	7.64	11.6	<0.2	20
120-2	Sample from ceiling at "Backslider"	8.99	7.29	<0.2	80
121-1	Sample from ledge in back passageway of "Wooden Bowl Room"	7.28	N.D.	<0.2	120
121-2	Same area as 121-1	7.51	4.04	<0.2	145
121-3	Sample from above plaque "Wooden Bowl Room"	7.91	6.52	<0.2	120
121-4	Sample on side wall of "Wooden Bowl Room"	7.38	6.34	<0.2	285
121-5	Sample near entrance to "Wooden Bowl Room"	7.40	11.2	<0.2	420
121-6	Sample 50 m beyond stairway below "Wooden Bowl Room"	8.54	4.26	<0.2	30
121-9	Water sample from "Richardson's Spring"	7.95	—	<0.2	1.5
121-10	Sample near "Richardson's Spring"	8.34	28.10	<0.2	26
122-1	Sample of reddish deposit near 2 fluorescent lights near "Blind Fish Aquarium"	7.78	10.4	<0.2	16

Sample	Description	pH	% Moisture	NO_2^-	NO_3^-
122-2	Sample on ledge near “Blind Fish Aquarium”	7.70	12.6	<0.2	100
122-4	Water sample from drippings at “Sidesaddle Pit”	7.50	—	<0.2	2.0
122-5	Sample across from “Sidesaddle Pit”	7.74	28.6	<0.2	4
122-6	Sample across dome cavity at “Sidesaddle Pit”	7.95	27.8	<0.2	5
122-8	Sample near floor at “College Heights Avenue”	7.05	18.6	<0.2	6
123-1	Same as 122-8; reddish clay	7.88	24.8	<0.2	70
123-2	Same area as 122-8; powdery sample	8.07	9.6	<0.2	10
123-3	Sample under ledge in “College Heights Avenue”	8.13	1.43	<0.2	10
123-4	Same as 123-3; much limestone	7.72	4.14	<0.2	13
123-5	Sample at “Flat Ceiling” behind fluorescent light	7.71	50.02	<0.2	220
123-6	Sample by rail at “Lover’s Leap Canyon”	7.83	N.D.	<0.2	240
123-7	Sample below “Lover’s Leap Canyon Trail”	8.20	N.D.	<0.2	90
123-8	Sample between “Flat Ceiling” and “Fairy Ceiling”	7.65	N.D.	<0.2	12.5
123-9	Sample from ledge 100 m beyond “Star Chamber”	7.08	10.8	<0.2	460
124-1	Sample 100 m from 123-9	8.07	4.76	<0.2	500
124-2	Sample 100 m from 124-1	7.80	N.D.	2	120
124-3	Same area as 124-2; gravel sample	8.81	N.D.	<0.2	93
124-4	Sample 100 m from 124-3	7.64	N.D.	<0.2	290
124-5	Sample 100 m from 124-4	8.13	5.37	<0.2	250
124-6	Same area as 124-5; other side of trail	8.16	N.D.	<0.2	10
124-7	Sample 100 m beyond 124-6	7.89	2.85	<0.2	310
124-8	Sample 100 m beyond 124-7	7.62	2.38	<0.2	405
125-1	Sample 100 m beyond 124-8	7.76	7.86	<0.2	430
125-2	Sample 100 m beyond 124-9	7.90	N.D.	<0.2	180
125-3	Sample near “Cataract Falls”	7.53	3.93	<0.2	330
125-4	Sample beyond waterfall at “Cataract Falls”	7.65	3.75	<0.2	410
125-5	Sample 100 m beyond 125-4	7.40	4.18	<0.2	300
125-6	Sample 100 m beyond 125-5	7.09	7.28	<0.2	356
125-7	Sample 100 m beyond 125-6	7.52	8.19	<0.2	450
125-8	Same area as 125-7	7.91	4.46	<0.2	410

126-1	Sample 30 m beyond 125-8	7.71	8.16	<0.2	440
126-2	Sample 150 m beyond 126-1; at "Chief City"	7.55	3.32	<0.2	460
126-3	Sample 100 m beyond 126-2	7.83	2.48	<0.2	410
126-5	Sample 100 m beyond 126-3	7.71	5.11	2	460
126-6	Sample above "Hains Dowe"	6.71	9.68	<0.2	290
126-7	Sample 100 m beyond 126-6	7.52	4.13	<0.2	440
126-8	Sample 100 m beyond 126-7	7.45	4.76	<0.2	150
126-9	Sample 100 m beyond 126-8; rocky sandy sample	7.70	1.60	<0.2	405
127-1	Sample 100 m beyond 126-9	8.05	5.00	<0.2	350
127-2	Sample 100 m beyond 127-1	7.13	9.82	<0.2	210
127-3	Sample 100 m beyond 127-2	7.81	7.0	<0.2	50
127-4	Sample 100 m beyond 127-3; base of wall	8.03	7.93	<0.2	10
127-5	Same area as 127-4	8.88	7.63	<0.2	115
128-1	Surface soil sample from "Backslider"	7.71	N.D.	<0.2	140
129-1	Surface soil sample from "Backslider"	7.09	N.D.	<0.2	27
130-1	Surface soil sample from "Backslider"	7.61	N.D.	<0.2	32
131-1	Surface soil sample from "Backslider"	7.90	N.D.	<0.2	260
132-1	Surface soil sample from "Backslider"	7.74	N.D.	<0.2	21
133-1	Sample from wall profile (0-5 cm) at "Backslider"; heavy clay	7.72	14.9	<0.2	40
133-2	Same as 133-1; 5-10 cm; heavy clay	7.61	16.7	<0.2	29
133-3	Same as 133-1; 10-15 cm; heavy clay	7.76	16.1	2	120
134-1	Same as 133-1; 20-25 cm; sandy	7.68	5.71	<0.2	19
135-1	Same as 133-1; 25-30 cm, base of profile; sandy	6.95	1.13	<0.2	28

(1) N.D. = Not Determined

Average NO_3^-: 222.8 ppm
range: 1 to 660 ppm
NO_2^-: range: <0.2 to 19.5 ppm
Average moisture: 8.21%
range: 1.13 to 28.6%
Average pH: 7.94
range: 5.95 to 8.99

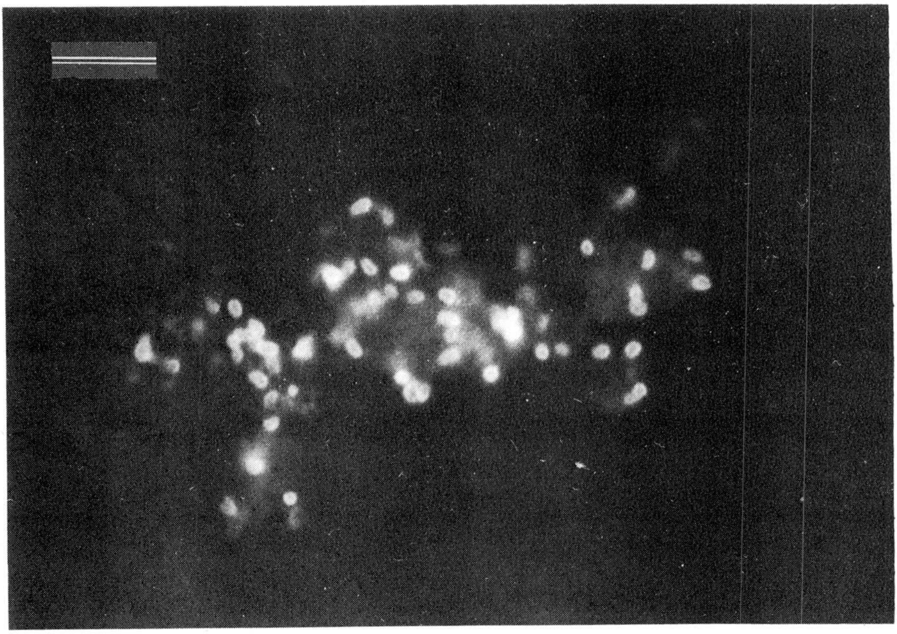


Fig. 2. *Nitrobacter* in Mammoth Cave sediments as stained by species specific fluorescent antibodies. Scale bar is 5 nm.

Since the fluorescent antibodies were species specific, the distribution of *N. agilis* and *N. winogradskyi* in the Mammoth Cave ecosystem were determined. The staining characteristics of *Nitrobacter* in Mammoth Cave sediments are shown in Figure 2. This black and white photomicrograph shows *Nitrobacter* as white cells, while in color photographs the cells would appear yellowish-green. The data in Table II indicate that 85% of the *Nitrobacter* population in Mammoth Cave was *N. agilis*. On the other hand, pure culture isolates obtained from a variety of agricultural soils were always *N. winogradskyi,* while only *N. agilis* was isolated from Mammoth Cave sediments (Table III).

The data summarizing nitrate concentrations and moisture content of the cave sediment samples are plotted with respect to *Nitrobacter* population densities in Figures 3 and 4, respectively. These data indicate that no strong correlation exists between the populations of *Nitrobacter* and either nitrate concentrations or sediment moisture. *Nitrobacter* densities in the cave sediments averaged 6.2 x 10^5 cells per gram of sediment, while soil samples taken above Mammoth Cave under a forest canopy had less than 10^3 *Nitrobacter* per gram of soil (Fliermans, unpublished data).

In order to determine if the presence of *Nitrobacter,* as observed in Mam-

Table 2. Population densities and species composition of chemoautotrophic nitrifiers in Mammoth Cave sediments.

Sample	Total *Nitrobacter* per gram sediment	#/Field	
		N. agilis	*N. winogradskyi*
115-2	2.3 x 10^4	16	4
118-3	6.1 x 10^4	37	7
127-2	2.2 x 10^3	18	0
133-1	1.8 x 10^4	19	4
123-1	4.4 x 10^4	35	4
117-4	1.8 x 10^4	11	4
125-8	7.5 x 10^4	63	10
125-7	5.0 x 10^4	22	20
121-9	4.2 x 10^4	24	13
126-3	3.1 x 10^4	13	14
123-5	2.5 x 10^4	15	8
132-1	2.5 x 10^4	14	3
114-2	4.1 x 10^4	17	17
122-2	1.7 x 10^4	9	4
124-7	2.2 x 10^4	8	6
123-3	1.2 x 10^4	6	3
118-8	1.2 x 10^4	17	2
117-2	1.2 x 10^4	6	4
126-7	1.5 x 10^4	10	3
124-6	6.7 x 10^5	300	39
125-1	7.4 x 10^6	48	2
128-1	9.1 x 10^5	60	15
122-6	5.3 x 10^4	290	15
127-4	1.3 x 10^4	2	10
130-1	5.2 x 10^4	32	1
121-3	1.9 x 10^4	6	10
123-7	2.1 x 10^4	14	2
126-5	8.5 x 10^6	67	0.3
115-6	3.6 x 10^5	27	0.2
120-2	5.4 x 10^4	25	2
Average	6.2 x 10^5	41.0	7.55
	% of Total *Nitrobacter*	84.5	15.5

Table 3. Immunofluorescence specificity test with chemoautotrophic nitrifiers isolated from various habitats.

Culture	Source	*Immunofluorescence Reaction* *N. agilis*-FA	 *N. winogradskyi*-FA
Nitrobacter			
Bearden 1	Minnesota Soil	Neg	3+
Bearden 2	Minnesota Soil	±	4+
Glencoe 1	Minnesota Soil	Neg	3+
Glencoe 2	Minnesota Soil	Neg	3+
Tara 1	Minnesota Soil	Neg	3+
Tara 2	Minnesota Soil	Neg	3+
F-A	Moroccan Soil	Neg	3+
F-B	Moroccan Soil	Neg	4+
Iceland 1	Iceland Soil	Neg	4+
133-2	Mammoth Cave, Ky.	3+	Neg
128-1	Mammoth Cave, Ky.	4+	Neg
125-8	Mammoth Cave, Ky.	4+	Neg
115-4	Mammoth Cave, Ky.	4+	Neg
123-1	Mammoth Cave, Ky.	3-4+	Neg
130-1	Mammoth Cave, Ky.	4+	Neg
122-6	Mammoth Cave, Ky.	3+	Neg

Absorbed *Nitrobacter* fluorescent antibodies were tested with pure cultures of autotrophic nitrifiers from diverse environments.

moth Cave was a widespread phenomenon in other saltpetre caves, samples were taken from 23 known saltpetre caves primarily in the southeastern United States. As shown in Table IV all but two of the caves had *Nitrobacter* present in sediment samples, as detected by immunofluorescence.

Leaching studies indicated that *Nitrobacter* populations in the cave sediments remained stable during the leaching process as compared to the change in the total bacterial population (Table V). Sediment samples from thirty different sites within Mammoth Cave were composited into a single sample and homogeneously mixed. Hydrometrical texture analyses of the pooled sample indicated that the mixture was 64% sand, 19.8% silt and 16.2% clay. The composite sample was then placed in a chromatographic column and continuously leached until free of nitrates and nitrites, using 400 ml of filter sterilized distilled water. The effluent was aseptically collected in 50 ml aliquots and the population densities of *Nitrobacter* and total bacteria were determined by direct microscopy (Fliermans and Schmidt, 1975). Before leaching, the total bacterial population, as measured directly with FITC staining, was 7.2×10^6 bacteria/g of sediment, and decreased by 57% to 4.1×10^6/g of sediment after 400 ml of filter sterilized distilled water had been passed through the sediment column. On the other hand, *Nitrobacter* populations, as measured by immuno-

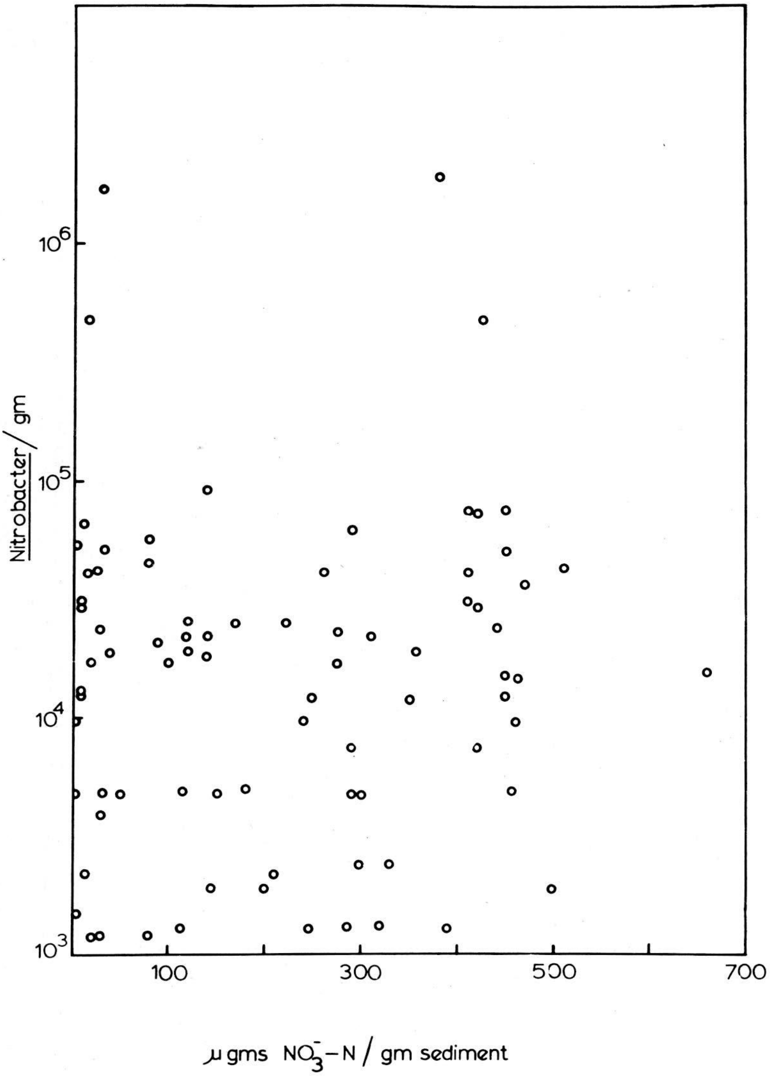


Fig. 3. Relationship between the number of *Nitrobacter* spp. per gram of cave sediments and the nitrate concentrations in the sediments.

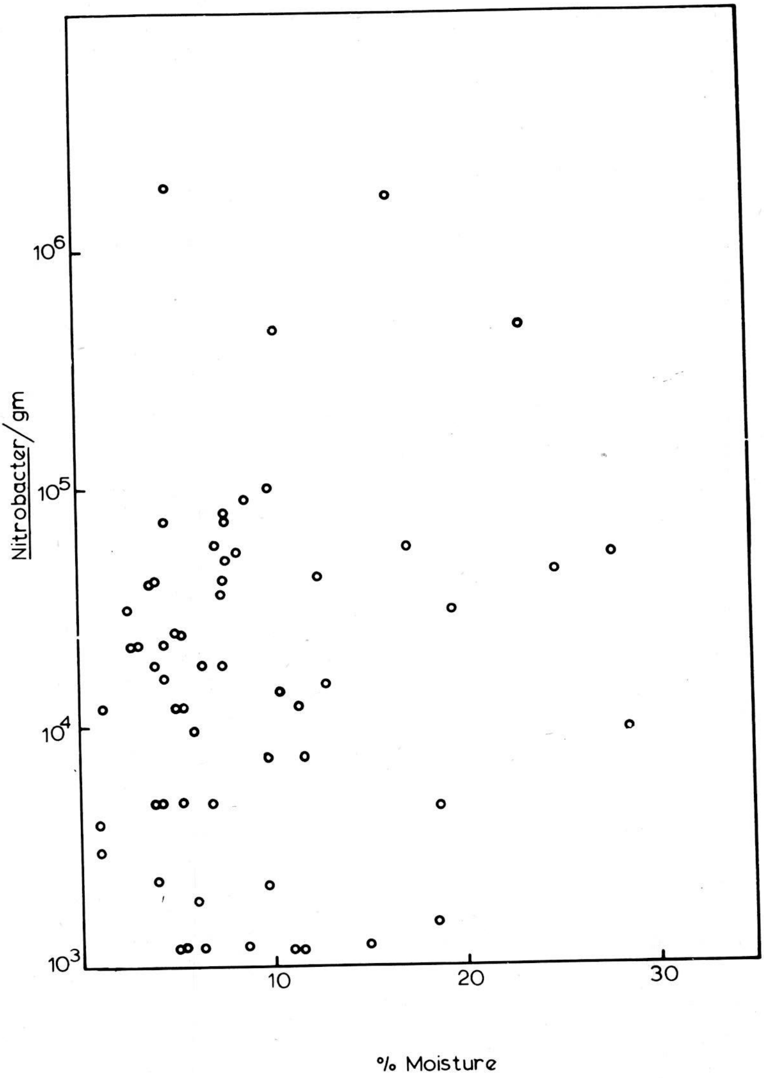


Fig. 4. Relationship between the number of *Nitrobacter* spp. per gram of cave sediments and the percent moisture in the sediments.

Table 4. Presence of *Nitrobacter* spp. in saltpetre caves as determined by immunofluorescence.

Cave	Location	*Nitrobacter*
Dan Boone Hut Cave	Bath Co., Ky.	—
Breathing Cave	Bath Co., Va.	+
Minor Saltpetre Cave	Lee Co., Va.	+
Perry Saltpetre Cave	Boutertate Co., Va.	+
Lawson Saltpetre Cave	Scott Co., Va.	+
Big Boone Cave	Van Buren Co., Tenn.	+
Petre Cave	Polaski Co., Ky	+
Crawford Cave	Randolph Co., W. Va.	+
Ellison's Cave	Walker Co., Ga.	+
Faust Saltpetre Cave	Wise Co., Va.	+
John Rogers Cave	Jackson Co., Ky.	+
Wind Cave	Wayne Co., Ky.	+
John Friends Saltpetre Cave	Garrett Co., Md.	+
Me Bane Saltpetre Cave	Pulaski Co., Va.	+
Saltpetre Cave	Buffalo River St. Park, Ark.	+
Greenville Saltpetre Cave	Logan Co., W. Va.	+
Madison Cave	Madison Co., Va.	—
Cave Mountain Cave	Grant Co., W. Va.	+
Henshaw's Cave	Warren Co., Tenn.	+
Carter Caves	Carter Co., Ky.	+
Dyers' Cave	Hardy Co., W. Va.	+
Saltpetre Cave	Mineral Co., W. Va.	+
Lobelia Saltpetre Cave	Greenbriar Co., W. Va.	+

fluorescence, were initially 4.8 x 10^4/g of sediment and showed no significant change to 5.2 x 10^4/g of sediment after leaching.

DISCUSSION

Although Mammoth Cave is a national park, it provides a unique speleological ecosystem for microbiological studies, since the touristic impact is restricted to about 10% of the known cave passages. Such an ecosystem is unique in that weathering occurs at a reduced rate since natural elements of rain, wind, sunlight, erosion, freezing and thawing are removed from the habitat. Air temperature in the deeper parts of the cave is relatively stable, fluctuating between 13.2 and 14.0° C with a mean of 13.6° C, while the relative himidity rarely drops below 80% and is generally between 95 and 100% (Barr and Kuehne, 1971). Light penetration into the cave is negligible and only where artificial lighting provides a source of energy do photosynthetic organisms occur. These organ-

Table 5. Effect of leaching on the removal of *Nitrobacter* spp. and other bacteria from Mammoth Cave sediments.

Volume Leached (ml)	Microorganisms/Microscope Field Total Bacteria[a]	 *Nitrobacter* spp.[b]
50	33.0	0.02
100	4.2	0.05
150	5.0	0.04
200	6.4	0.06
250	7.7	0.06
350	8.5	0.02
400	6.3	0.06
	Microorganisms/gm sediment	
Before Leaching	7.2×10^6	4.8×10^4
After Leaching	4.1×10^6	5.2×10^4

[a] Calculations based on 10 microscope fields.
[b] Calculations based on 50 microscope fields.

isms are primarily heterocystic filamentous bluegreen algae and diatoms (Fliermans, unpublished data). Moisture content of the cave sediments varied substantially from one site to the next within the cave. Mammoth Cave has five different passage levels with the lowest one being in contact with the underground Echo River. In general, moisture levels are highest in the lower region of the cave, although the majority to sediments contained less than 10% moisture. Isolated packets of high sediment moisture was apparent where seepage from natural springs arose.

The classical approach for the mining of saltpetre relied on the observation of a variety of physical phenomena within the cave. Some of these observations are consistent with the growth conditions required by the chemautotrophic nitrifier, *Nitrobacter*. Faust (1967) described the following ecological phenomena which were generally observed in saltpetre formations:

1. Caves must contain alkaline sediments with a stable year-round temperature of 11-14° C.
2. Free flowing air circulation must occur and running water or flood waters must not reach the saltpetre deposits.
3. Saltpetre sediments disturbed by running a sharp object through them became smooth in 2 to 5 days.
4. Sediments which were leached free of nitrates and subsequently returned to the cave ecosystem would regenerate comparable levels of nitrate in 3 to 5 years.
5. Saltpetre deposits are generally found in areas low in organic matter.

Nitrobacter spp. have a pH range of 6.5 to 8.5 with an optimum for growth between 7.5 and 8.0 (Watson, 1975), thus the slightly alkaline conditions of Mammoth Cave sediments are close to the pH optimum required for *Nitrobacter* growth. Pure cultures of the nitrifiers are optimally adapted to a temperature near 25 to 30° C with a range from 5 to 40° C. Therefore, the mean cave temperature of 13.6° C for Mammoth Cave may not be optimal for *Nitrobacter* growth unless these bacteria are adapted to a different temperature optima *in situ.* Additionally, saltpetre deposits are found where air circulation occurs and water drainage is absent. Since the nitrifying bacteria are strict aerobes, they require oxygen as a terminal electron acceptor, and thus air circulation may help maintain the necessary aerobic conditions. The prevention of high water levels in the caves facilitates the formation of saltpetre deposits, since either seepage or flooding conditions promote leaching of the soluble nitrate ions from the cave sediments. In addition, saturated conditions produce anaerobic environments which prevent the growth of the chemoautotrophic nitrifiers.

The phenomenon of disturbing the sediments with a sharp object and having the sediments return to a smooth surface cannot be explained microbiologically. Since these sediments are at a low moisture content any disruption may result in a moisture equilibration with the high relative himidity of the cave and thus the saltpetre deposits swell due to water of hydration and cause a smoothing of the disturbed sediments.

The process of nitrate regeneration is interesting, since historically saltpetre sediments were often leached free of nitrates, returned to the cave ecosystem and a regeneration of saltpetre to initial nitrate concentrations occurred in 3 to 5 years. Laboratory leaching experiments with 300 g of Mammoth Cave sediments indicated that the nitrates were easily removed from the sediments but the nitrifying bacteria were not. Total bacterial populations before leaching were 7.2 x 10^6/g of sediment measured by direct FITC staining and decreased by 57% after 400 ml of distilled water had been leached through the sediments. On the other hand, *Nitrobacter* populations, as measured by immunofluorescence were 4.8 x 10^4/g of sediment and showed no significant change to 5.2 x 10^4/g. Thus, it appears that leaching of the sediments selectively maintains the *Nitrobacter* populations while removing some of the other bacteria. Likewise, leaching of the sediments appeared to promote the oxidation of nitrite to nitrate in that much higher levels of nitrite were oxidized after leaching than before (Fliermans, unpublished data). Such an increase in nitrite oxidation may result from the removal of nitrate which serves an end product inhibitor for *Nitrobacter* spp.

In order for nitrification to occur and deposits of saltpetre to form, a supply of inorganic nitrogen must be available. Since the nitrifiers in Mammoth Cave are chemoautotrophs, their metabolic activity is not affected directly by the concentration of organic matter. However, preliminary micro-kjeldahl studied indicated that these cave sediments were very low in organic matter (Fliermans, unpublished data), which is probably due to the lack of photosynthesis and thus the deposition of plant debris and humus material in cave ecosystems. Mammoth Cave is an old geological structure and the bacteriological events observed in the cave are a result, in part, of this long period of time. The low levels of total organic matter (0.02 to 0.04%) may be the result of a continuous but very slow decomposition process. Many saltpetre caves have had at one time large populations of bats living in the cave which may have been a supply of organic material. It is possible that these guano deposits were eventually decomposed through deaminization and/or ammonification and NH_4^+ released, which in turn was used as substrate for the nitrifiers. The process of saltpetre formation may be near termination in that very little organic matter is now being deposited naturally in Mammoth Cave due to the absence of extensive bat populations.

Although the stochiometry of nitrogen transfer through the various components of the cave ecosystem remains unknown, the detection of a specific group of chemoautotrophic nitrifying bacteria, *Nitrobacter,* has been shown in saltpetre cave sediments. The population densities present in Mammoth Cave may be sufficient to account for the levels of saltpetre found in the sediments. Caverns such as Mammoth Cave, with stable parameters of temperature, pH, light, moisture and organic nutrients, may provide or at one time provided unique habitats for the chemoautotrophic nitrifiers.

ACKNOWLEDGMENTS

Information contained in this article was developed during the course of work under grant 1,245,00 from the National Geographic Society and grant GB 29636A1 from the National Science Foundation.

We thank C. Hill for valuable discussions and L. McKenzie of the National Park Service for guidance in the caves. Our thanks to the Cave Research Foundation and R. A. Fliermans for help in locating and obtaining samples from many of the saltpetre caves.

SUMMARY

Mammoth Cave, a large limestone cavern in Mammoth Cave National Park in the Central Kentucky karst, was first mined for saltpetre in 1808 and was a major source of nitrates used in the manufacture of gunpowder during the War of 1812. The mechanism of saltpetre formation is unknown, although hypotheses encompassing both biotic and abiotic functions have been suggested.

Present studies were conducted in various saltpetre caves using species specific fluorescent antibodies in order to determine if the chemoautotroph, *Nitrobacter,* were present. Population densities and species distribution of *Nitrobacter* were studied in relation to chemical and physical parameters for over 200 sediment samples from Mammoth Cave. Both the isolation and immunofluorescence data indicate that *Nitrobacter* are present in relatively high population densities in Mammoth Cave sediments, and that such bacteria are common among saltpetre caves in the southeastern United States. Immunofluorescence data further indicates that *N. agilis* dominates the *Nitrobacter* population in Mammoth Cave. The possibility that *Nitrobacter* is the etiological agent for saltpetre formation is suggested.

RÉSUMÉ

"Mammoth cave", une vaste caverne calcaire du parc national de Mammoth cave dans le karst du Kentucky central, a d'abord été exploitée pour le salpêtre en 1808; elle a été la principale source de nitrate utilisé dans la fabrication de la poudre pendant la guerre de 1812. Le mécanisme de la formation du salpêtre est inconnu, quoique des hypothèses comportant à la fois des arguments biotiques et abiotiques aient été suggérées.

Les présentes recherches ont été conduites dans diverses grottes à salpêtre, en utilisant des anticorps fluorescents spécifiques, afin de déterminer si le chimioautotrophe *Nitrobacter* était présent. La densité de population et la distribution du genre *Nitrobacter* ont été étudiées, en rapport avec des paramètres physique et chimique, sur plus de 200 échantillons de sédiments de "Mammoth cave". Les données établies par isolement et fluorescence indiquent que Nitrobacter est représenté par une densité de population relativement élevée dans les sédiments de "Mammoth cave" et qu'une telle bactérie est commune dans le salpêtre des cavernes du Sud-Est des Etats-Unis. Les résultats de l'immunofluorescence indiquent de plus que *Nitrobacter agilis* domine parmi la population de *Nitrobacter* de "Mammoth cave". La possibilité que Nitrobacter soit l'agent étiologique de la formation du salpêtre est suggérée.

REFERENCES

BARR, Thomas C. Jr., 1971. Ecological studies in the Mammoth Cave System of Kentucky. II. the ecosystem, Ann. Spéléol. 26: 47-96.

BOHLOOL, B. B. and E. L. SCHMIDT. 1968. Nonspecific Staining: Its Control in Immunofluorescence Examination of Soil. Science 162: 1012-1014.

BROWN, S. 1809. A Description of a Cave on Crooked Creek with Remarks and Observations on Nitre and Gun-Powder. Trans. Am. Phil. Soc. 6: 235.

CAUMARTIN, V. 1963. Review of the Microbiology of Underground Environments. American Caver 25: 1-14.

CLARK, C. and E. L. SCHMIDT. 1967. Growth Response of *Nitrosomonas europaea* to Amino Acids. J. Bact. 93: 1302-1308.

CLARKE, W. 1924. Nitrates. U. S. Geological Survey Bull. 770: 254-259.

FAUST, Burton. 1967. Saltpetre Mining in Mammoth Cave, Kentucky. Jr. Spelean History, 1: 3-9.

FLIERMANS, C. B., B. B. BOHLOOL and E. L. SCHMIDT. 1974. Autecological Study of the Chemoautotroph *Nitrobacter* by Immunofluorescence. Appl. Microbiol. 27: 124-129.

FLIERMANS, C. B. and E. L. SCHMIDT. 1975. Fluorescence Microscopy: Direct Detection, enumeration and Spatial Distribution of Bacteria in Aquatic Systems. Arch. Hydrobiol. 76: 33-42.

GOLDMAN, M. and R. K. CARVER, 1961. Microfluorimetry of Cells Stained with Fluorescent Antibody. Expt. Cell Res. 23: 265-280.

GOUNOT, A. M. 1967. La Microflore des limons agrileux souterrains: Son activité productrice dans la biocenose cavernicole. Ann. Spéléol. 22: 23-143.

HESS, W. H. 1900. The Origin of Nitrates in Cavern Earths. J. Geol. 8: 129-134.

PRAMER, D. and E. L. SCHMIDT. 1964. Experimental Soil Microbiology. Burgess Publishing Co., Minneapolis, Minn.

PRIESTLEY, J. 1809. Observations on the Discovery of Nitre in Common Salt, Which Had Been Frequently Mixed with Snow. Trans. Am. Phil. Soc. 6: 129.

SCHMIDT, E. L. 1973. Fluorescent Antibody Techniques for the Study of Microbial Ecology. *In:* T. Rosswal (ed.) Modern Methods in the Study of Microbial Ecology. Bull. Ecol. Res. Comm. (Stockholm) 17: 67-76.

SHINN, M. B. 1941. Test for Nitrate Nitrogen. Industrial and Engineering Chemistry, Analytical Edition 13: 33-35.

STRICKLAND, J. D. H. and T. R. PARSONS. 1968. A Practical Handbook of Seawater Analysis. Fish. Res. Bd. Can., Bull. 167.

WATSON, S. W., 1974. Gram-negative chemolithotrophic bacteria. Family I. Nitrobacteraceae. *In:* Bergey's manual of determinative bacteriology (William and Wilkins). 450-456.

WIEBE, W. J. 1971. Perspectives in microbial ecology. *In:* E. P. Odum, fundamentals of Ecology (W. B. Saunders). 484-497.

Int. J. Speleol. 9 (1977), pp. 21-41.

Recherches sur les Ostracodes Entocytheridae. Données sur *Sphaeromicola cebennica juberthiei* nov. ssp. et *Sphaeromicola cirolanae* Rioja

par

Dan. L. DANIELOPOL*

Investigations on the Entocytheridae Ostracods. Data on Sphaeromicola cebennica juberthiei nov. ssp. and Sphaeromicola cirolanae Rioja.

SUMMARY

Sphaeromicola cebennica juberthiei n. ssp., a cavernicolous ostracod found in the South of France (Système de Cent Fons and Avencas, Herault), is described here. The new subspecies differs from *Sph. cebennica cebennica* (found in a cave from Ardèche) by the smaller carapace and a reduced distal bristle on the "finger" of the male copulatory organ. The affinities and differences between the European *Sphaeromicola* and *Sph. cirolanae* from Mexico are discussed. The genus *Sphaeromicola* is divided into two groups of species: the group *topsenti* and the group *cirolanae*. Some observations on the mating process of *Sph. cebennica juberthiei* are presented. A precocious sexual behaviour of the female in the last post-embryonic instar is recorded. This type of behaviour seems to be a characteristic of the Entocytheridae.

La famille des *Entocytheridae* comprend actuellement quatre sous-familles, contenant tous les Ostracodes commensaux des Crustacés Malacostracés: les *Entocytherinae* peuplant les eaux douces de l'Amérique du Nord et de l'Amérique Centrale (Hart, 1962, Hart et Hart, 1969, Hobbs Jr., 1971), les *Sphaeromicolinae* répandus dans les eaux souterraines continentales du Sud de l'Europe (Remy, 1948a) et au Mexique (Rioja, 1951 et Hobbs Jr., 1971)** (une seule espèce appartenant à cette sous-famille est épigée, vivant dans la Méditerrannée et dans l'Océan Atlantique (Danielopol, 1971b)), les *Notocytherinae* vivant dans les eaux douces épigées d'Australie, de la Tasmanie, de la Nouvelle-Zélande et de la Nouvelle-Guinée (Hart et Hart, 1969 et 1970), enfin la sous-famille des *Microsyssitrinae* représentée par une seule espèce trouvée dans

---

* Limnologisches Institut, Österreichische Akademie der Wissenschaften, Berggasse 18, A-1090 Wien, Austria.

** Chappuis (1953) et Delamare-Deboutteville (1960) ont présenté la répartition géographique des *Sphaeromicola* européennes.

l'Océan Indien (Hart, Nair et Hart, 1967). La large distribution géographique des Entocytheridae (fig. 1) témoigne de l'ancienneté du ce groupe.

Depuis la description détaillée de *Sphaeromicola topsenti* l'espèce type du genre *Sphaeromicola* (Paris, 1920), une dizaine de mémoires sont parus, contenant des données morphologiques et biogéographiques sur les *Sphaeromicolinae,* connues actuellement par sept espèces (cf. bibliographie). Hubault (1938) et Remy (1948b) ont considéré que les Sphaeromicolinés connues alors (cinq espèces troglobies d'Europe et une espèce épigée marine) représentaient un groupe homogène représenté par un seul genre, *Sphaeromicola.* A cette opinion s'est rallié, plus tard, aussi Rioja, 1951. En étudiant cinq espèces de Sphaeromicolinés i.e. *Sph. topsenti, Sph. stammeri, Sph. cebennica, Sph. cirolanae,* espèces troglobies et *Sph. dudichi,* espèce epigée, j'ai constaté que cette dernière était du point de vue morphologique tellement différente des autres *Sphaeromicola* que j'ai dû la considérer comme appartenant à un genre nouveau *Hartiella,* type d'une lignée phylétique que j'ai nommée tribu des *Hartiellini* (Danielopol, 1971)*. Les *Sphaeromicola* troglobies restent placées dans la tribu des *Sphaeromicolini.* Dans le présent travail je décris une nouvelle sous-espèce de *Sphaeromicola cebennica* et examine les affinités et les différences entre les *Sphaeromicola* troglobies d'Europe et d'Amérique Centrale. Ces données permettrons de cerner de plus près la question de l'homogénéité phylétique du genre *Sphaeromicola,* car comme le remarquait E. G. Racovitza il y a 50 ans "suivre pas à pas, stade par stade, dans l'espace et dans le temps, les modifications d'un groupe homogène semble à priori être la meilleure méthode pour constater les modalités de l'évolution et en même temps le meilleur moyen de bien définir et délimiter des problèmes restreints, et relativement simples, par lesquels devrait commencer l'étude de la très complexe et très difficile question des causes immédiates de cette évolution". La dernière partie du travail présente quelques observations sur le processus d'accouplement chez les Entocythéridés.

I. SUR UNE NOUVELLE SOUS-ESPÈCE DE SPHAEROMICOLA CEBENNICA DU KARST NORD-MONTPÉLLIERAIN

Lors d'un court séjour au Laboratoire Souterrain du C.N.R.S. à Moulis, en 1970, Monsieur C. Juberthie a eu l'amabilité de me confier l'étude de quelques exemplaires de *Sph. cebennica* recueillis sur des *Sphaeromides raymondi,* Isopodes trouvés dans le système karstique de Cent Fonds dans la Vallée de l'Hérault (voir à ce sujet Rouch, Juberthie-Jupeau et Juberthie, 1968). D'autre part Mme Juberthie-Jupeau a mis a ma disposition quelques exemplaires de *Sphaeromides raymondi* en provenance de la résurgence de l'Avencas, située elle aussi toujours dans la vallée de l'Hérault à cinq kilomètres du système de

---

* D'après Hobbs III (1975) les *Hartiella* représentent une sous-famille à part, équivalente à celle des *Entocytherinae* et des *Sphaeromicolinae.*

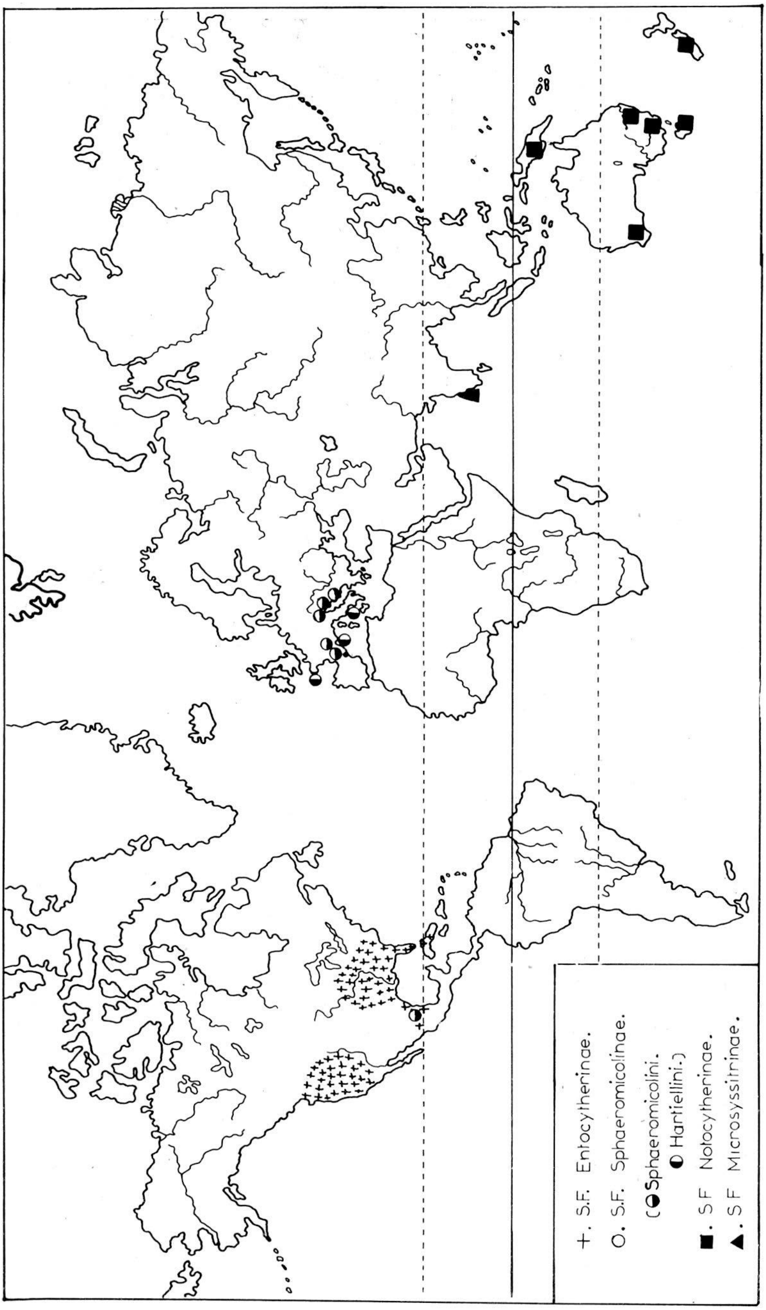


Fig. 1. Répartition géographique des représentants de la famille *Entocytheridae* Hoff.

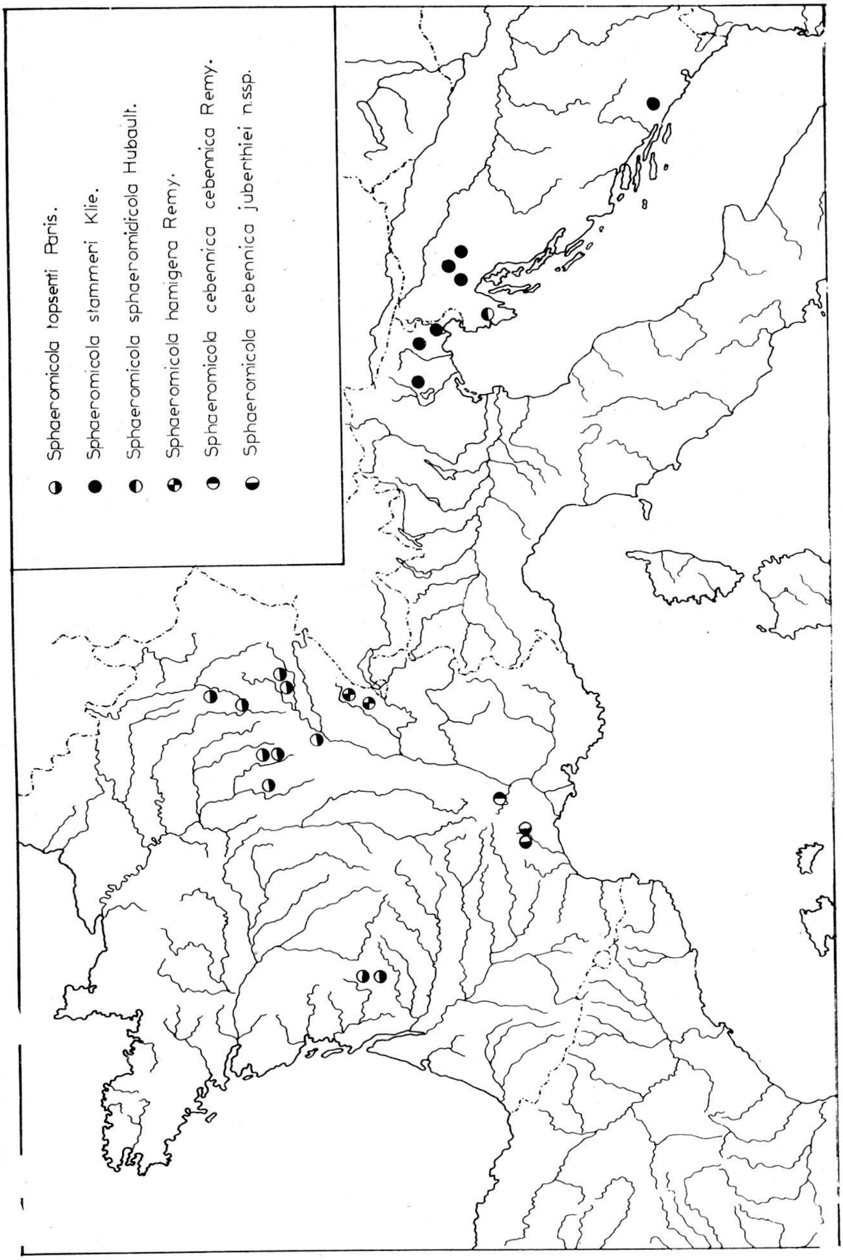


Fig. 2. Répartition géographique des espèces du genre *Sphaeromicola* Paris.

Cent Fons (fig. 3). Enfin en 1972 M. J.-Y. Bertrand m'a offert un nouveau lot de Sphaeromicola de Cent Fons.

Remy (1948a) mentionne pour la première fois *Sphaeromicola cebennica* dans le karst de l'Ardèche (grotte de la Dragonnière).

Trois ans plus tard Remy cite la présence de cette espèce dans la résurgence de Cent Fons. A cette date le spécialiste français avait eu en mains un tube contenant deux sortes d'Isopodes (*Sphaeromides raymondi* et *Faucheria*

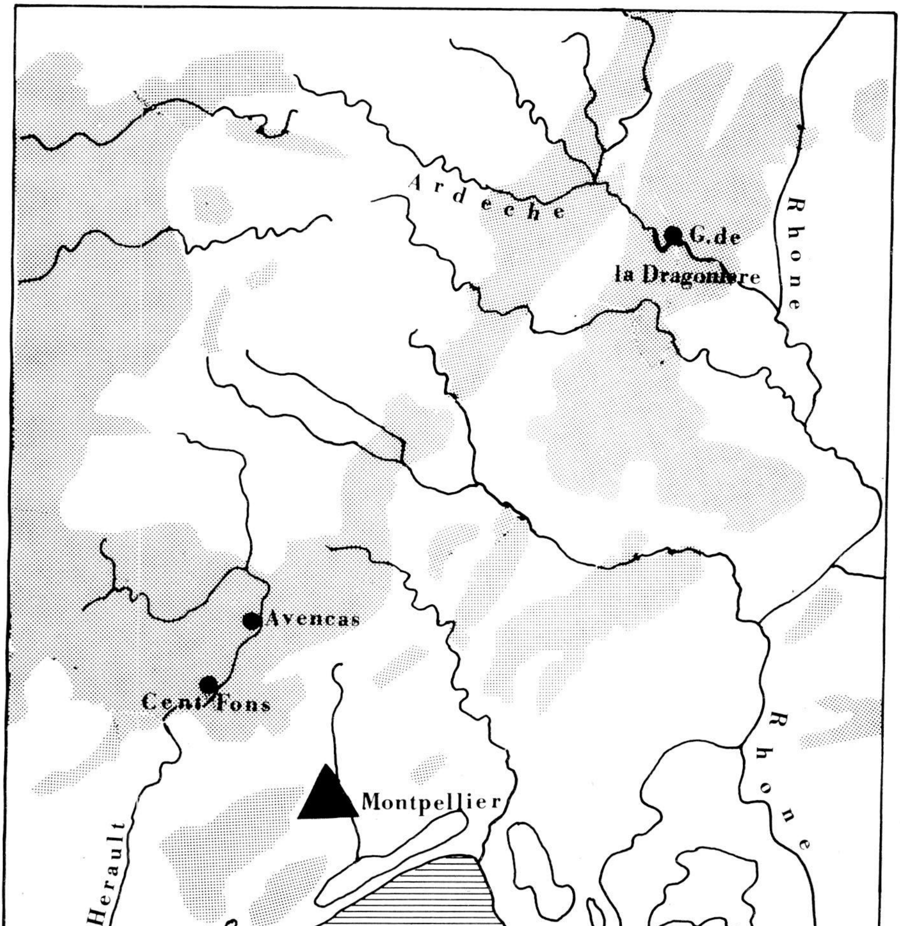


Fig. 3. Répartition géographique de *Sphaeromicola cebennica cebennica* Remy et *Sph. cebennica* n. ssp. (●) dans les régions karstiques de l'Ardèche et de la région nord-montpellièraine. (à remarquer l'isolement des noyaux karstiques des deux régions mentionnées).

faucheri); les *Sphaeromicola cebennica* gisaient au fond du tube ce qui avait mis en embarras Remy qui ne savait pas si les Ostracodes provenaient des Sphaeromides ou des *Faucheria*. Il inclinait à croire qu'ils avaient été fixés aux premiers. Le matériel recueilli par l'équipe du Laboratoire souterrain de Moulis vient confirmer cette opinion. Aucun *Sphaeromicola* n'a été à cette date recueilli sur des *Faucheria*. Du point de vue biogéographique il était intéressant de savoir si les *Sph. cebennica* du karst nord-montpellierain sont identiques à ceux de l'Ardèche de par le fait que les noyaux karstiques sont isolés actuellement entre eux comme le montre la figure 3 (d'après Balazuc, 1956). Le matériel examiné (18 mâles, 18 femelles adultes et 15 juvéniles provenant de Cent Fons et 1 mâle, 2 femelles et 4 juvéniles en provenance de l'Avencas) m'a permis d'individualiser une nouvelle sous-espèce que je dédie à Monsieur C. Juberthie.

1. Sphaeromicola cebennica juberthiei n. ssp. - Description

MALE

Carapace (fig. 4): bord dorsal largement arqué, bord antérieur atteignant presque la hauteur du bord postérieur, bord ventral droit. Valve droite dépassant du côté dorsal la valve gauche. Vu du côté ventral le bord ventral est droit dépourvu de l'expansion médiane connue chez les Podocopides. Les valves

Tableau 1. Dimensions de la carapace de *Sph. cebennica juberthiei* n. ssp. (exemplaires adultes de Cent Fons).

Caractères	Mâle (N = 18)	Femelle (N = 18)
Longueur moyenne (L en μ)	292	316
Intervalle de confiance pour L (P = 0,95)	289-296	311-320
Valeurs extrêmes pour L	286-306	306-333
Différences entre ♂ et ♀ (Test-t)	t — 8,5577 Difference significative (P < 0,001)	
Hauteur/Longueur moyenne (H/L en %)	55	59
Intervalle de confiance pour H/L en % (P = 0,95)	55-56	57-60
Valeurs extrêmes pour H/L en %	53-58	53-66
Différences entre ♂ et ♀ (Test-t)	t — 3,4127 Différence significative (P < 0,005)	
Largeur = 1 (estimation fonction L)	1 < 1/3L	1 ≤ 1/3L

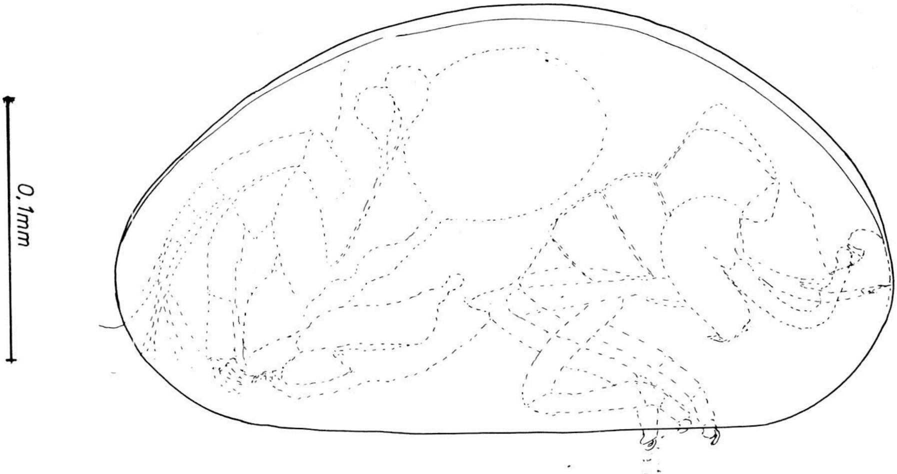


Fig. 4. *Sphaeromicola cebennica juberthiei* n. ssp. — mâle, vue générale.

sont translucides faiblement minéralisées. Il est à remarquer que les valves appartenant à un juvénile au 5-ème stade de développement post-embryonnaire asséché en vue de leur examen au microscope électronique à balayage se sont déformées complétement, signe d'une très faible minéralisation de la paroi externe. Par contre une valve appartenant à un mâle adulte a résisté au

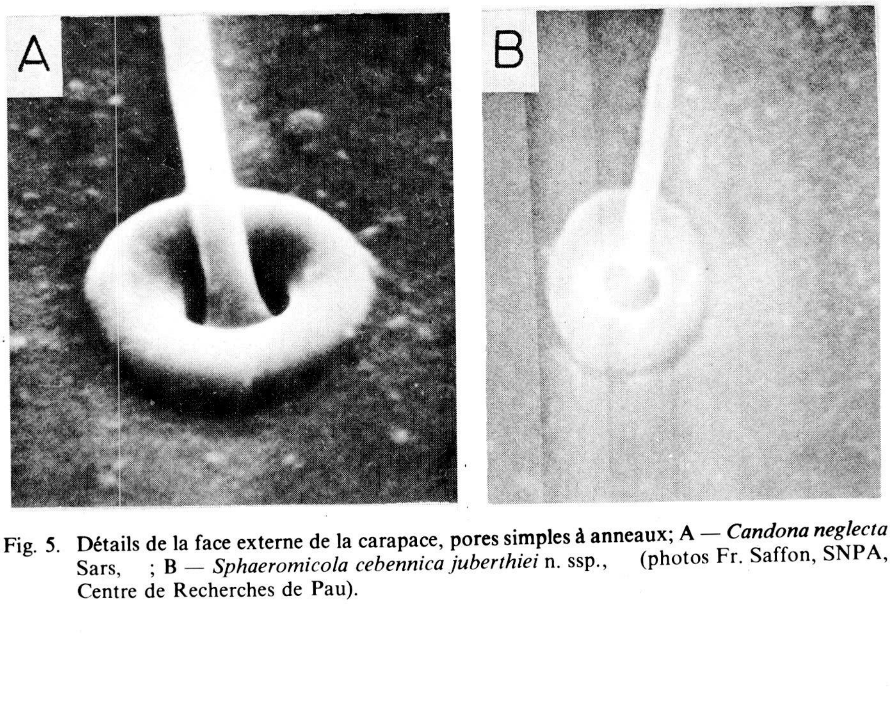


Fig. 5. Détails de la face externe de la carapace, pores simples à anneaux; A — *Candona neglecta* Sars, ; B — *Sphaeromicola cebennica juberthiei* n. ssp., (photos Fr. Saffon, SNPA, Centre de Recherches de Pau).

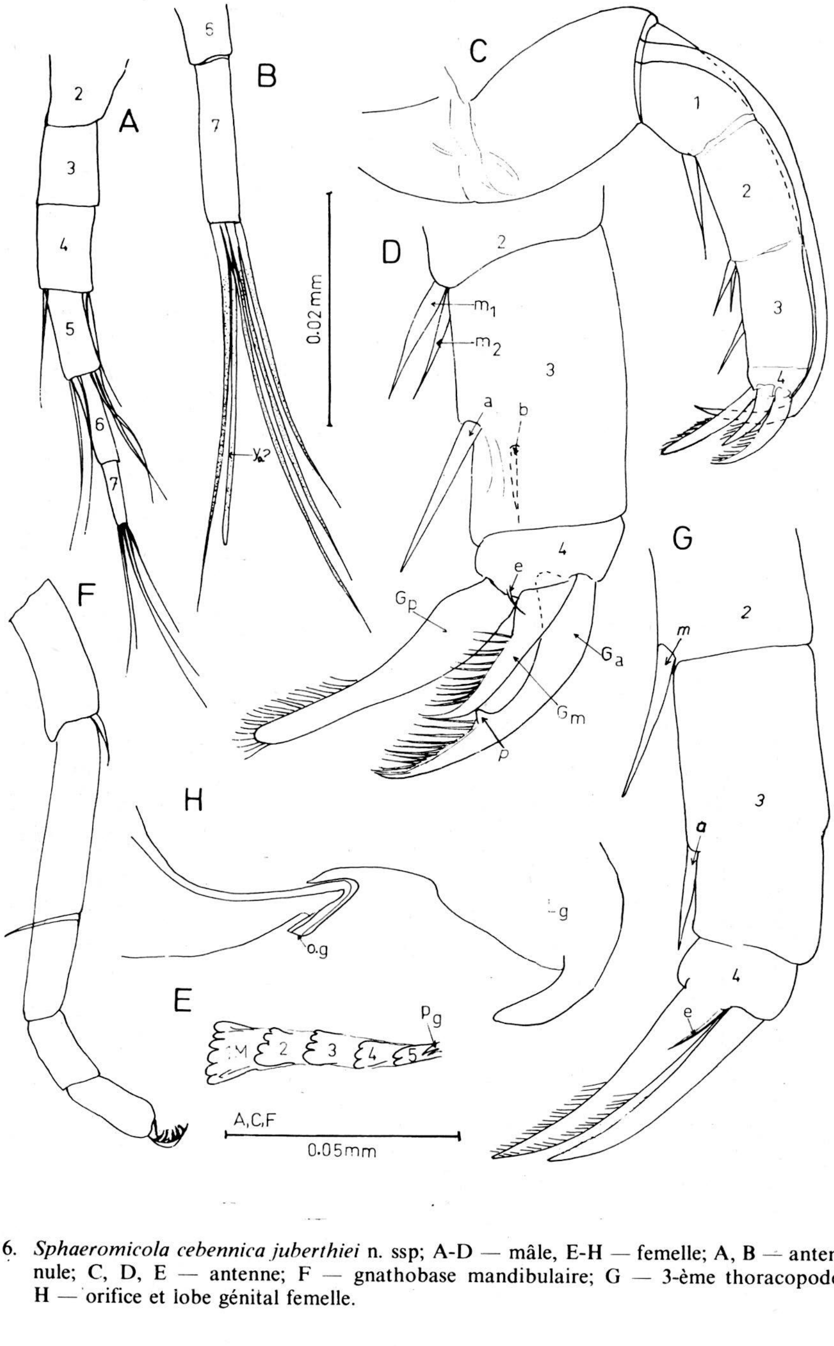


Fig. 6. *Sphaeromicola cebennica juberthiei* n. ssp; A-D — mâle, E-H — femelle; A, B — antennule; C, D, E — antenne; F — gnathobase mandibulaire; G — 3-ème thoracopode; H — orifice et lobe génital femelle.

traitement d'assèchement, ce qui indique une meilleure (plus forte) minéralisation. Les valves possèdent des poils sensoriels simples qui sortent de pores entourés d'un anneau proéminent (fig. 5B) tout comme chez les Podocopides à carapace normalement minéralisée (se reporter à notre fig. 5A, aux données de Puri et Dickau; 1969 et à celles de Sandberg et Plusquequelec, 1969). La longueur moyenne de la carapace est de 292 μ, la hauteur représente en moyenne 55% de la longueur et la largeur atteint moins d'un tiers de la longueur (pour plus de détails voir tableau 1).

Antennule (fig. 6A, B): les 3 premiers articles sont dépourvus de poils, le 4-ème du côté distal présente 2 poils longs qui atteignent le tiers proximal de l'article suivant, le 5-ème article porte 4 poils qui atteignent l'extrémité distale du 7-ème article, le 6-ème article, dépourvu de poils, est légérement plus long que les articles qui l'encadrent. L'article distal (fig. 6B) est muni de 5 poils dont l'un pourrait être un aesthetasc de type "Ya". Les poils antennulaires sont finement poilus à leur tour.

Antenne (fig. 6C,D): endopodite à 4 articles: le premier muni d'un poil qui atteint presque l'extrémité distale de l'article suivant, le 2-ème article endopodial avec 2 poils (ml, m2) égaux qui ne dépassent pas en longueur la moitié de l'article suivant, le 3-ème article endopodial (fig. 6D) avec 2 poils (a, b) et l'article antennaire distal est muni de 3 griffes et d'un poil mince (e). La griffe postérieure (Gp), longue est finement pectinée. La griffe médianne (Gm) plus courte (atteignant presque la moitié de la griffe postérieure) est armée d'une seule rangée de longs denticules. La griffe antérieure (Ga), forte atteint les deux tiers de la longueur de la griffe postérieure; elle est munie d'une dizaine de longs denticules placés sur le tiers distal et délimités du côté proximal par une protubérance (p).

Gnathobase mandibulaire (fig. 6E): formée par 5 dents, dont la première (1M), mobile, a du côté distal 6 lobes. Les dents 2 et 3 ont 4 cuspides, la 4-ème dent a seulement 3 cuspides et enfin la 5-ème dent en a 2 ou 3 et près de cette dent il y a 2 poils minces (P.g.).

Palpe maxillaire: un seul article pourvu d'un poil en spatule et un poil mince "normal".

Thoracopodes (fig. 6G): portent un poil sur le premier article endopodial qui dépasse en longueur d'un tiers la longueur des 2 articles suivants, la griffe distale est armée d'environ 5-6 épines.

Organe copulateur: péniferum (fig. 7B) présentant du côté distal un lobe A et un lobe B; ces deux lobes petits, digitiformes et courbés, s'entrecroisent formant une sorte de pince. A l'intérieur du péniferum on aperçoit le manchon (ma) qui présente une languette (l) proximale. Le manchon, court, n'atteint pas l'extrémité du péniferum. Les muscles M1 et M2 sont bien visibles. Les

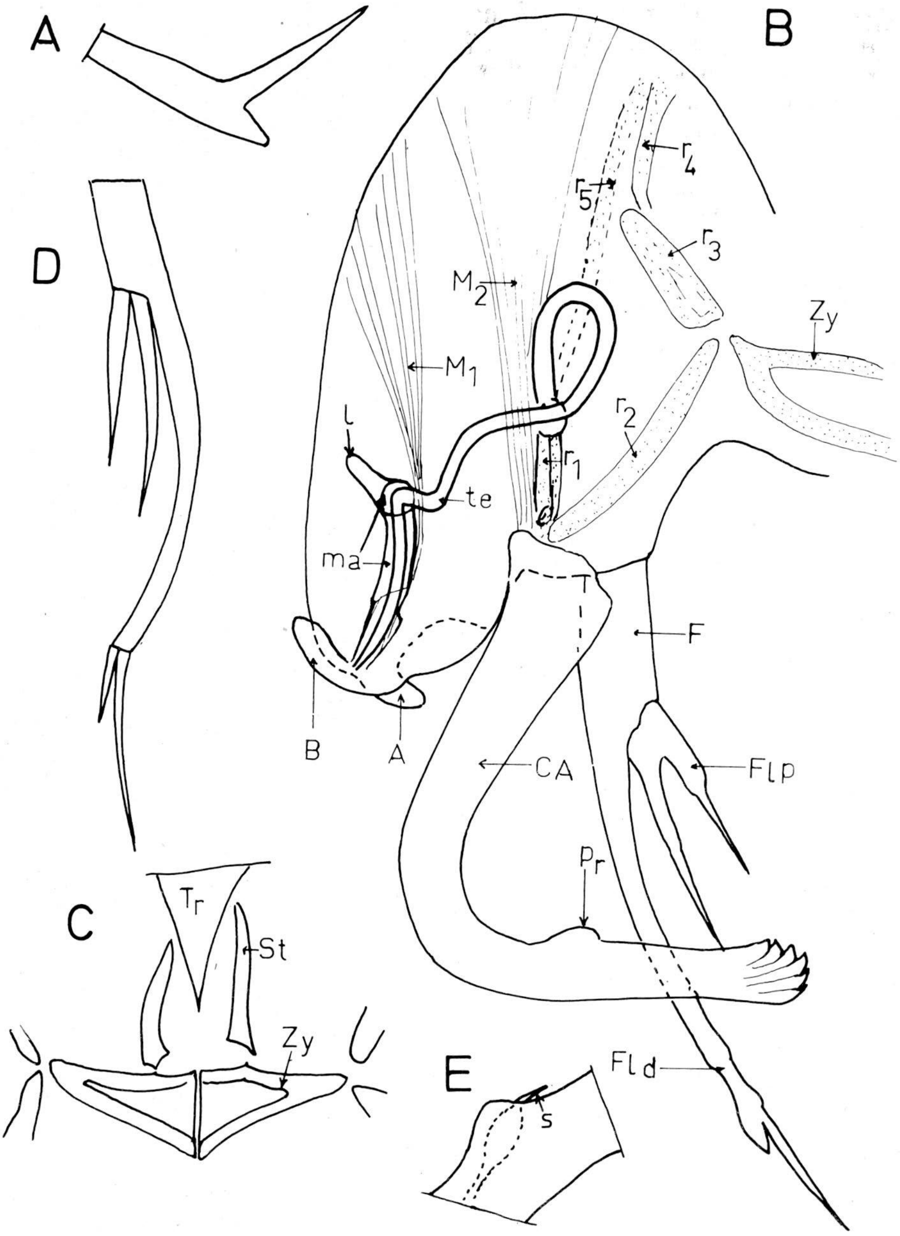


Fig. 7. *Sphaeromicola cebennica juberthiei* n. ssp., ♂, A, B, C, E — organe copulateur, détails; A — extrémité distale du flagelle; B — organe copulateur, vue médiale; C — pièces de l'articulation dorso-postérieure de l'organe copulateur; E — la protubérance "Pr" du crochet accessoire. D — *Sph. cebennica cebennica* Remy, flagellum (d'après Remy, 1948a).

rainures sclérifiées du péniferum sont difficilement visibles. On aperçoit toutefois sur la face latérale la rainure "r1" qui est placée entre l'extrémité proximale du tube copulateur et le bord du crochet accessoire; une rainure "r2" fait la liaison entre le zygum et la rainure "r1"; une rainure "r3" de forme annelée va du zygum jusqu'à la rainure "r4" sur la face médiale; cette dernière fait la liaison entre la "r3" et la paroi du péniferum; une rainure "r5" du côté latéral est parallèle avec la "r4". Le crochet accessoire (CA) a la moitié distale courbée á angle presque droit. L'extrémité du crochet possède 6 petits denticules. Sur le tiers distal du crochet il y a une forte protubérance (Pr). Le flagelle (F) a deux bras: le bras proximal (Flp) porte 2 poils inégaux, l'un représentant deux tiers de la longueur du second, le bras distal (Fld) long muni à son extrémité d'un poil court atteignant le 1/4 de la longueur de la tige et d'un moignon conique (fig. 7A et B). Le squelette interpénien (fig. 7C) est formé par un tropis (Tr) triangulaire. Les 2 sterynx (St) minces du côté dorsal s'élargissent du côté ventral près de leur articulation avec le zygum (Zy). Ce dernier est divisé par une mince bande du côté médian.

Extrémité du corps (fig. 4A, 8C): une expansion lamellaire (Ec) esarté tout près du bord dorsal de la valve, elle est pliée du côté ventral et possède 2 petits lobes orientés dorsalement.

FEMELLE

Carapace: semblable à celle du mâle à l'exception du fait qu'elle est plus grande et à bord dorsal plus arqué. Dimensions: longueur moyenne 316μ, hauteur moyenne représentant 59% de la longueur, largeur par contre d'environ un tiers de la longueur (pour plus de détails voir le tableau 1).

Antenne (fig. 6E, 8E, H): exopodite typique pour les Cythéracés, toutefois près du poil fileur on remarque (fig. 8E) un poil minuscule (pe). L'endopodite à 4 articles diffère de l'endopodite du mâle par l'absence d'un poil "m2" sur le 2-ème article endopodial et du poil "b" sur le 3-ème article. L'article distal possède 2 griffes, l'antérieure glabre et mobile la postérieure fusionnée à l'article est finement pectinée sur le bord interne. Près de ces deux griffes ont aperçoit difficilement 2 poils minces (e1, e2). Le poil "e2" pourrait être le vestige de la 3-ème griife bien développée chez le mâle.

Maxille (fig. 8G): semblable à celle du mâle.

Thoracopodes: pas de poils sur le premier article endopodial, à l'inverse de ce qui existe chez le mâle.

Lobe génital (fig. 6H): du côté distal une expansion digitiforme, à l'extrémité de laquelle s'ouvre l'orifice génital qui est dépourvu d'une structure sclérifiée comme on en trouve chez la majorité des Cythéracés (voir par exemple chez les *Metacyprinae*).

Femelle au dernier stade du développement post-embryonnaire *(femelle "mi-*

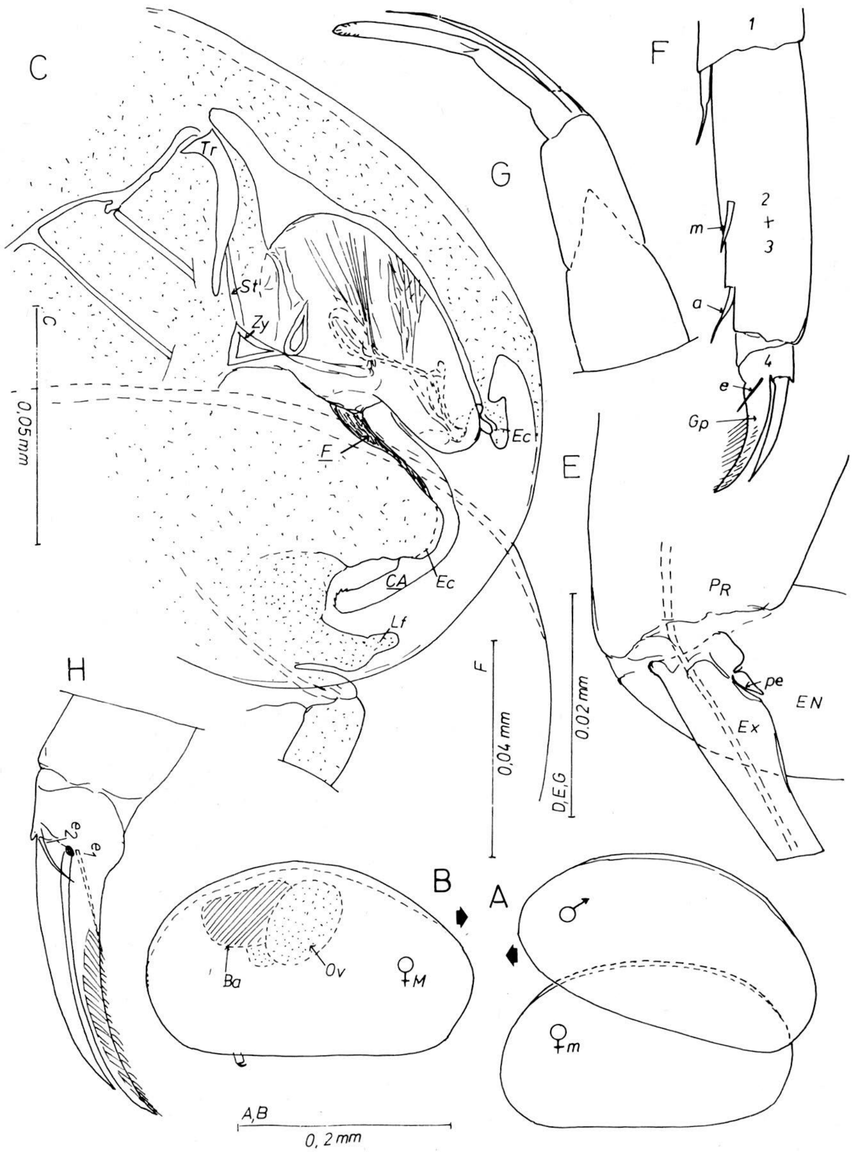


Fig. 8. *Sphaeromicola cebennica juberthiei* n. ssp; A, C — mâle et femelle "minor" en position d'accouplement; B — femelle "major" (a-ovocyte; b-bol alimentaire); D-E — femelle "major", antenne; D — extrémité distale de l'endopodite; E — extrémité proximale de l'exopodite (S-sillon; pe-poil exopodial; PR — protopodite; EX — exopodite; EN — endopodite); F, G — femelle "minor"; F — endopodite de l'antenne; G — maxille; H — femelle adulte (major), antenne, extrémité distale.

nor") — L'antenne possède seulement 3 articles endopodiaux (l'article 2 n'est pas séparé du suivant). La carapace un peu plus petite que celle du mâle adulte (longueur variant entre 260-268μ, pour 4 exemplaires fixés en position d'accouplement). On n'observe pas d'ovocytes à l'intérieur du corps, le lobe génital est à peine esquissé et il n'est pas traversé par un tractus génital.

Ovocytes (fig. 8B): visibles seulement chez la femelle adulte (femelle "major"), ils sont de forme ovale et mesurent entre 90,6-115μ de long, sur 56-75μ de large (n = 4); le rapport longueur maximale ovocyte longueur de la carapace varie entre 1/2,7 et 1/3,1. Chez toutes les femelles examinées je n'ai trouvé au maximum que 2 ovocytes par individu. Les exemplaires de l'Avencas ressemblent à ceux de Cent Fons, à une exception près, le mâle possède sur le crochet accessoire une protubérance "Pr" pourvue d'un petit poil (s).

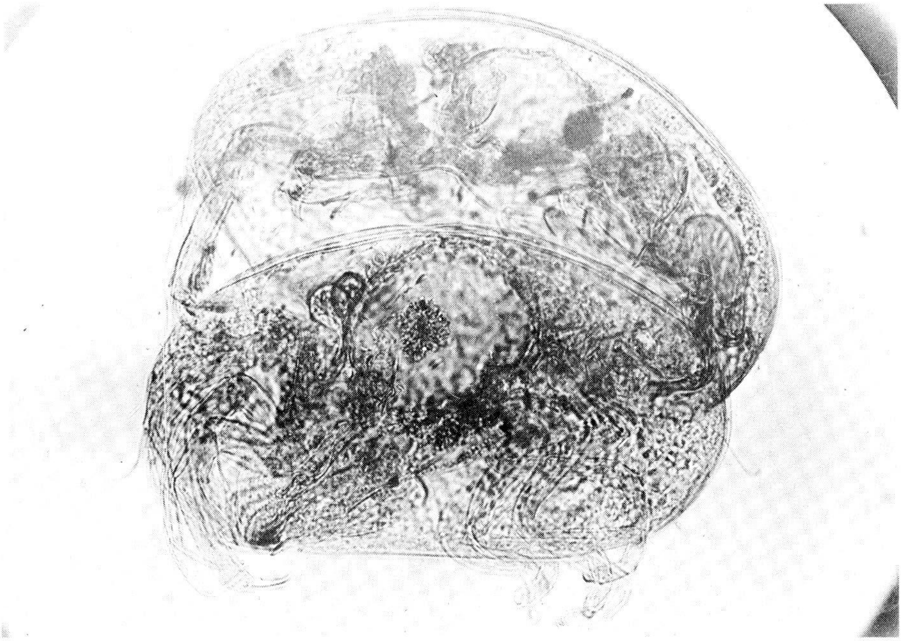


Fig. 9. *Sphaeromicola cebennica juberthiei* n. ssp., mâle et femelle "minor" en position d'accouplement (photo J. Durand, Laboratoire Souterrain du CNRS, Moulis).

2. Discussion

Sphaeromicola cebennica juberthiei n. spp. de la région nord-montpelliéraine est représentée par des populations qui habitent un noyeau karstique isolé géographiquement du noyau karstique d'ou provient *Sphaeromicola cebennica cebennica* (voir fig. 3), dans l'Ardèche.

La nouvelle sous-espèce diffère de la sous-espèce type par la taille de la carapace et par un détail de l'organe copulateur du mâle. *Sph. cebennica cebennica* possède des carapaces qui atteignent chez le mâle les longueurs suivantes: 430, 438, 446, 492 μ et les "femelles adultes en copulation", 438, 453, 461, 492 μ (Données citées d'après Remy, 1948a, p. 130). En ce qui concerne les femelles il est clair que Remy n'a pas fait attention aux femelles immatures en position d'accouplement. Il en ressort toutefois que les femelles de *Sph. cebennica cebennica* sont plus grandes que celles de la sous espèce nouvellement décrite. Chez la sous- espèce type le flagelle de l'organe copulateur du mâle a "à son extrémité distale deux soies divergentes pointues, inégales, la plus longue égale à 2/5 environ de la tige" (Remy, 1948a, p. 132 et fig. 7D de ce travail), tandis que chez les mâles de *Sph. cebennica juberthiei* le flagelle possède un moignon et une soie plus courte (environ 1/4 de la longueur de la hampe).

En examinant la position du flagelle chez ldes mâles de *Sph. c. juberthiei* accouplés à des femelles "minor" (fig. 8C) on observe que cette structure vient en contact avec le lobe "Ec" de la femelle et il est certain que les extrémités du flagelle ont une fonction sensorielle. Il est à remarquer que le principe de diversification de *Sphaeromicola cebennica* est semblable à celui d'autres Ostracodes Podocopides (voir par exemple le genre *Elpidium,* Danielopol, 1975) i.e. différences d'une part des structures à rôle sensoriel de l'organe copulateur du mâle, d'autre part dans la taille de la carapace.

La description d'une nouvelle sous-espèce de *Sphaeromicola cebennica* est intéressante aussi car elle démontre que des populations géographiquement isolées telles les populations du noyau karstique de l'Ardèche et de celui de la région nord-montpelliéraine assignées à une même espèce possèdent des différences morphologiques significatives. Or presque toutes les espèces de *Sphaeromicola* sont connues par plusieures populations isolées entre elles (voir fig. 1 et 2). Les données présentées ici suggèrent la nécessité d'une révision de la morphologie détaillée des populations appartenant aux différentes espèces de *Sphaeromicola*; il n'est pas exclu que certaines populations réprésentent des unitées taxonomiques bien individualisées. J'ai en effet eu la possibilité d'examiner une trentaine d'espèces d'*Entocytherinae* dont la moitie appartenait aux genres *Ankylocythere, Dactylocythere, Uncynocythere* et *Donnaldsoncythere,* provenant des Etats Unis et j'ai coustater que les différences interspécifiques à l'intérieur de ces genres sont bien plus petites qu'entre certaines espèces de *Sphaeromicola,* comme par exemple entre *Sph. stammeri, Sph. sphaeromidicola* et *Sph. topsenti.*

II. AFFINITÉS ET DIFFÉRENCES ENTRE LES *SPHAEROMICOLA* D'EUROPE ET *SPHAEROMICOLA CIROLANAE* DE L'AMÉRIQUE CENTRALE

J'ai montré ci-dessus (voir aussi fig. 1) la répartition disjointe des espèces du genre *Sphaeromicola* i.e. d'une part les espèces européennes, d'autre part l'espèce américaine, *Sph. cirolanae* connue de plusieurs grottes du Mexique dans la région de San Luis Potosi et Tamaulipas, sur le flanc Ouest de la Sierra de El Abra (pour plus de détails voir Rioja, 1951 et Hobbs Jr., 1971). Gràce à l'amabilité de Dr. H. H. Hobbs Jr. (Washington) j'ai eu la possibilité d'examiner 5 mâles, 9 femelles et 4 juvéniles de *Sph. cirolanae* de la Cueva de la Florida (Tamaulipas) recueillis sur des Isopodes *Speocirolana pelaezi* Bolivar.

L'espèce mexicaine, tout comme les *Sphaeromicola* d'Europe, possède des valves faiblement calcifiées, il n'y a ni lamelle calcaire interne, ni canaux marginaux radiaires et la charnière est faiblement développée. L'antennule possède l'avant-dernier article de la même longueur que le suivant (fig. 10A). L'antenne du mâle porte sur l'avant-dernier article 2 poils (a, b, fig. 10B). L'antenne de la femelle adulte (fig. 10C) porte sur l'article distal 2 griffes et un poil "e" (à l'encontre des *Hartiellini* et des *Entocytherinae* ou les femelles portent 3 griffes (femelles "triunguis"). L'une des griffes est pectinée, l'autre est glabre. La gnathobase mandibulaire est munie de 5 dents, la première à 6 cuspides. La maxille a un seul endite et le palpe, uniarticulé, est pourvu de 2 poils.

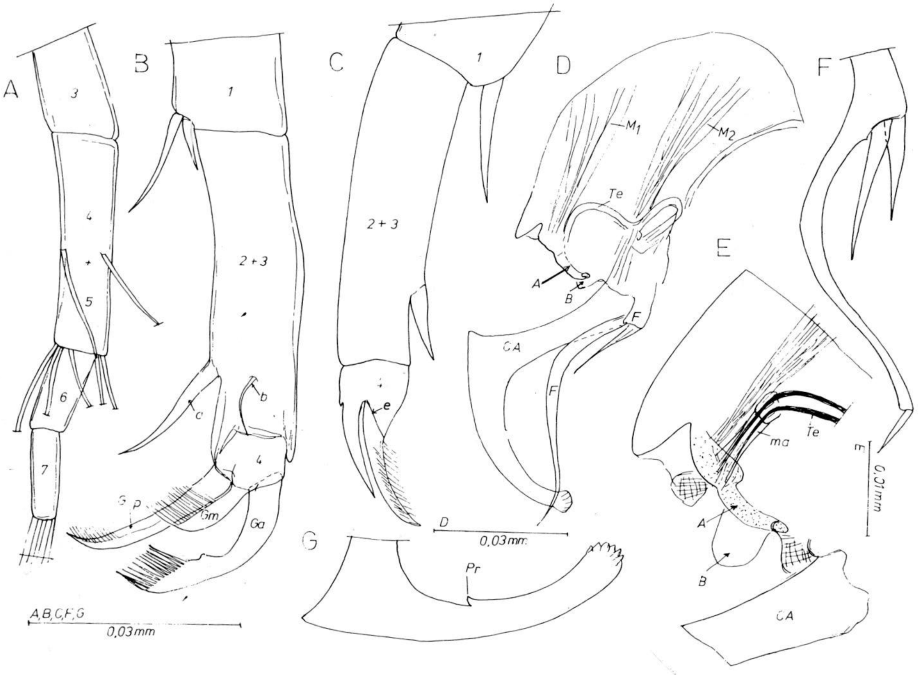


Fig. 10. *Sphaeromicola cirolanae* (Rioja); A, B, D-G — mâle; C — femelle; A — antennule; B, C — antenne; D-G — organe copulateur; D — vue générale; E — Détail de la région distale du péniferum; F — flagellum; G — extrémité distale du crochet accessoire.

Les thoracopodes ayant les griffes distales en forme de peigne, grâce au developpement d'une rangée fines de denticules. L'organe copulateur a un flagelle à 2 bras, l'un long, l'autre court (fig. 10D et E). Le crochet accessoire long et coudé (fig. 10D et G). Le péniferum à 2 lobes distaux, A et B (fig. 10 D et E). *Sph. cirolanae* ressemble à *Sph. stammeri* par la présence de 2 poils sur le premier article endopodial de l'antenne; par le faible développement des lobes A et B du peniferum de l'organe copulateur du mâle elle ressemble à *Sph. stammeri, Sph. hamigera* et *Sph. cebennica;* par la présence d'une protubérance sur le crochet accéssoire du mâle *Sph. cirolanae* ressemble à *Sph. sphaeromidicola* et *Sph. cebennica.* Tenant compte des similitudes morphologiques entre les Sphaeromicola d'Europe et celle du Mexique et tenant compte aussi de la distribution géographique de ce groupe autour du bassin méditerranéen et près de la Mer des Caraïbes (fig. 1) je pense que ce groupe peuplait à l'origine la Téthys (voir à ce sujet aussi les remarques que j'ai faites en 1971 et l'analyse biogéographique de McKenzie, 1973). Etant donné que l'ouverture de l'Atlantique du Nord a eu lieu au plus tard à l'Eocène (Thenius, 1975) il faut admettre que les *Sphaeromicola* marins peuplaient la Téthys avant cette époque.

Sphaeromicola cirolanae diffère des espèces européennes par les caractères suivants: l'antennule a le 4-ème article fusionné au 5-ème; ce dernier porte 5 poils au lieu de 4. L'antenne tout aussi bien chez le mâle qu chez la femelle porte seulement 3 articles endopodiaux, dus à la fusion du 2-ème et 3-ème articles (fig. B. C), au lieu de 4 articles comme chez les espèces européennes; Enfin les griffes distales des thoracopodes portent chez *Sph. cirolanae* seulement 4 denticules tandis que les espèces européennes en portent entre 5 et 8. Tenant compte de ces données je propose de diviser le genre Sphaeromicola en deux groupes d'espèces: l'un réunira les Sphaeromicola européennes et il sera nommé groupe *topsenti* d'après l'espèce type du genre, tandis que le second groupe sera représenté pour le moment seulement par *Sph. cirolanae* et portera le nom de cette espèce.

III. REMARQUES SUR L'ACCOUPLEMENT CHEZ LES ENTOCYTHERIDAE

Jusqu'aux investigations de Hobbs Jr (1971) il était admis que les *Entocytheridae* possèdent deux types de femelles: 1. un type "minor" ou "nubile" ayant une taille presque égale à celle du mâle et n'ayant pas d'ovocytes visibles; 2. un type "major" ou "gravide", ayant une taille plus grande que celle du mâle et les ovocytes visibles par la transparence des valves. Rioja (1943) pensait que ces deux types de femelles représentaient, ou bien un cas de poecilogynie comme Cuénot l'avait cité chez plusieurs Insectes ou bien une continuation de la croissance de la femelle (qui muerait encore une fois après avoir atteint l'etat adulte). A cette dernière hypothèse souscrivait aussi Hoff (1943): "The only adequate explanation for the two kinds of females seems that a moult occurs between the time of copulation and the time of development of the eggs within the ovary" p. 281. Remy (1948a), en décrivant *Sph. cebennica cebennica* mentionne (p. 130) que les femelles adultes sont celles qu'on trouve en état d'accouplement.

Récemment Hobbs Jr. (1971) a montré que la femelle "minor" représente des juvéniles au dernier stade du développement post-embryonnaire, tandis que les femelles "major" sont des femelles adultes. Jusqu'à présent, d'après cet auteur un seul exemple de couple entre Entocythéride mâle et femelle "major" (adulte) avair été observé, la majorité des couples connus étant formés entre mâles et femelles "minor".

Les observations sur l'accouplement chez les Ostracodes sont rares du fait que ce processus se déroule très rapidement et pour une courte durée. J'ai étudié le comportement sexuel et toutes les phases de la réproduction chez le Cytheridae *Limnocythere sancti-patrici* et chez les Cyprididae *Candona dancaui, Pseudocandona pratensis, Ps. rouchi* n. sp. et *Mixtacandona* sp.* Dans tout ces cas le processus d'accouplement consiste dans un appariement du mâle et de la femelle adulte (avec fixation du mâle à la femelle surtout par l'intermédiaire de l'organe copulateur et en moindre mesure par l'antenne et les palpes préhensiles des thoracopodes 1); l'érection des hémipenis se fait très rapidement ils pénétrent entre les valves de la femelle et ils se fixent sur le lobe génital; le tube copulateur est introduit dans l'orifice génital de la femelle et l'insémination a lieu; après cette phase les couples le plus souvent se défont et reprénent leur activité normale. Parfois les mâles peuvent chevaucher des femelles immatures. Dans ces cas l'hémipenis n'arrive pas à se fixer au lobe génital et le mâle ne se maintient fixé à la femelle qu'un temps éphémère; cela est dû aussi au fait que les femelles immatures ne cessent pas de bouger et n'acceptent pas le mâle (à l'encontre de ce qui se passe chez les femelles adultes). Wohlgemuth (1914) chez *Heterocypris incongruens* et Theisen (1966) chez 3 espèces de Cythérides ont montré que les femelles sont fécondées immédiatement après avoir subi la dernière mue.

Parmi le matériel de *Sphaeromicola cebennica juberthiei* il y avait 4 mâles fixés en position d'accouplement à des femelles "minor" (fig. 8A, C et 9). De tels couples montrent que le crochet accessoire de l'organe copulateur mâle se fixe au pli postéro-dorsal existant à l'intérieur de la carapace de la femelle "minor" et non pas au lobe génital (donc l'organe copulateur du mâle ne vient pas en contact avec la région génitale de la femelle et il n'y a pas transmission de spermatozoïdes). Les griffes distales de l'antenne du mâle ne se fixent pas toujours fermement au creux antéro-dorsal de la carapace femelle comme on le remarque dans la figure 9. Le fait que la femelle au dernier stade du développement post-embryonnaire accepte la fixation du mâle sur son côté dorsopostérieur est l'indice de l'existence d'un comportement sexuel précoce chez celle-ci. L'affirmation de Hoff (1943) qu'une femelle fécondée pourrait de nouveau muer gardant après les oeufs qui se développent normalement, est difficilement acceptable. L'exuvie de l'ostracode mué garderait automatiquement les spermatozoïdes et/ou les oufs. Les observations que j'ai pu faire d'une

---

* Observations faites au Laboratoire Souterrain du C.N.R.S., à Moulis et à l'Institut Limnologique de Vienne.

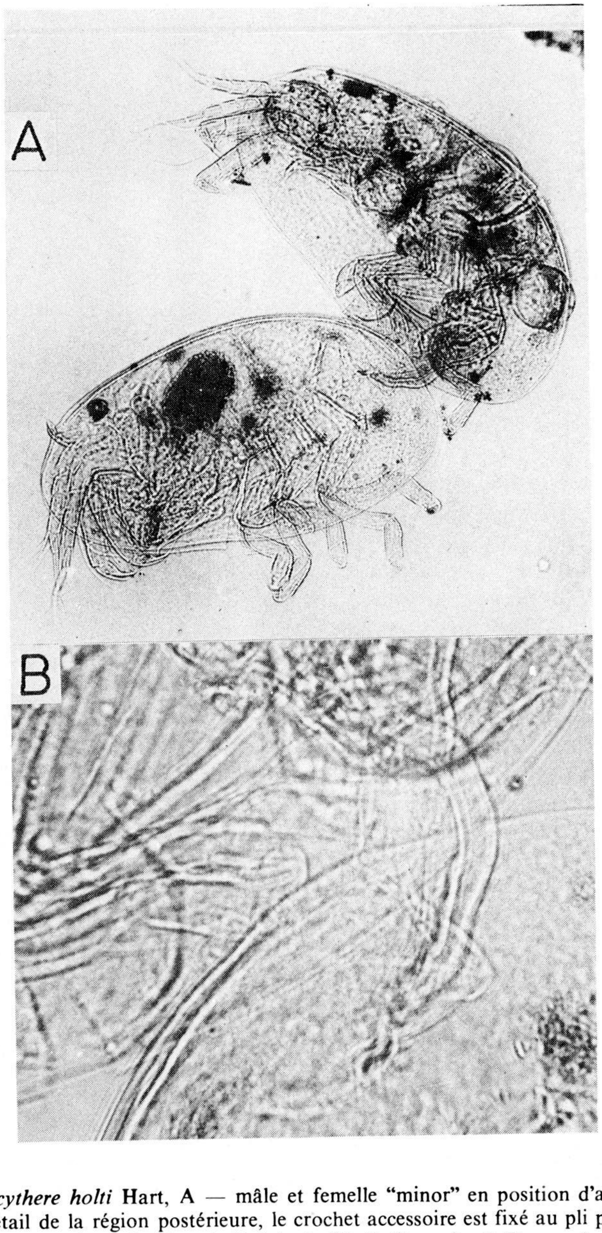


Fig. 11. *Uncinocythere holti* Hart, A — mâle et femelle "minor" en position d'accouplement; B — détail de la région postérieure, le crochet accessoire est fixé au pli postéro-dorsal (photo Tr. Orghidan, Institut de Spéologie "E. G. Racovitza", Bucarest).

part sur les Podocopides vivants cités ci-dessus, d'autre part sur le matériel fixé de *Sph. cebennica juberthiei* m'ont suggéré une autre interprétation du processus d'accouplement et de fécondation chez ce dernier: i.e. le mâle se fixe fermement à la femelle immature par le crochet accessoire au lobe postéro-dorsal restant ainsi accouplé jusqu'à la mue de la femelle immature. La femelle adulte sortant du côté antéro-dorsal le mâle se décroche de l'exuvie et se fixe cette fois-ci à l'adulte provoquant très rapidement l'insémination après quoi il se sépare de celle-ci. Le type d'accouplement décrit chez *Sph. cebennica juberthiei* semble être une des originalités des Entocytheridae. La figure 11 (A et B) montre un cas semblable chez *Uncinocythere holti* Hart (Entocytherinae), le crochet accessoire s'y fixe aussi au pli postéro-dorsal d'une femelle "minor".

Récemment Whatley et Stephens (1976) ont montré que chez certains groupes de Cythérides le dimorphisme sexuel apparaît à un stade plus précoce que chez la majorité des autres Ostracodes. Nous sommes avec les Entocytheridae en présence d'un cas de comportement sexuel précoce de la femelle et il serait intéressant de voir si cela est lié à l'apparition d'un dimorphisme sexuel précoce.

IV. REMERCIEMENTS

Je suis extrémement reconnaissant à toutes les personnes qui m'ont aidé à réaliser ce travail: M. et Mme C. Juberthie (Moulis), M. J-Y. Bertrand (Paris), qui m'ont confié l'étude du matériel de *Sphaeromicola cebennica,* MM H. Hobbs Jr. et W. C. Hart Jr. (Washington, D.C.) qui m'ont cédé des *Sphaeromicola cirolanae* et une collection de 30 espèces d'Entocytherinae. M. C. Plesa (Cluj) qui à mis à ma disposition du matériel de *Sph. topsenti* et *Sph. stammeri*; MM H. J. Oertli et Fr. Saffon ainsi que le Centre de Recherches de la SNPA (Pau) grâce auxquels j'ai examiné les Sphaeromicola au microscope éléctronique à balayage; MM J. Durand (Moulis) et Tr. Orghidan (Bucarest) qui ont photographié les Entocytherides en position d'accouplement; Mme J. Rey (Toulouse), Mlle N. Gourbault (Paris) et M. J-P. Henry (Dijon) qui m'ont proccuré les photocopies de plusieurs travaux rares sur les Sphaeromicola. M. le Prof. R. Husson (Dijon) a bien voulu revoir et corriger le manuscrit.

RÉSUMÉ

On décrit *Sphaeromicola cebennica juberthiei* n. ssp., Ostracode cavernicole, du Sud de la France (stations karstiques du système de Cent Fons et de l'Avencas, Herault). La nouvelle sous-espèce diffère de *Sph. cebennica cebennica* (connue d'une grotte de l'Ardèche) par la taille de la carapace plus petite et par la réduction d'un des poils distaux du flagelle de l'organe copulateur du mâle. Les affinités et différences entre les *Sphaeromicola* d'Europe et *Sphaeromicola cirolanae* du Mexique sont discutées et le genre *Sphaeromicola* est divisé en deux groupes d'espèces: gr. *topsenti* et gr. *cirolanae*; suivent quelques observations sur l'accouplement chez *Sph. cebennica juberthiei.* Un comportement sexuel précoce de la femelle, au dernier stade du developpement post-embryonnaire, est signalé. Ce type de comportement est vraisemblablement une caractéristique des Entocytheridae.

BIBLIOGRAPHIE

BALAZUC, J., 1956. Spéléologie du département de l'Ardèche. *Rass. Spéléol. Ital. Mem.* 2.

CHAPPUIS, P. A., 1953. Sur certaines reliques marines dans les eaux souterraines. *Ier Congrès Int. Spéléologie,* 3, 3: 47-53.

DANIELOPOL, D. L., 1971a. Quelques remarques sur le peuplement ostracodologique des eaux souterraines d'Europe. In Oertli, H. J. (ed.): Paléoécologie des Ostracodes. *Bull. Centr. Rech. SNPA-Pau* 5 Suppl.: 179-190.

DANIELOPOL, D. L., 1971b. Définition de la tribu des Hartiellini nov. et quelques remarques sur la poecilogynie de *Hartiella dudichi* (Ostracoda, Entocytheridae, Sphaeromicolinae). *Trav. Inst. Spéol. "Emile Racovitza",* 10: 189-207.

DANIELOPOL, D. L., 1975. Remarques sur la diversification morphologique de trois nouvelles espèces d'*Elpidium* (Ostracoda) à Cuba. *Bull. Amer. Paleontol.* 65: 47-60.

DELAMARE-DEBOUTTEVILLE, C., 1960. Biologie des eaux souterraines littorales et continentales 740 pp, Hermann Paris.

HART, C. W., 1962. A revision of the Ostracods of the family Entocytheridae. *Proc. Acad. Nat. Sc. Philad.* 114: 121-147.

HART, C. W. and HART, D., 1969. Evolutionary trends in the Ostracods family Entocytheridae, with notes on the distributional patterns in the Southern Appalachians. In Holt, P. C. (ed.): *The distributional History of the Biota of the Southern Appalachians Part I Invertebrates* 179-190. Blacksburg.

HART, C. W. and HART, D., 1970. A new Ostracod (Enthocytheridae, Notocytheridae) on New Guinea Crayfish. *Zool. Mededel. Leiden,* 44, 19: 279-283.

HART, C. W., NAIR, N. B. and HART, D., 1967. A new Ostracod (Ostracoda: Entocytheridae) commensal on a wood-boring marine isopods from India. *Notul. Nat.* 409: 1-11.

HOFF, C. C., 1943. Two new Ostracods of the Genus *Entocythere* and Records of Previously Described Species. *J. Washington Acad. Sc.* 33: 276-286.

HOBBS, H. H., Jr., 1971. The enthocytherid Ostracods of Mexico and Cuba. *Smithson. Contr. Zool.* 81: 1-55.

HOBBS, H. H., III, 1975. Distribution of Indiana Cavernicolous Crayfishes and their Ecto-Commensal Ostracods. *Int. J. Speleol.* 7: 273-302.

HUBAULT, E., 1937. *Monolistra hercegovinensis* Absolon, Sphéromien cavernicole d'Herzégovine et *Sphaeromicola stammeri* Klie son commensal. *Arch. Zool. Exp. et Gén.* 78: 357-373.

HUBAULT, E., 1938. *Sphaeromicola sphaeromidicola* nov. sp. commensal de *Sphaeromides virei* Valle en Istrie et considérations sur l'origine de diverses espèces cavernicoles périméditerranéennes. *Arch. Zool. Exp. et Gén.* 80: 11-24.

KLIE, W., 1930. Über eine neue Art der Ostracodengattung *Sphaeromicola. Zool. Anz.* 88: 272-276.

KLIE, W., 1938. *Sphaeromicola dudichi* n. sp. (Ostr.) ein Kommensale des Bohramphipoden Chelura terebrans. *Zool. Anz.* 121: 317-322.

McKENZIE, K. G., 1973. The biogeography of some cainozoic Ostracoda in: Organisms and Continents through time. *Special Papers in Paleontology* 12: 137-153.

PARIS, P., 1920. Ostracodes (Première Série). Biospeologica. *Arch. Zool. Exp. et Gen.* 58: 475-487.

PURI, H. S. and DICKAU, B. E., 1969. Use of normal pores in taxonomy of Ostracoda. *Trans. Gulf. Coast. Assoc. Geol. Soc.* 19: 353-367.

REMY, P. A., 1943. Nouvelle station du *Sphaeromien* troglobie *Caecosphaeroma (V.) burgundeum* Dollfus var. *rupis-fucaldi* Hubault et de l'Ostracode commensal *Sphaeromicola topsenti* Paris. *Bull. Soc. Zool. de France.* 68: 35-38.

REMY, P. A., 1946. *Sphaeromicola stammeri* Klie var. *hamigera* n. var. Ostracode commensal du Sphaeromide obscuricole *Caecosphaeroma* (C.) *virei* Dollfus. *Coll. Mus. Zool. Nancy* 1: 7-9.

REMY, P. A., 1948a. Description de *Sphaeromicola cebennica* n. sp. ostracode cytheride commensal de l'isopode cirolanide cavernicole *Sphaeromides raymondi* Dollfus. *Bull. Mens. Soc. Linneene Lyon.* 17, 7: 129-132.

REMY, P. A., 1948b. Sur la distribution géographique des Sphaeromidae, Monolistrini et des Sphaeromicola leurs Ostracodes commensaux. *Bull. Mus. Hist. Nat. de Marseille.* 8, 1: 1-4.

REMY, P. A., 1951. Stations de crustaces obscuricoles. *Arch. Zool. Exp. et Gén.* 88: 217-230.

RIOJA, E., 1943. Estudios carcinológicos 15, Polimorfismo femenino de los Ostracodos del género Entocythere. *Anal. Inst. de Biologia,* 14, 2: 567-585.

RIOJA, E., 1951. Estudios carcinológicos 25. El hallazgo del género *Sphaeromicola* en América (Ostracodos, Citeridos) y descripción de una nueva especie. *Anal. Inst. de Biologia.* 22, 1: 169-179.

ROUCH, R., JUBERTHIE-JUPEAU, L. et JUBERTHIE, C., 1968. Essai d'étude du peuplement de la zone noyée d'un karst. *Ann. Spéléol.* 23: 717-733.

SANDBERG, Ph. A. and PLUSQUELEC, P. L., 1969. Structure and polymorphysm of normal pores in cytheracean Ostracoda (Crustacea). *J. Paléont.* 43: 517-521.

THEISEN, B. F., 1966. The life History of Seven Species of Ostracods from a Danish Brackish-Water Locality. *Meddel. Dan. Fisk. Havunders.* 4: 215-270.

THENIUS, E., 1975. Biogeography in the view of the earth sciences. The Paleogeography as the basis of a historical biogeography. Verh. Dtsch. Zool. Ges. 1974: 358-372. Stuttgart.

WHATLEY, R. C. and STEPHENS, J. M., 1976. Precocious sexual dimorphism in fossil and recent Ostracoda. *Int. Symp. Ecology and Zoogeography of recent and fossil Ostracoda.* Abstracts: 40. Facultas Verlg. Wien.

WOHLGEMUTH, R., 1914. Beobachtungen und Untersuchungen über die Biologie der Süsswasserostracoden, ihr Vorkommen in Sachsen und in Böhmen, ihre Lebensweise und ihre Fortpflanzung. *Int. Rev. Ges. Hydriobiol. Hydrogr.,* Biol. Suppl. 6: 1-72.

Int. J. Speleol. 9 (1977), pp. 43-58.

Variation among Populations of the Troglobitic Amphipod Crustacean *Crangonyx antennatus* Packard Living in Different Habitats I. Morphology

by

Gary W. DICKSON*

INTRODUCTION

Intraspecific morphological variation in response to habitat differences has been observed in freshwater amphipod populations of epigean species (Minckley and Cole, 1963; Pinkster, 1971, 1972; Pinkster et al., 1970; Strong, 1972) and hypogean species (Ginet, 1960; Holsinger, 1967, 1969; Holsinger and Culver, 1970). Variable morphological characters include body length, allometric relationships between body and appendage lengths, setation and integument coloration. Environmental factors, such as current velocity, available food, temperature, predators, competitors and other biotic and abiotic components are considered responsible for these variations.

In the present study morphological variation was recorded among different populations of the troglobitic (i.e., obligatory cavernicole) amphipod *Crangonyx antennatus*. Certain features of the subterranean environment make it attractive for the study of population variation. Caves represent relatively constant, partitioned habitats which lack the major fluctuations of temperature and humidity normally associated with epigean habitats. Because of the lack of significant autotrophism and relative scarcity of food materials, species diversity and population sizes in caves are generally quite small compared to epigean communities.

C. antennatus offers several unique advantages for the study of population variation in a troglobitic species: 1) it is one of the most common and widespread aquatic troglobites in the southern Appalachian region of the eastern United States (Holsinger, 1969, 1972), and populations are relatively large in the caves investigated in Lee Co., Virginia; 2) it populates two distinctly different subterranean habitats, mud-bottom pools and small gravel-bottom streams, which permits a direct comparison of populations in a small portion

---

* Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23508, U.S.A. Present address: Dept. of Zoology, University of Georgia, Athens, Georgia 30602, U.S.A.

of the overall species range under different environmental conditions; and 3) this species has been extensively studied ecologically (Holsinger and Holsinger, 1971; Holsinger, 1975a; Holsinger and Dickson, in press; Dickson, 1975, in press; Dickson and Kirk, in press).

In the present study, *C. antennatus* cave populations were examined from mud-bottom pool and small gravel-bottom stream habitats to determine: 1) if body length differences at maturity are evident between populations living in the two distinct habitats; 2) if variation is present in first antennal segment number to body length ratio and integument coloration; and 3) possible influences of environmental differences on population variation.

METHODS AND MATERIALS

Populations of *C. antennatus* were sampled seasonally during 1974 and 1975 in six caves located in the Powell Valley of Lee Co., Virginia. Three of the amphipod populations sampled inhabited caves containing mud-bottom pools (Roadside No. 1, Molly Wagle and Sweet Potato caves) and three of the populations were sampled in caves containing small gravel-bottom streams (Cope, Spangler and Gallohan No. 2 caves). Pool habitats receive water through ceiling drips and seepage, whereas stream habitats receive water from subterranean and/or direct surface recharge. The benthic substrate in the mud-bottom pools is characterized by mud sediments containing relatively large populations of bacteria and fungi, in contrast to stream habitats which contain bottom gravels (1-3 cm diameter) and sparse benthic sediments with small microbial populations (Dickson, 1975; Dickson and Kirk, in press). Because of the evidence for potentially well-developed dispersal ability (Holsinger, 1969, 1975a) and high migration rates of *C. antennatus* (Culver, 1973a, in press), the hydrology of the Powell Valley (Holsinger, 1975b) and the close proximity of the investigated caves (within a 5 km radius), the populations sampled were not considered to be genetically isolated.

Ten sample sites of 0.09 m^2 were randomly selected in stream and pool areas of each cave during seasonal sampling periods. All *C. antennatus* observed within the sample sites, including any present in the top 5 cm of gravels in the stream habitats, were collected until 25 amphipods were obtained in each cave. Amphipods were collected with a modified 15 ml asepto syringe and preserved in 70% ethyl alcohol for later examination in the laboratory.

Coloration of the integument was recorded in each cave prior to preservation (because of alcohol bleaching), along with specific information from each of the random plots including the number of *C. antennatus,* presence and number of potentially competitive species (isopods, *Asellus recurvatus* and *Lirceus usdagalun*), substrate type, presence and type of detritus and current velocity. Average water temperature, evidence of flooding and the number of potential predators (salamander, *Gyrinophilus porphyriticus* and crayfish, *Cambarus bartonii*) within 1 m^2 of the sample plots were also recorded.

In the laboratory, amphipods were measured from the base of the first an-

Table 1. Environmental parameters of the sampled *C. antennatus* habitats in six Lee Co., Virginia caves. Seasonal collections listed as: I-August, II-November, III-February and IV-June. An environmental parameter not present in a habitat is indicated by N.P.

Cave	Habitat	Overall[1] food rating	Current[2] velocity rating	Density *C. antennatus* (no./m²)				Density isopods (no./m²)				Predator[3] sightings				Average water temperature (C°)			
				I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Gallohan No. 2	stream	1	1	16.7	5.6	3.3	7.8	3.3	4.4	24.4	14.4	0	0	0	0	13.5	12.5	11.7	11.6
Spangler	stream	2	2	130.0	30.0	15.6	18.9	24.4	30.0	16.7	44.4	0	0	0	0	14.5	11.5	11.1	13.3
Cope	stream	3	3	14.4	12.2	11.1	6.7	16.7	0	3.3	5.6	3	2	4	2	13.4	10.4	12.2	—
Sweet Potato	pool	4	N.P.	34.4	64.4	24.4	71.1	3.3	0	1.1	3.3	1	0	0	0	12.5	11.0	11.7	12.2
Molly Wagle	pool	5	N.P.	—	20.0	26.7	41.1	N.P.				0	0	0	0	—	9.9	9.4	10.0
Roadside No. 1	pool	6	N.P.	251.1	164.4	140.0	128.9	N.P.				1	0	0	0	12.0	9.9	8.9	10.0

---

[1] Based on quantitative (Dickson, 1975; Dickson and Kirk, in press) and qualitative comparisons of the amount and type of available food (small mammal dung, mud sediments and plant detritus) for *C. antennatus* in each habitat. (1-lowest, 6-highest).

[2] Based on average current velocity in habitats determined by calibrated pitot tube. Ratings: 1) <3.5 cm/sec, 2) 3.5 cm/sec and 3) >3.5 cm/sec.

[3] The number of potential amphipod predators observed within 1 m² of sample plots.

tennae to the base of the telson as an indication of total body length (Holsinger, 1967). Sexual maturity was determined in the male by the presence of calceoli (small, paddle-shaped structures) on the second antennae in addition to papillae and in the female by long marginal setae on the oostegites which may be retained during successive molts (Hynes, 1954; Holsinger, 1967). The number of first antennal segments (in addition to the first peduncular segment) was also recorded for each amphipod. Because of sexually dimorphic size differences between mature male and female *C. antennatus,* statistical analyses were conducted separately for each sex.

RESULTS

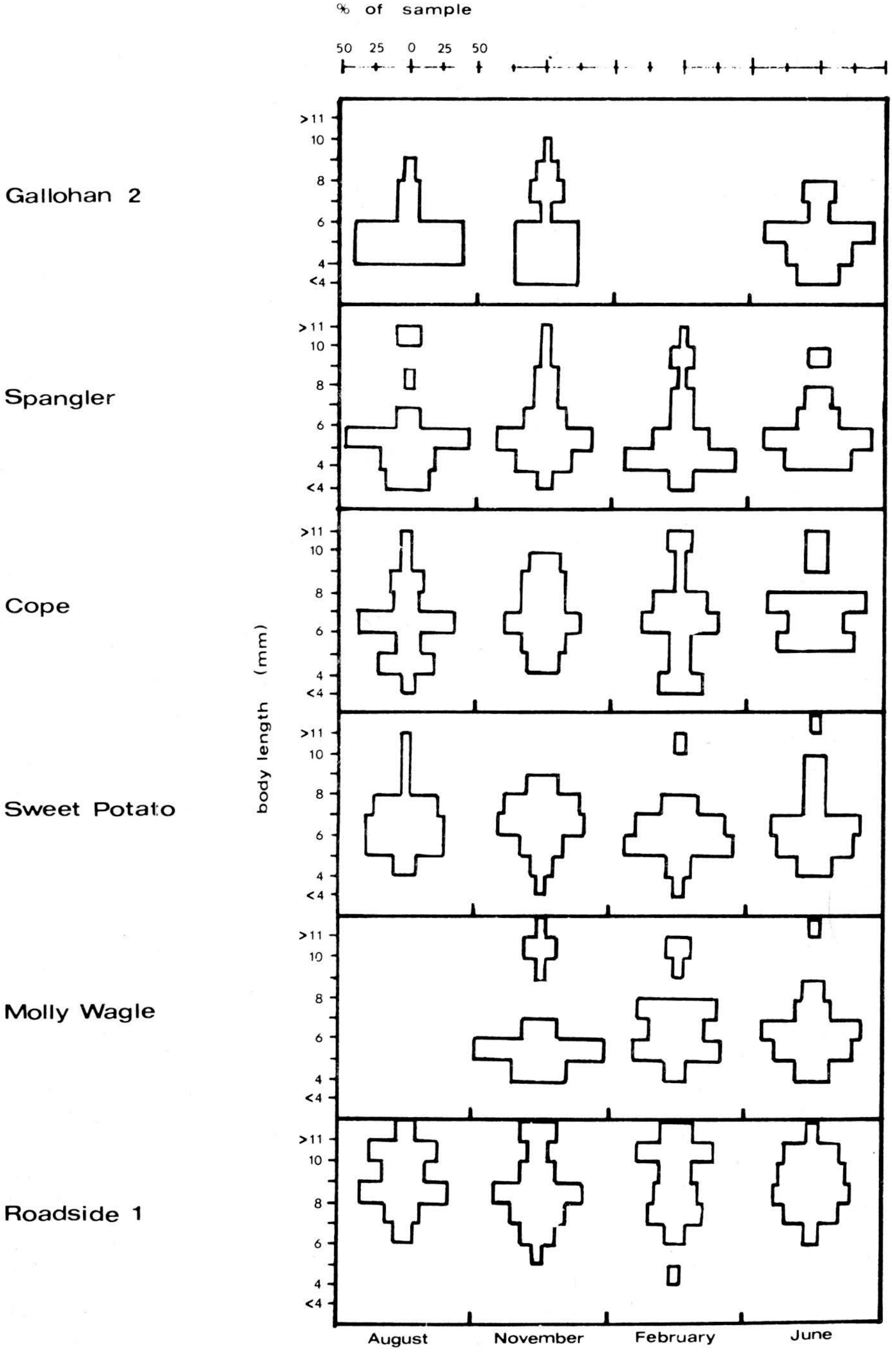
The environmental parameters recorded for each cave are presented in Table 1. In general, mud-bottom pool habitats are characterized by greater quantities of available food, higher *C. antennatus* densities, lower water temperatures, lower isopod densities and fewer predators than the small gravel-bottom stream habitats.

Size class distributions, based on body lengths of populations from each of the six caves is shown in Figure 1. The pool habitats are characterized by a higher proportion of individuals located in larger size classes than in the stream habitats. This trend is also apparent in size class distributions of both males (Fig. 2) and females (Fig. 3).

Mean body lengths of both mature males and females (Table 2) were found to be significantly ($P<0.001$) greater in pool habitats than in stream habitats (Table 3; Figs. 2 and 3). No significant ($P>0.05$) seasonal differences in mature body lengths were observed between habitats in either sex (Table 3). Although a significant ($P<0.01$) correlation was found between mature body lengths of males and females with both the overall food rating and *C. antennatus* densities, no significant ($P>0.05$) relationship to either isopod densities or predator sightings was observed (Table 4). Mature male body length was found to be inversely correlated ($P<0.05$) with average water temperatures and positively correlated to current velocity rating (Table 4). Finally, a significant correlation was noted between overall habitat food ratings and both the average water temperature ($r=-0.658$, $n=22$, $P<0.01$) and *C. antennatus* densities ($r=0.663$, $n=23$, $P<0.01$).

Examination of newly released young from both habitats revealed that the number of first antennal segments appears to be a fixed developmental feature at hatching (no. of segments=6, n=48). The number of first antennal segments of pool-dwelling amphipods was found to be significantly ($P<0.001$) greater

Fig. 1. Size class distribution in random samples of *C. antennatus* collected from each cave seasonally. Vertical axis represents amphipod body lengths; horizontal axis, percent frequency. Due to adverse conditions, sampling could not be conducted in Molly Wagle Cave in August and Gallohan No. 2 in February.


% of sample
50 25 0 25 50
>11
10
8
6
4
<4
body length (mm)
Gallohan 2
Spangler
Cope
Sweet Potato
Molly Wagle
Roadside 1
August
November
February
June

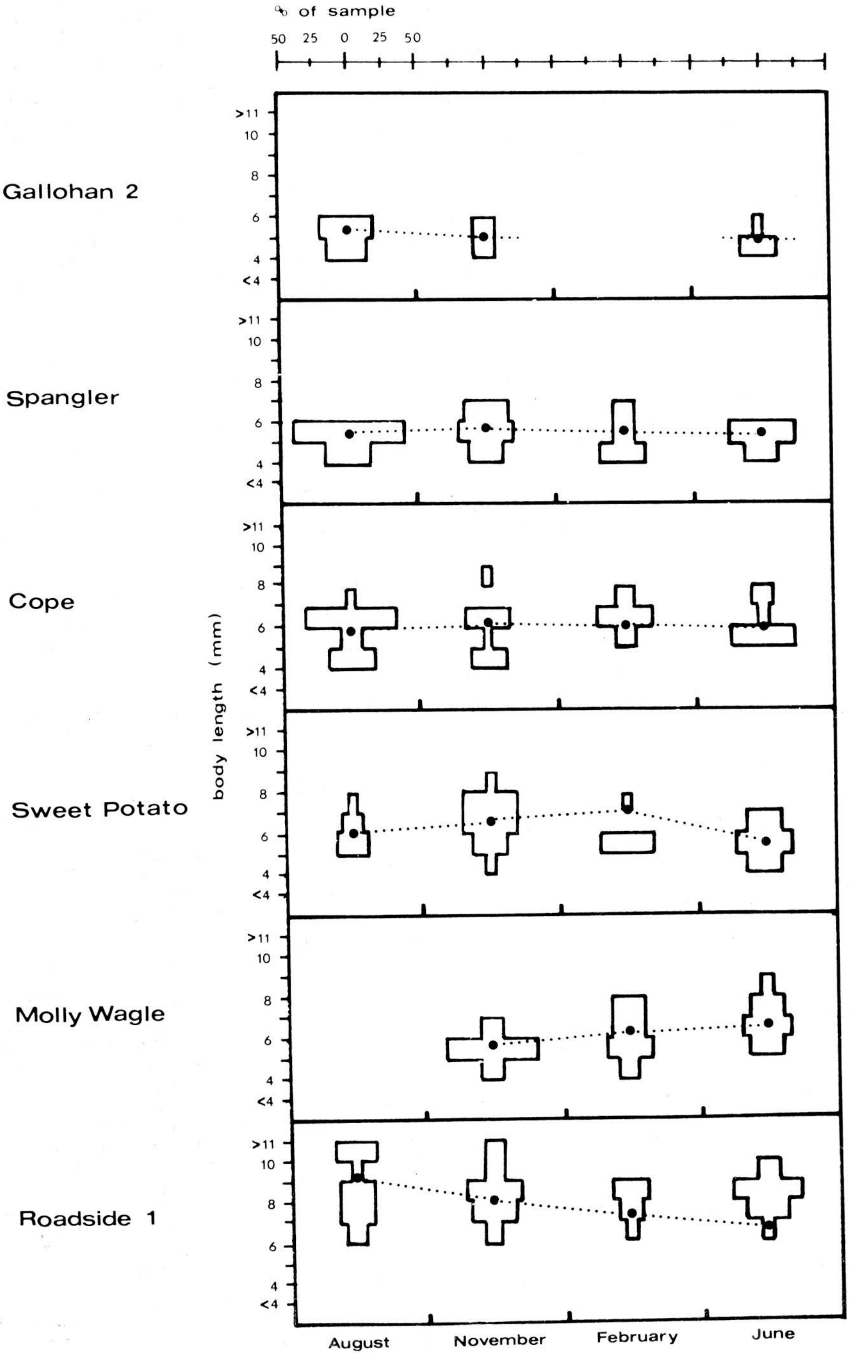
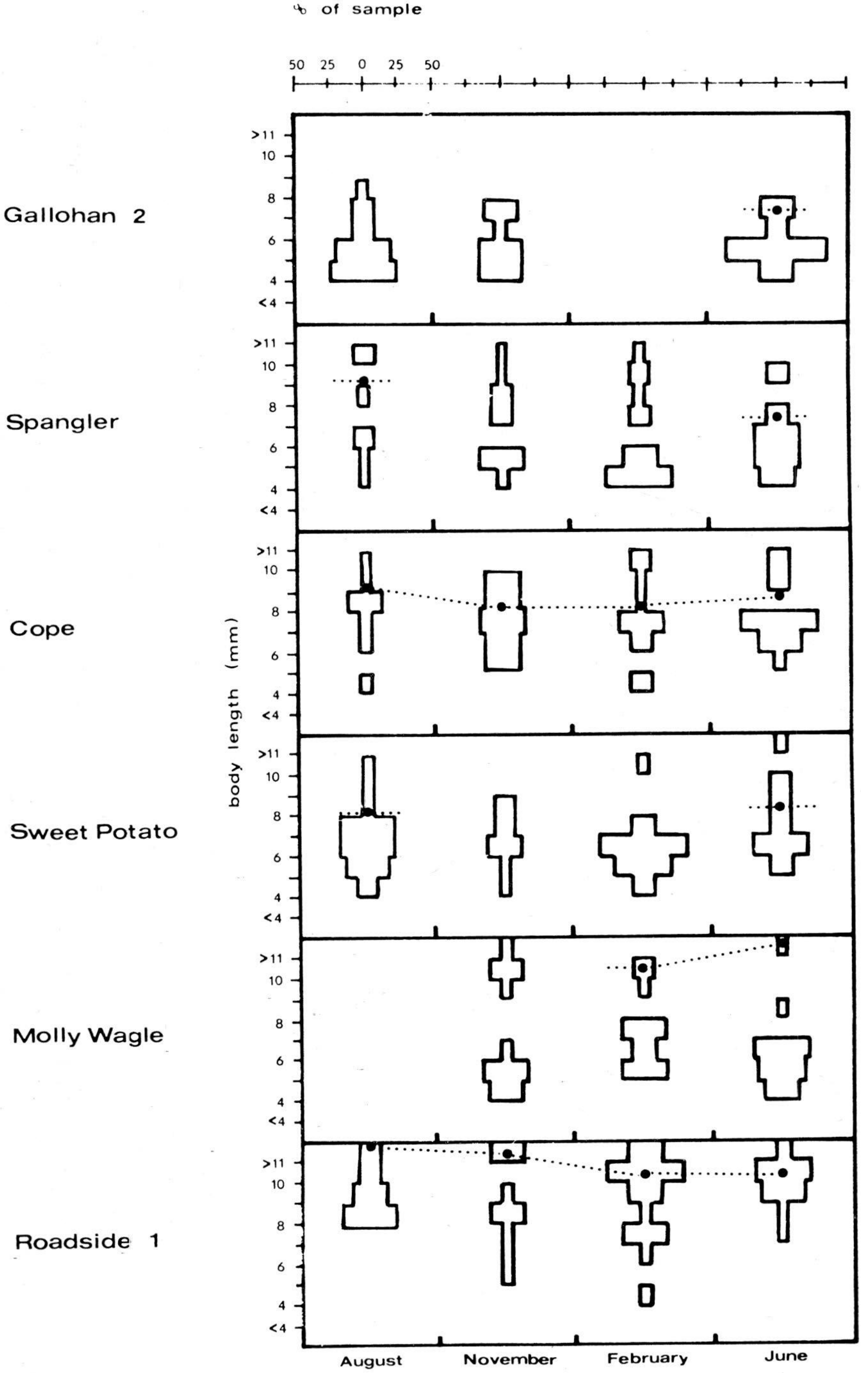


Fig. 2. Size class distribution of males in random samples of *C. antennatus* collected from each cave seasonally. Vertical axis represents amphipod body lengths; horizontal axis, percent



cave seasonally. Vertical axis represents amphipod body lengths; horizontal axis, percent frequency. Broken lines represent average mature female body length. The absence of broken lines indicates that no mature females were observed in the seasonal collection.

Table 2. Mean mature body lengths (mm) of *C. antennatus* from seasonal random samples of six cave populations in Lee Co., Virginia.

	August		November		February		June	
	male	female	male	female	male	female	male	female
Gallohan No. 2	5.4 n = 5 SE = 0.15	—	5.1 n = 3 SE = 0.33	—	No collection		4.9 n = 4 SE = 0.13	7.3 n = 1
Spangler	5.4 n = 10 SE = 0.12	9.5 n = 2 SE = 1.15	5.9 n = 10 SE = 0.21	—	5.6 n = 4 SE = 0.34	—	5.2 n = 4 SE = 0.25	7.3 n = 1
Cope	6.0 n = 14 SE = 0.23	9.1 n = 1	6.3 n = 6 SE = 0.49	8.1 n = 3 SE = 0.68	6.2 n = 9 SE = 0.25	8.2 n = 3 SE = 0.70	5.9 n = 4 SE = 0.26	8.8 n = 5 SE = 0.50
Sweet Potato	6.1 n = 6 SE = 0.30	8.2 n = 3 SE = 0.44	6.6 n = 14 SE = 0.25	—	6.4 n = 3 SE = 0.65	—	5.6 n = 11 SE = 0.18	8.3 n = 2 SE = 0.20
Molly Wagle	No collection		5.8 n = 10 SE = 0.15	—	6.4 n = 10 SE = 0.23	10.5 n = 1	6.8 n = 9 SE = 0.25	11.9 n = 1
Roadside No. 1	9.1 n = 12 SE = 0.35	12.4 n = 1	8.2 n = 15 SE = 0.30	11.4 n = 3 SE = 0.12	7.6 n = 6 SE = 0.24	10.4 n = 8 SE = 0.32	6.9 n = 13 SE = 0.39	10.4 n = 5 SE = 0.22

Table 3. Comparison of mature *C. antennatus* body lengths between stream and pool habitats and seasonal collections. The number of first antennal segments is compared between stream and pool populations. Significance is determined by one-way analysis of variance (model-I).

	F ratio	Numerator df	Denominator df	P
Mature male body lengths between habitats	70.7	1	194	<0.001 Sig.
Mature male body lengths between seasons	1.2	3	196	>0.05 N.S.
Mature female body lengths between habitats	173.8	1	34	<0.001 Sig.
Mature female body lengths between seasons	0.4	3	36	>0.05 N.S.
First antennal segment number between habitats	476.8	1	511	<0.001 Sig.

than that of stream-dwelling ones (Table 3). Linear regression analyses (model-II, Bartlett) of the number of first antennal segments to body length produced different slopes in each of the six cave populations (Fig. 4). The three stream populations exhibited greater positive slopes than the three pool populations, with the two extremes, Gallohan No. 2 and Roadside No. 1 caves, being significantly ($P<0.001$) different (Fig. 4).

Live amphipods, observed in the field and in the laboratory, exhibited a distinct difference in body integument coloration from the two habitats. Stream-dwelling amphipods are brownish in color in contrast to pool-dwelling ones which are whitish in color.

DISCUSSION

It has been determined that significant morphological variation exists among populations of *C. antennatus* living in different cave habitats in a small section of the overall range. Environmental differences between habitats apparently influence this variation.

Table 4. Correlation of mature male and female *C. antennatus* body lengths with environmental parameter values (See Table 1).

	Overall food rating	Current velocity rating	Population density *C. antennatus*	Population density isopods	Predator sightings	Average water temperature
Mature male body length	$r=0.853$ $n=22$ $P<0.01$ Sig.	$r=0.903$ $n=11$ $P<0.01$ Sig.	$r=0.801$ $n=22$ $P<0.01$ Sig.	$r=-0.487$ $n=15$ $P>0.05$ N.S.	$r=-0.474$ $n=6$ $P>0.05$ N.S.	$r=-0.488$ $n=21$ $P<0.05$ Sig.
Mature female body length	$r=0.823$ $n=14$ $P<0.01$ Sig.	$r=0.488$ $n=7$ $P>0.05$ N.S.	$r=0.716$ $n=15$ $P<0.01$ Sig.	$r=-0.250$ $n=9$ $P>0.05$ N.S.	$r=-0.440$ $n=6$ $P>0.05$ N.S.	$r=-0.473$ $n=14$ $P>0.05$ N.S.

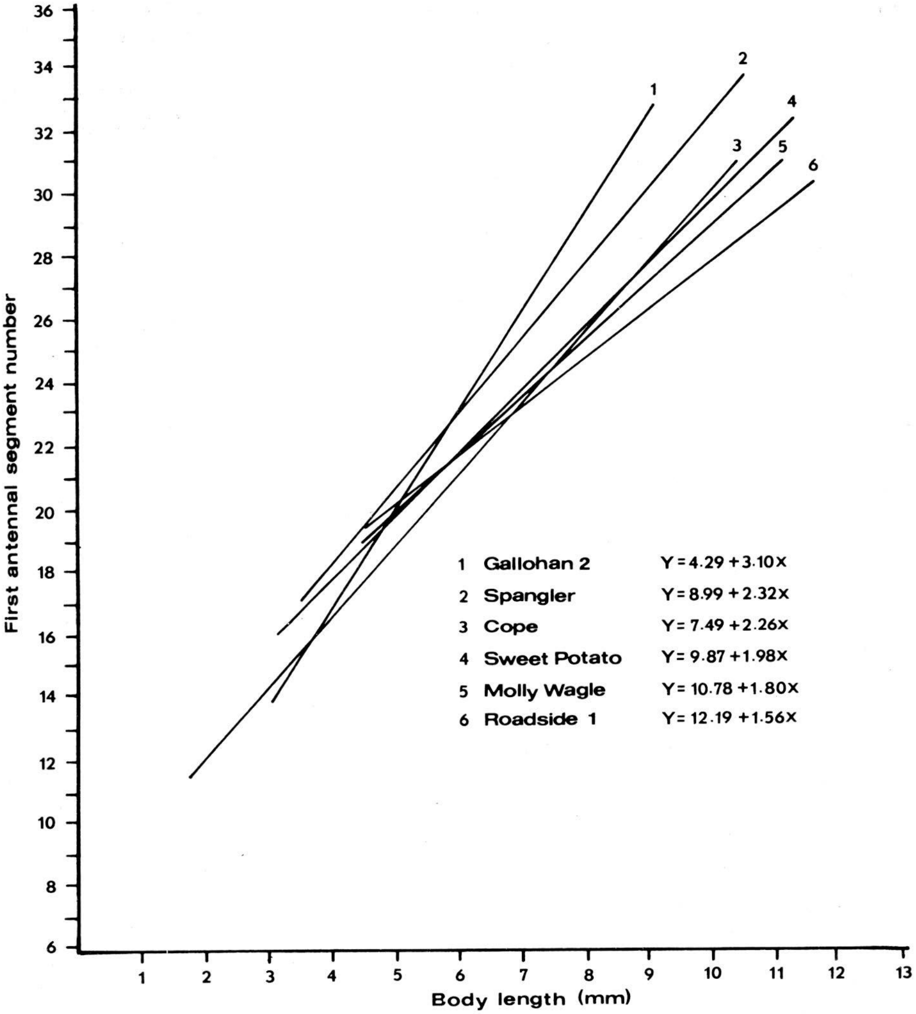


Fig. 4. Regression (model-II, Bartlett) of *C. antennatus* body length plotted against the number of first antennal segments in each of the six caves. A significant difference was found between Gallohan No. 2 and Roadside No. 1 cave populations (F(1,171)=18.9, P<0.001).

The amount of available food within each habitat appears to be the most important environmental factor affecting mature body length in *C. antennatus*. Results from both this study (Table 4) and a laboratory investigation of morphological variation in juvenile *C. antennatus* reared on different substrates (Dickson, in press) indicate a direct relationship between available food and body length. Similar observations on the amount of habitat food affecting body length were made in cave populations of another troglobitic crustacean, the crayfish *Orconectes pellucidus* (Jegla et al., 1965).

Population densities of *C. antennatus* and average water temperature of the habitats, although indicating a direct relationship with mature amphipod body lengths (Table 4), represent factors which are also correlated with overall food ratings of the habitats and thus probably have no major individual effects on mature body size. This interpretation is supported by studies on populations of other freshwater crustaceans. Population density was not observed to affect mature body lengths in populations of the copepod *Cyclops strenuus strenuus* (Elgmork and Halvorsen, 1976) and the crayfish *Orconectes pellucidus* (Jegla et al., 1965). In a laboratory investigation of the amphipod *Gammarus pulex,* decreased water temperatures were related to decreased biomass production and smaller size (Nilsson, 1974).

Current velocity and incidence of flooding are two of the most frequently cited causes for the selection of small size in stream invertebrates (Pennak, 1953; Houston, 1960; Patrick, 1970). This variation is explained as an adaptation to flowing water habitats through both the decrease of surface area exposed to water currents and the ability of organisms to move deeper into the benthic gravels. Based on observations by Culver (1973a) on the presence of apparently smaller mature *C. antennatus* in benthic gravels of Cope and Gallohan No. 2 caves, size variation in this species was initially thought to represent an adaptation to flowing waters. After comparison of all of the populations investigated, current velocity does not appear to be a primary factor in the selection of smaller body lengths in *C. antennatus,* as larger amphipod body lengths were correlated with greater average current velocities (Table 4).

Severe depression of population densities of *C. antennatus* has been noted due to intense competition with the isopods, *A. recurvatus* and *L. usdagalun* (Culver, 1973a). This effect was also observed in the present study; however, no evidence was found to indicate that population densities of potentially competitive isopods were a major cause of mature body length variation in *C. antennatus* (Table 4). The presence of relatively large populations of amphipod predators also was not found to be associated with smaller *C. antennatus* in the habitats studied (Table 4). These results support Culver's (1973b) observations that the salamander *G. porphyriticus* does not exhibit feeding selectivity for larger prey over smaller prey, thus indicating the apparent absence of predator-influenced selection pressure for small body size.

Another morphological feature found to vary in *C. antennatus* populations was the relationship between body length and the number of first antennal segments. The first pair of antennae in amphipods plays an important role in chemoreception (Kaestner, 1970). The length of the first antennae frequently seems to be an allometric function of body length (Holsinger, 1967; Holsinger and Culver, 1970) and the number of segments added during each molt can vary between individuals of the same species (Geisler, 1944).

The number of first antennal segments in *C. antennatus* was found to be significantly greater in pool populations than in stream populations (Table 3).

This was initially thought to represent an allometric growth function involving additional antennal segments being associated with larger amphipods. After examination of regression slopes representing this linear relationship in the six populations (Fig. 4), it becomes apparent that another factor is also affecting this relationship. The regression lines of the three stream populations are found to exhibit greater positive slopes than the pool populations, indicating that a greater number of first antennal segments per unit body length are associated with the smaller stream amphipods. Although this might be caused by an increase in the number of molts in stream habitat amphipods, the associated increase in metabolic activity which is necessary for ecdysis (Waterman, 1960) would not be expected to occur more frequently in relatively food-poorer habitats. If it is assumed that the chemosensory structures located on the first antennae are utilized to locate food, this population variation could represent a selective adaptation to increasingly food-poor habitats through the accumulation of additional sets of chemoreceptors located on each segment. Although not significant, a trend was observed in juvenile *C. antennatus* in which amphipods reared on food-poor substrates possessed a greater number of first antennal segments than individuals reared on food-rich substrates (Dickson, in press). Increase in numbers of sensory structures in relation to less available habitat food has also been noted in cave populations of amblyopsid fish (Poulson, 1963).

The integumentary pigment of amphipods is composed of various carotenoid-protein complexes which must be obtained from food sources (i.e., leaf and woody detrital material) because of the inability to synthesize them (Beatty, 1949; Kaestner, 1970). Some troglobitic crustaceans are thought to have genetically lost the ability to utilize carotenoids in integumentary pigmentation, while in others it depends on exposure to food materials containing these substances (Beatty, 1942; Maguire, 1961; Wolfe and Cornwell, 1964). The distinct variation in integument coloration between stream and pool populations is apparently due to differences in the type of available food. Populations inhabiting streams possess a brownish integument, probably due to the availability of carotenoid materials in stream detritus. Pool populations possess a whitish integument, apparently because of the lack of allochthonous plant material and the loss of carotenoids from drip water due to percolation through limestone as reported by Beatty (1942). Similar results were found in a laboratory study which correlated integument coloration with food type in young *C. antennatus* (Dickson, in press).

Strong (1972) found through laboratory breeding experiments that morphological variation in isolated populations of the amphipod *Hyalella azteca* was inherited. However, two factors tend to indicate that morphological variation in *C. antennatus* is environmentally rather than genetically induced. The populations of *C. antennatus* investigated are not believed to be isolated genetically for reasons stated under Methods and Materials; and, second, the significant morphological variation observed in juvenile *C. antennatus* from a single clutch under different environmental conditions (Dickson, in press) indicates the presence of morphological flexibility within amphipods from a single habitat.

Studies by Culver (1973a, in press) indicating that *C. antennatus* experiences the most intense competition and exhibits the highest migration rates of all of the aquatic cave crustaceans in Lee Co., Virginia and the evidence for its high dispersal potential between caves (Holsinger, 1969, 1975) suggests that this species has evolved increased vagility to escape competitive pressures. Variability encompassed within the genome and the accompanying ability to adapt to various environmental conditions would enhance this species survival in the habitats encountered during dispersal.

ACKNOWLEDGMENTS

I am indebted to Dr. John R. Holsinger for ideas and assistance during the entire project and for making helpful comments on the manuscript. Dr. David C. Culver provided valuble advice concerning initial aspects of the study. I wish to thank George D. Corbett, Jean Dickson, James A. Estes, Stephen W. Hetrick and David Wapinski for their help in the field.

SUMMARY

Populations of the troglobitic (i.e., obligatory cavernicole) amphipod *Crangonyx antennatus* living in two distinct aquatic habitats were examined for possible morphological variation. Collections were made seasonally for one year in six Lee Co., Virginia caves, three with mud-bottom pools and three with small gravel-bottom streams. Environmental parameters thought to influence population variation were recorded for each of the six caves. Body length of mature amphipods was found to be greater in the mud-bottom pool habitats, whereas stream amphipods possessed more first antennal segments per unit body length. Variation was also observed in integument coloration; stream amphipods were characterized by a brownish integument and pool amphipods a whitish integument. Differences in the type and amount of available food in the two habitats is considered the most important environmental parameter affecting morphological variation. The population variation noted between habitats is believed indicative of the adaptive flexibility of this vagile troglobitic species.

RÉSUMÉ

Une éventuelle variation morphologique a été recherchée chez *Crangonyx antennatus*, Amphipode troglobie (c'est-à-dire cavernicole obligatoire), dans des populations vivant dans deux biotopes aquatiques distincts. Des récoltes saisonnières ont été faites pendant une année dans six grottes du Lee Co. (Virginie), trois d'entre elles présentant des flaques d'eau à fond argileux, les trois autres des cours d'eau à lits de graviers. Les paramètres du milieu susceptibles d'influencer la variation de la population ont été enregistrés dans chacune des six grottes. Les Amphipodes matures des flaques d'eau ont une taille plus grande, tandis que chez ceux des eaux courantes le nombre des premiers segments antennaires est plus élevé, corrélativement à la longueur du corps. La couleur du tégument montre aussi une variation: les Amphipodes des eaux courantes sont caractérisés par un tégument brunâtre, ceux des flaques par un tégument blanchâtre. D'après les différences observées dans le type et la quantité de nourriture disponible dans les deux biotopes, on doit considérer le facteur nourriture comme le paramètre le plus important de la variation morphologique. La variation des populations des deux biotopes montre, selon toute vraisemblance, la souplesse d'adaptation de cette espèce troglobie.

REFERENCES

BEATTY, R. A. 1942. The pigmentation of cavernicolous animals. I. Carotenoid pigments in the cave environment. J. exp. Biol. 18: 144-152.

— 1949. The pigmentation of cavernicolous animals. III. The carotenoid pigments of some amphipod Crustacea. J. exp. Biol. 26: 125-130.

CULVER, D. C. 1973a. Competition in spatially heterogeneous systems: An analysis of simple cave communities. Ecology 54 (1): 102-110.

— 1973b. Feeding behavior of the salamander *Gyrinophilus porphyriticus* in caves. Int. J. Speleol. 5: 369-377.

— In press. The evolution of aquatic cave communities. Amer. Natur.

DICKSON, G. W. 1975. A preliminary study of heterotrophic microorganisms as factors in substrate selection of troglobitic invertebrates. Nat. Speleol. Soc. Bull. 37 (4): 89-93.

— In press. The importance of cave mud sediments in food preference, growth and mortality of the troglobitic amphipod crustacean *Crangonyx antennatus* Packard (Crangonyctidae). Crustaceana.

— and P. W. KIRK, Jr. In press. Distribution of heterotrophic microorganisms and their relation to detritivore populations in Virginia caves. *In* M. K. Roane and R. A. Paterson (eds.), The distributional history of the biota of the southern Appalachians. Part IV. Algae and fungi. University Press of Virginia, Charlottesville, Va.

ELGMORK, K. and G. HALVORSEN. 1976. Body size of free-living copepods. Oikos 27 (1): 27-33.

GEISLER, Sister F. S. 1944. Studies on the postembryonic development of *Hyalella azteca* (Saissure). Biol. Bull. 86: 6-22.

GINET, R. 1960. Écologie, éthologie et biologie de *Niphargus* (Amphipodes, Gammaridés hypogés). Ann. Spéléol. 15: 127-376.

HOLSINGER, J. R. 1967. Systematics, speciation, and distribution of the subterranean amphipod genus *Stygonectes* (Gammaridae). U. S. Nat. Mus. Bull. 256: 1-176.

— 1969. Biogeography of the freshwater amphipod crustaceans (Gammaridae) of the central and southern Appalachians. pp. 19-50. *In* P. C. Holt (ed.), The distributional history of the biota of the southern Appalachians. Part I. Invertebrates. Virginia Polytechnic Inst. Press, Blacksburg, Va.

— 1972. The freshwater amphipod crustaceans (Gammaridae) of North America. Biota of freshwater ecosystems, Identification manual No. 5, U. S. Environmental Protection Agency, 89 pp.

— 1975a. Observations on the dispersal of the cavernicolous amphipod crustacean *Crangonyx antennatus* Packard. Bull. Assoc. Southeastern Biologists 22: 58.

— 1975b. Descriptions of Virginia caves. Virginia Division of Mineral Resources. Bull. 85: 1-450, 7 plts.

— and D. C. CULVER. 1970. Morphological variation in *Gammarus minus* Say (Amphipoda, Gammaridae), with emphasis on subterranean forms. Postilla 146: 1-24.

— and C. H. HOLSINGER. 1971. Observations on the population ecology of the cavernicolous amphipod crustacean *Crangonyx antennatus* Packard. Virginia J. Sci. 22 (3): 97.

— and G. W. DICKSON. In press. Burrowing as a means of survival in the troglobitic amphipod crustacean *Crangonyx antennatus* Packard (Crangonyctidae). Hydrobiologia.

HOUSTON, K. 1960. Size of *Gammarus pulex* in fast and slow-running streams. Report Dauntsey's School Nat. Hist. Soc. 1959-1961, pp. 11-13.

HYNES, H. B. N. 1954. The ecology of *Gammarus dueboni* Lilljeborg and it's occurrance in fresh water in western Britain. J. Anim. Ecol. 23: 38-84.

JEGLA, T. C., T. L. POULSON and M. R. COOPER. 1965. Interpopulation variations in a cave crayfish *(Orconectes pellucidus).* Amer. Zool. 5 (4): 639.

KAESTNER, A. 1970. Invertebrate zoology. Vol. 3, Crustacea. New York Interscience, N.Y. 523 pp.

MAGUIRE, B., Jr. 1961. Regressive evolution in cave animals and it's mechanism. Texas J. Sci. 13: 363-370.

MINCKLEY, W. L. and G. A. COLE. 1963. Ecological and morphological studies on gammarid amphipods (*Gammarus* spp.) in spring-fed streams of northern Kentucky. Occasional Papers of the C. C. Adams Center for Ecol. Studies 10: 1-35.

NILSSON, L. M. 1974. Energy budget of a laboratory population of *Gammarus pulex* (Amphipoda). Oikos 25: 35-42.

PATRICK, R. 1970. Benthic stream communities. Amer. Scientist 58 (5): 546-549.

PENNAK, R. W. 1953. Fresh-water invertebrates of the United States. Ronald Press, New York. 769 pp.

PINKSTER, S. 1971. Members of the *Gammarus pulex*-group (Crustacea-Amphipoda) from North Africa and Spain, with description of a new species from Morocco. Bull. Zool. Mus. Univ. Amsterdam 2 (7): 45-61.

— 1972. On members of the *Gammarus pulex*-group (Crustacea-Amphipoda) from western Europe. Bijdragen tot de Dierkunde 42 (2): 164-191.

— , A. L. DENNET, B. STOCK and J. H. STOCK. 1970. The problem of European freshwater populations of *Gammarus dueboni* Lilljeborg, 1852. Bijdragen tot de Dierkunde 40 (2): 116-147.

POULSON, T. L. 1963. Cave adaptation in amblyopsid fishes. Amer. Midl. Nat. 70 (2): 257-290.

STRONG, D. R. 1972. Life history variation among populations of an amphipod *(Hyalella azteca).* Ecology 53 (6): 1103-1111.

WATERMAN, T. H. 1960. The physiology of Crustacea. Vol. I. Metabolism and growth. Academic Press, New York. 670 pp.

WOLFE, D. A. and D. G. CORNWELL. 1964. Carotenoids of cavernicolous crayfish. Science 144 (3625): 1467-1469.

Int. J. Speleol. 9 (1977), pp. 59-63.

Nouvelles données sur l'écologie et la biogéographie de *Niphargus nadarini* Alouf (Crustacés, Amphipodes)

par

N. J. ALOUF*

New data about the biogeography and the ecology of Niphargus nadarini Alouf (Crustacea, Amphipoda)

SUMMARY

Some new samples made between 1971 and 1975 show that the area of extension of *Niphargus nadarini* Alouf is the Biqâ's plain. New data about the ecology of this species is given.

Lors de la description de *Niphargus nadarini* Alouf (1972), cette espèce n'était connue que des puits du village Hawsh Hâlâ-Rayack et son statut écologique restait à définir. Des prospections ultérieures, en vue d'élargir son aire de répartition, permirent sa découverte dans les puits d'une autre localité et dans les eaux de deux résurgences. Le moyen de récolte dans les puits était la balance classique posée préalablement pour quelques heures et le filet de Surber pour l'eau de surface des résurgences.

DESCRIPTION DES STATIONS

Puits

Le village Talia est à 13 km au N.NE. de Hawsh Hâlâ. Les coordonnées sont: X: 2214, Y: 1847, altitude 1034 m feuille de Rayack au 1/50.000°. Les puits sont profonds de 37 m. L'eau, dont la température est de 17.5° en Septembre, se rencontre à 35 m du sol. Les récoltes furent effectuées le 1/9/71 et le 14/9/71. Les *Niphargus* capturés sont grands de taille, probablement adultes,

---

* Faculté des sciences. Université libanaise. Hadath Beyrouth. Liban.
Adresse actuelle: Nicolas Alouf V 8, Mail Service of Lebanon, LARNACA (Cyprus).

et répondent à la description du type de *N. nadarini,* excepté un individu qui avait une épine latérale sur un lobe du telson.

Résurgences

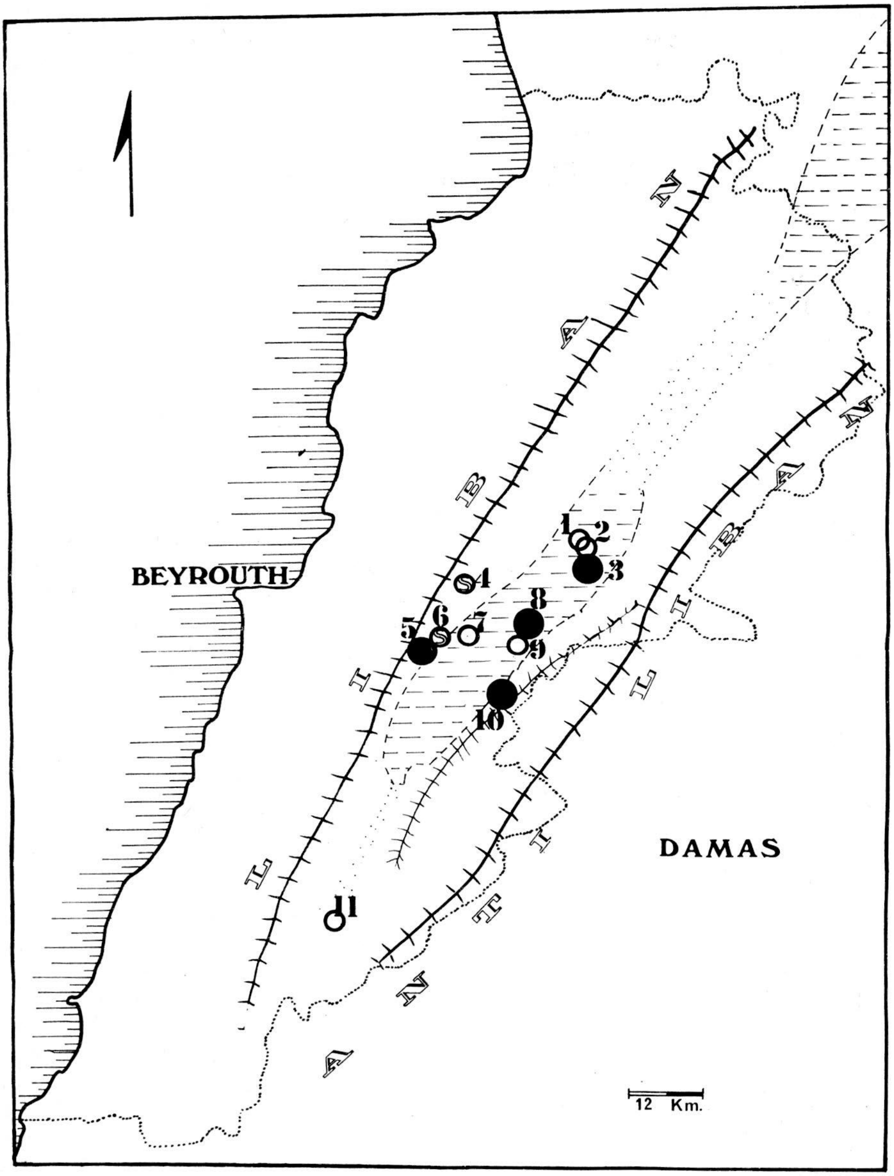
La résurgence Shamsîne est à 15 km au S.SE. de Hawsh Hâlâ. Les coordonnées sont: X: 1708, Y: 2005, altitude 871 m, feuille de Rachaya Nord au 1/50.000°. Cette station a été prospectée durant toute l'année 1974 (Alouf, à paraître) et la majeure partie de l'année 1975, dans le but de connaître la faune de surface. Les prélèvements avaient lieu à deux endroits: A 1 ou 2 mètres du point de sortie de l'eau et dans la mare qui y fait suite. La première station hébergeait des *Niphargus* durant une partie de l'année, tandis qui la seconde en était dépourvue. Cette résurgence est du type trop-plein, les points de sortie de l'eau se trouvent disséminés le long de la ligne de contact entre la plaine alluviale et le karst Nummulithique aquifère. La température est stable, les extrêmes observés sont de 14.8 et de 15.8°C. Les relevés sont faits vers midi et ont lieu entre le 15 et 20 de chaque mois. Tous les *Niphargus* capturés sont des jeunes, petits de taille.

Détail des données

1974	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
t°C	15.4	15.2	15.4	15.4	15.4	15.7	15.8	14.8	14.8	15.6	15.5	15.3
Nb.	0	0	1	1	2	3	5	0	0	0	0	0
1975												
t°C	15.3	14.9	14.9	14.9	15.7	15.6	15.7	15.6	15.4	—	15.8	—
Nb.	1	1*	3	37	14	8	0	0	1	—	1	—

* Une récolte, dans le milieu interstitiel à une vingtaine de centimètres de profondeur, a donné 12 *Niphargus.*

Répartition géographique de *Niphargus nadarini* au Liban.
— L'espace avec des tiretés indique l'emplacement du lac au néogène.
— Les ronds pleins indiquent les stations à résultats positifs.
— Les ronds vides indiquent les stations à résultats négatifs.
N° 1 — Puits à Hawsh Barada.
N° 2 — Puits à Magdalûn (Majdaloun).
N° 3 — Puits à Talia.
N° 4 — Résurgence Bardauni.
N° 5 — Résurgence Râs-l-'Ayn à Qabb Ilyâs.
N° 6 — Source Shtaura.
N° 7 — Puits à Sa'dnâyil.
N° 8 — Puits à Hawsh Hâlâ.
N° 9 — Puits à Turbol (Tourbol).
N° 10 — Résurgence Shamsîne.
N° 11 — Puits dans l'ancienne mine de bitume à Hâsbayyâ.


BEYROUTH
DAMAS
L I B A N
A N T I L I B A N
1
2
3
4
5
6
7
8
9
10
11
12 Km.

La résurgence Râs-l-'Ayn à Qabb Ilyâs, dont les coordonnées sont: X: 1580, Y: 2067, altitude 990 m, est à 19 km au S.W. de Hawsh Hâlâ. Elle a été prospectée durant la seconde moitié de l'année 1973, toute l'année 1974 et la première moitié de l'année 1975. C'est, aussi, une source de trop-plein. Les prélèvements, en quête de faune de surface, ont lieu au milieu du cours engendré, lors de la saison des pluies, par les eaux des sources, formant la résurgence, et par l'eau de ruissellement. La température de l'eau du ruisseau au niveau des sources varie entre 8.5, Février 1975, et 15.2°C, Septembre 1975. Celle des sources va de 12.5, Février 1975, et 14.5°C en été. La faune spécifique au milieu souterrain, qu'on peut capturer dans la zone des sources, est pratiquement absente, du fait que l'eau de ruissellement "camoufle" la source, surtout en période de crue. Toutefois, au début du mois de Mai 1975, il y avait un jeune *Niphargus* dans une récolte prise à un endroit proche d'un point de sortie de l'eau. Malheureusement, les prospections ont dû cesser à cause de l'état d'insécurité dans le pays.

CONSIDERATIONS ECOLOGIQUES

Les modalités de la colonisation du milieu souterrain par *N. nadarini* ne nous sont pas connues de même que la limite des marges que cet animal peut supporter. Ainsi, les maxima et les minima de température observés montrent un écart de 5° pour l'ensemble des stations et qui se réduit à 1 ou 2° seulement si chaque station est prise à part. Les données actuelles sont, encore, assez fragmentaires pour émettre des hypothèses sur la variabilité physiologique en fonction de la température (Ginet et Mathieu 1968). *N. nadarini* vit dans des milieux à température relativement élevée, 12 à 18°, par comparaison avec les espèces européennes qui ont leur optimum entre 8 et 14° (Ginet 1960).

Les localités de Hawsh Hâlâ-Rayack, Talia et Shamsîne sont sur le versant ouest de l'Anti Liban. Les puits et la résurgence sont alimentés par les chutes atmosphériques, pluies et surtout neiges, qui arrosent cette chaine de montagne. Les puits sont creusés dans des dépots alluviaux quaternaires qui reposent en discordance, à une profondeur indéterminé, sur du Nummulithique. Ces dépots qui forment la plaine de la Biqâ' *s.s.*, sont le produit de remblayement d'un ancien lac, d'origine marine, qui exista au néogène (Dubertret 1953). La résurgence de Râs-l-'Ayn est à quelques km de la plaine alluviale sur le versant est du Liban. Le terrain est karstique.

Bien que d'autres localités présentent ces mêmes conditions, il n'a pas été possible de rencontrer *N. nadarini* dans d'autres résurgences et puits de la région (voir carte). Ces résultats négatifs peuvent être dûs, soit à des accidents géologiques, géomorphologiques ou humains, soit à de mauvaises méthodes de prospection. Ce *Niphargus* semble être une espèce des eaux interstitielles colonisant les karsts et les alluvions quaternaires. Toutefois, si la balance classique, moyen de récolte dans les puits, permet l'obtention d'adultes, le filet de Surber ne permet la capture que d'animaux à la dérive qui sont, surtout, des jeunes incapables de s'accrocher au substrat lors de périodes de crue. Ceci serait valable aussi bien pour la faune aquatique d'origine épigée (Décamps et Rouch 1973) que pour la faune du milieu interstitiel.

L'ensoleillement, très intense dans cette région, est nettement défavorable au rayonnement et à la colonisation de certains milieux, *i.e.* sources et ruisselets de sources, que le *Niphargus* est capable d'occuper dans certaines régions où les conditions climatiques sont assez proches de celles du Liban, telle que la Corse (Stock 1972). Cette confination au milieu souterrain peut être, aussi, imputée au fait que la zone en question héberge, au Liban, des Gammares et qu'une certaine exclusive, quoique toute relative, entre ces deux groupes d'Amphipodes semble être la régle avec, toutefois, des exceptions (Ginet, Magné et Carrayat 1963).

Quoiqu'il en soit, il est certain que cette espèce a envahit le domaine limnique, au plus tard, au Nummulithique, date de la dernière incursion marine au Liban. Son centre de rayonnement serait la vallée du Litani à l'emplacement de ce qui fut le lac durant le néogène. Les conditions paléoécologiques qui y régnèrent rappellent les conditions qui dominaient dans le bassin pannonique, centre de rayonnement de *Niphargus* en Europe (Dubertret 1955, Ruffo et Krapp Schickel 1969 et Sket 1970).

RÉSUMÉ

De nouvelles récoltes faites entre 1971 et 1975 ont permis d'étendre l'aire de répartition de *Niphargus nadarini* Alouf à l'ensemble de la plaine de la Biqâ'. De nouvelles données sur l'écologie de cette espèce sont données.

BIBLIOGRAPHIE

ALOUF, N. J. - 1972. Description d'un nouvel Amphipode Gammaridé des eaux souterraines du Liban. *Niphargus nadarini* n. sp. Ann. Spéléol. 27, 547-558.

— à paraître. Cycle de reproduction de deux formes parentes de Gammares (Crustacés, Amphipodes) du Liban.

DUBERTRET, L. - 1953. Carte géologique du Liban au 1/50.000°. Feuille de Zahlé avec notice explicative. Rép. Libanaise Min. Trav. Publ. Beyrouth.

— 1955. Carte géologique du Liban au 1/200.000° avec notice explicative. Rép. Libanaise Min. Trav. Publ. Beyrouth.

GINET, R. - 1960. Ecologie, éthologie et biologie de *Niphargus* Ann. Spéléol. 15, 1-254.

GINET, R., MAGNÉ, J. et CARRAYAT, J. J. - 1963. Observations sur l'écologie et la systématique de quelques Amphipodes d'eaux souterraines et de sources dans le bassin d'Aquitaine. Bull. Soc. Hist. Nat. Toulouse. 98, 426-451.

GINET, R. et MATHIEU, J. - 1968. Comparaison des températures létales supérieures de *Niphargus longicaudatus* (Crust. Amphipodes) hypogés et épigés. Ann. Spéléol. 23, 425-440.

RUFFO, S. et KRAPP SCHICKEL, G. - 1969. Hydrobiologie der Randhöhlen. Teil II. Troglobionte Amphipoden aus Karstsiphonen von Istrien. Int. Revue Ges. Hydrobiol. 54, 685-696.

SKET, B. - 1970. Über Struktur und Herkunft der unterirdischen Fauna Jugoslawiens. Biol. Vestnik Ljubljana. 18, 69-78.

STOCK, J. H. - 1972. Les Gammaridae (Crustacés Amphipodes) des eaux douces et saumâtres de Corse. Bull. Zool. Mus. Univ. Amsterdam. 2, *19*, 197-220.

Int. J. Speleol. 9 (1977), pp. 65-71.

Stenasellus escolai n. sp., Crustacea Isopoda Asellota des eaux souterraines d'Espagne méridionale

par

Guy MAGNIEZ*

SUMMARY

Stenasellus escolai n. sp., Crustacea Isopoda Asellota from the subterranean waters of Southern Spain.

This new species was captured in the phreatic waters of Guadalquivir valley, where it lives together with another, very small-sized Stenasellid: *St. bragai* Magniez, previously described.

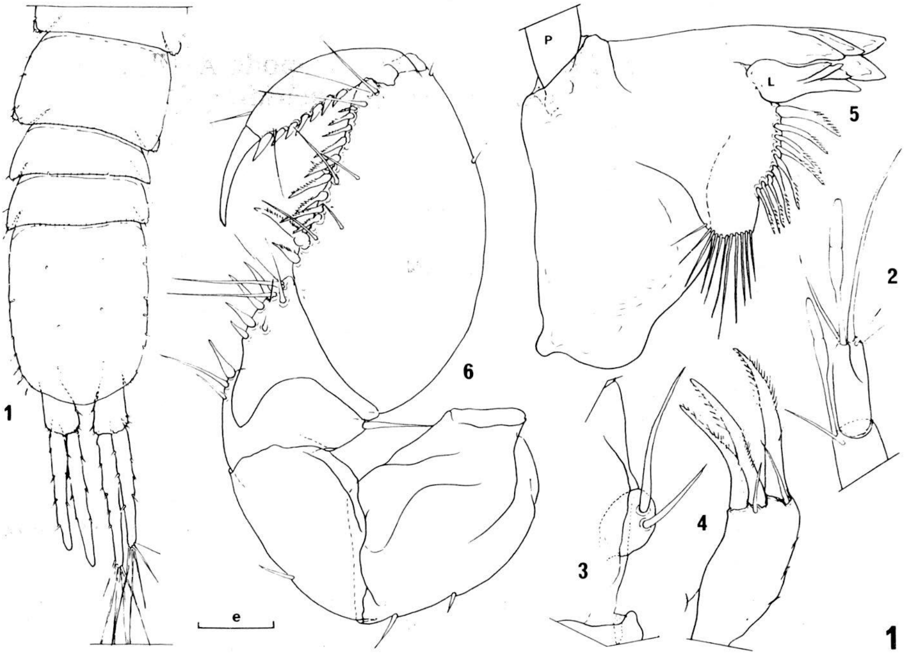
It belongs to the phyletic line of *St. breuili* Racovitza, which colonizes the subterranean waters of the Iberic peninsula.

ORIGINE DU MATÉRIEL: Ce nouveau Sténasellide a été capturé par Monsieur Claude Bou, en août 1972, au cours de prospections des eaux souterraines d'Espagne méridionale par le procédé des sondages tubés Bou-Rouch. La station est située sur le Guadalquivir, à environ 10 km en amont du confluent du fleuve avec le Guadiana Menor, un peu en aval du barrage supportant la route de Torreperogil à Peal-de-Becerro, province de Jaèn (renseignements aimablement communiqués par mon collègue Cl. Bou que je remercie de la confiance qu'il me témoigne en mettant ce matériel à ma disposition). Nous dédions cette forme au Dr. O. Escolà, de Barcelone, en raison de ses importantes contributions à la biospéléologie espagnole.

LOT CAPTURÉ: 3 ♂ adultes de 4,5; 4,7 et 5 mm; 1 ♀ de 4 mm immature (sans oostégites); 1 jeune de 1,5 mm sans péréiopodes VII (1er stade libre); 2 jeunes de 1,7 mm à péréiopodes VII rudimentaires (2ème stade libre) et 1 jeune de 1,9 mm à péréiopodes VII parfaits, mais plus courts que les VI (3ème stade libre). Les dimensions de ces trois stades larvaires libres, correspondant aux trois premières intermues postmarsupiales, sont donc pratiquement identiques à celles relevées chez *Stenasellus virei.*

---

* Laboratoire de Biologie Animale et Générale, Université de Dijon, 6, Bd. Gabriel, 21000 Dijon, France.

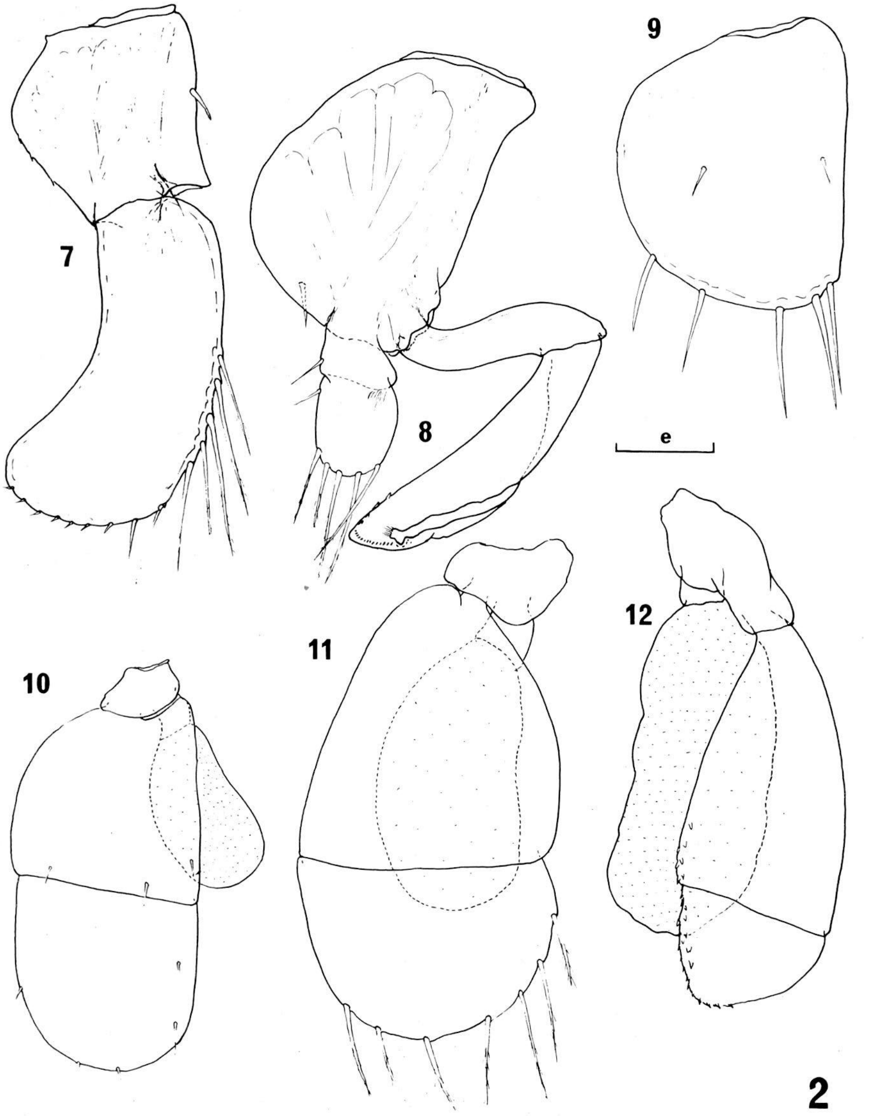


Figures I:
1. Péréionite VII, pléon et uropodes de la ♀ de 4 mm; e = 333 μ.
2. Extrémité d'une antennule du ♂ de 5 mm; e = 50 μ.
3. Article III de la hampe et *squama* d'une antenne du même; e = 50 μ.
4. Lobe interne de la maxillule gauche du même; e = 33 μ.
5. Mandibule gauche du même (P = palpe, supprimé; L = *lacinia mobilis*); e = 50 μ.
6. Péréiopode I gauche du même; e = 100 μ.

CARACTÈRES GÉNÉRAUX: *St. escolai* n. sp. est un *Stenasellus* de taille moyenne, dont les ♀ adultes devraient atteindre quelques 7-8 mm. L'aspect général rappelle celui de *St. virei virei*: téguments bien indurés, corps grêle (largeurs respectives de la tête, des péréionites I, IV, VII et du pléotelson: 640, 650, 700, 680 et 620 μ, chez la ♀ de 4 mm), mais appendices robustes, avec une chétotaxie générale bien développée.

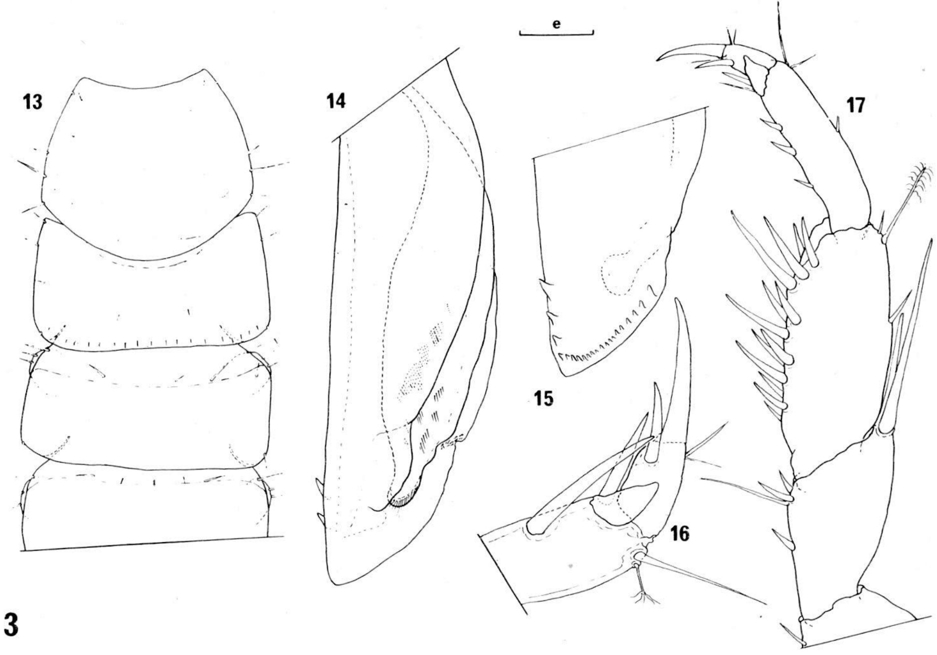
Céphalon subtrapézoïdal, plus large que long, à bord rostral bien concave. Régions pleurales des péréionites étroites, coxopodites des péréiopodes II-VII aussi développés que ceux de *St. virei* et débordant légèrement les péréionites (fig. 1, 13). Pléonites I et II bien développés en longueur. Pléotelson allongé, à pointe caudale effacée (fig. 1).

APPENDICES CÉPHALIQUES: Fouet de l'antennule (fig. 2) comptant jusqu'à 9 articles avec 6 lames olfactives de 95-100 μ sur les distaux (fouet de 2



Figures II:

7. Pléopode I gauche du ♂ de 5 mm; e = 100 μ.
8. Pléopode II gauche, face tergale, du même; e = 100 μ.
9. Pléopode II droit de la ♀ de 4 mm; e = 100 μ.
10. Pléopode III droit du ♂ de 5 mm; e = 200 μ.
11. Pléopode IV droit du même; e = 100 μ.
12. Pléopode V gauche du même; e = 100 μ. Les endopodites des pléopodes III, IV et V, charnus et respiratoires, sont figurés en pointillés.



Figures III:
13. Céphalon et premiers péréionites, face dorsale, de la ♀ de 4 mm; e = 200 μ
14. Extrémité de l'endopodite, face tergale, du pléopode II gauche du ♂ de 5 mm. Les sétules tapissant la paroi interne et bordant l'orifice efférent ont été schématisées; e = 33 μ.
15. Le même, face sternale, montrant l'armature de denticules chitineux distaux. L'orifice efférent, tergal, est indiqué en pointillés; e = 33 μ.
16. Extrémité du péréiopode VII gauche de la ♀ de 4 mm; e = 50 μ.
17. Péréiopode V gauche du ♂ de 5 mm, montrant la puissante armature sternale du carpopodite; e = 100 μ.

articles, avec une seule L.O. chez le jeune au premier stade; 3 articles + 1 L.O. au deuxième stade et 4 articles + 2 L.O. au troisième).

Antennes atteignant la moitié de la longueur du corps, *squama* bien développée, portée par le troisième article de la hampe (fig. 3) et munie de 2 tiges lisses; fouet de 25-27 articles chez le ♂ adulte, 22-23 chez la ♀, 10 seulement aux 1er et 2ème stades et 11 au 3ème stade.

Mandibules typiques, massives (fig. 5) avec palpe triarticulé normal; article distal muni d'un peigne de 8 tiges lisses croissant distalement (la distale atteint 120-130 μ).

Maxillules normales, à lobe externe portant 11 fortes épines barbelées distales de 30-50 μ, disposées sur deux rangs et quelques sétules sur la marge interne; lobe interne (fig. 4) avec 3 fortes tiges rameuses et 2 soies simples sur la marge distale; marge externe portant quelques sétules.

Maxilles trilobées normales; lobes externe et moyen portant chacun une rangée distale de 7 lames falciformes, dentelées sur leur marge interne, de longueur décroissant de l'extérieur (70 μ) vers l'intérieur (20 μ); lobe interne entier, portant distalement une quinzaine de tiges rameuses polymorphes (30-55 μ), implantées sur deux rangs, ainsi que de fines soies sur la marge interne.

MAXILLIPÈDES: Typiques de *Stenasellidae,* sans épipodite; endite avec 2 crochets rétinaculaires.

PÉRÉIOPODES I: Assez semblables à ceux de *St. virei* (fig. 6). Propodite bien renflé, à marge sternale fortement armée de lames dentelées; marge interne du dactylopodite munie d'une rangée continue de lames simples.

PÉRÉIOPODES II À VII: Robustes et assez trapus, à chétotaxie bien développée, surtout au niveau de la marge sternale des carpopodites (fig. 17). Dactylopodites avec une seule épine sternale. Longueurs respectives des péréiopodes I-VII chez le ♂ de 5 mm: 1,41 - 1,47 - 1,49 - 1,45 - 1,59 - 1,87 et 2,20 mm. Longueurs relatives à celle de P.II: 0,96 - 1 - 1,014 - 0,98 - 1,08 - 1,27 et 1,49. Une paire de papilles génitales longues et grêles insérées à l'angle interne des coxopodites VII chez les ♂.

PLÉOPODES II ♀ : Petits et indépendants (fig. 9), à marges externe et distale régulièrement courbes.

PLÉOPODES I ♂ : Protopodite subtrapézoïdal avec un crochet rétinaculaire sur la marge interne. Rame longue et étroite, à marge externe très concave. Une rangée continue de tiges plumeuses sur la marge interne-distale et de sétules sur la marge distale (fig. 7).

PLÉOPODES II ♂ : Protopodite subquadrangulaire avec une forte épine submarginale sternale externe (fig. 8). Exopodite biarticulé avec marge distale du second article munie de longues soies plumeuses. Endopodite avec article proximal long et grêle; article distal falciforme armé distalement d'une rangée sternale d'épines (20 environ), décroissant vers l'intérieur (fig. 15). Orifice efférent subterminal-tergal, petit et à marge garnie de fines soies faisant saillie à l'extérieur. Parois de la gouttière interne garnies de rangées de sétules dirigées distalement (fig. 14).

PLÉOPODES III: Exopodite (opercule) allongé; endopodite charnu, très petit (fig. 10).

PLÉOPODES IV: Exopodite lamelleux à suture non oblique; article distal muni de longues soies marginales plumeuses (fig. 11); endopodite petit et charnu.

PLÉOPODES V: Exopodite induré, biarticulé, en massue, plus long que l'endopodite; une aire glandulaire couverte d'écailles cuticulaires sur la marge interne-distale. Endopodite charnu arqué, respiratoire comme les endopodites III et IV (fig. 12).

UROPODES: Biramés normaux, pratiquement aussi longs que le pléotelson (fig. 1).

REMARQUES:

1. Les détails de structure (épines distales sur l'organe copulateur, constitution des pléopodes I ♂, des pléopodes IV et V) montrent que *St. escolai* appartient à la lignée ibérique de *St. breuili* (cf. Magniez 1974a, p. 42-43), qui comprend aussi *St. galhanoae* Braga de puits de l'Algarve et *St. bragai* Magniez du sous-écoulement du Guadalquivir.

2. *St. escolai* n. sp. et *St. bragai* vivent ensemble dans le même biotope: la nappe fluviale du Guadalquivir. Le fait que les deux espèces aient été capturées ensemble en 1972 et que tous les stades de l'une et l'autre soient représentés dans le lot provenant d'un même pompage indique qu'il y a cohabitation parfaite entre elles, peut-être dans des interstices de tailles différentes, au sein des alluvions (cf. Magniez 1974b, p. 148, fig. 22). Jusqu'à présent, trois cas de cohabitation de 2 espèces de Sténasellides ont été signalés: *Magniezia africana* (Monod) + *Parastenasellus chappuisi* (Remy) dans un puits de Man (Côte-d'Ivoire), *M. guineensis* (Braga) + *P. chappuisi* dans un puits de Gabu (Guinée-Bissau) et *Mexistenasellus wilkensi* + *M. parzefalli* Magniez dans une grotte du Mexique. A chaque fois, l'association comprend une espèce robuste et fortement armée, dont ont peut penser que les moeurs carnassières sont développées et une seconde espèce plus faible, aux péréiopodes I grêles, sans doute plus polyphage. L'association de la nappe du Guadalquivir ne fait pas exception, puisque nous y trouvons une forme robuste aux péréiopodes I puissants (fig. 6): *St. escolai* n. sp., accompagnant une forme naine et gracile: *St. bragai* (Magniez 1976, fig. 5-6).

3. Dans une publication précédente (Magniez 1974a), j'ai noté (p. 43) la découverte de *St. escolai* n. sp., ainsi que sa localisation géographique (p. 48, fig. 10, n° 25, *Stenasellus* sp. nov. 2).

Manuscrit terminé en octobre 1975.

RÉSUMÉ

Cette nouvelle espèce à été capturée dans la nappe fluviale du Guadalquivir où elle vit en compagnie d'un autre. Sténasellide de très petite taille: *St. bragai* Magniez, précédemment décrit. Elle appartient à la lignée de *Stenasellus breuili* Racovitza, répandue dans les eaux souterraines de la péninsule ibérique.

BIBLIOGRAPHIE

BRAGA, J. M. 1962, Sur la distribution géographique des *Stenasellus* de la péninsule ibérique et description d'une espèce nouvelle de ce genre. *An. Fac. Ciên. Porto,* 44, 9-28.

MAGNIEZ, G. 1974a. Données faunistiques et écologiques sur les *Stenasellidae* (*Crustaceae Isopoda Asellota* des eaux souterraines). *Int. J. Speleol.,* 6, 1-80.

MAGNIEZ, L. 1974b. Observations sur *Stenasellus virei* dans ses biotopes naturels (*Crustacea Isopoda Asellota* des eaux souterraines). *Ibid.,* 6, 115-171.

MAGNIEZ, G. 1975. Observations sur la biologie de *Stenasellus virei* (*Crustacea Isopoda Asellota* des eaux souterraines). *Ibid.,* 7, 79-228.

MAGNIEZ, G. 1976. *Stenasellus bragai* n. sp., *Crustacea Isopoda Asellota* des eaux souterraines d'Espagne méridionale. *Ibid.,* 8, 285-290.

Int. J. Speleol. 9 (1977), pp. 73-86.

Books received

BULLITT, Alexander Clark. Rambles in the Mammoth Cave during the year 1844, by a visitor. *Johnson Reprint Corp.,* New York, 1973. 101 pages. $7.50. (Obtainable from Zephyrus Press, 417 Maitland Ave., Teaneck, New Jersey, U.S.A.

This 100 page volume (plus six engravings) is one of the earliest accounts of the exploration of what is now the largest cave in the world. Although written 133 years ago it is still amazingly accurate. So accurate in fact, that many of the later publications dealing with Mammoth Cave are based extensively on this work. It was originally published anonymously but the actual author was well known to all at Mammoth Cave. There is a twenty-eight page introduction by Harold Meloney in this reprint edition that summarizes the early days of exploration at Mammoth Cave and details the literary history of the volume. Surprisingly, by 1844, a half dozen volumes had already been written about the cave. But it is Bullitt's book that has become recognized as the most definitive work about original exploration of the famous cave. The physical reproduction of the text is excellent and the binding worthy of the classic this is.

FORD, T. D. and C. H. D. CULLINGFORD, editors. The Science of Speleology, Academic Press, London, 1976. 593 pages. £14.00.

This is a sequel to the well-known text, British Caving. Rather than concentrating on the location and description of caves of Britain, it expands the scientific section and updates research in this area in a series of fourteen discrete chapters, all written by different authors.

Cave surveys (B. M. Ellis) assumes that the reader has had no previous experience. The geology of caves (T. D. Ford) concentrates on the geochemistry of limestone, the origin of caves and cave sediments. Geomorphology (G. T. Warwick) surveys karst terrains and development of cave passages, plus the hydrology of cave systems and age of caves. Caves in rock of volcanic origin (C. Wood) reviews the formation of caves in igneous rocks of all types in a chapter that covers material absent from most general speleological texts. The erosion of limestones (T. C. Atkinson and D. I. Smith) relates surface and subsurface solutions to the physiography of limestone areas. The hydrology of limestone terrains (D. I. Smith, T. C. Atkinson and D. P. Drew) deals mainly with groundwater flow. Chemistry of cave waters (R. G. Picknett, L. G. Bray and R. D. Stenner) is a lengthy chapter concerned with "the solutional effects of natural waters on cave-bearing rocks (limestone, with gypsum and anhydrite), and with the deposition of solids from the resulting solutions. Cave minerals, and speleothems (W. B. White) includes a checklist of all cave minerals, description of nine forms of speleothems, a complete mineralogy of carbonate deposits, evaporite minerals and minerals of possible organic origin. Physics of caves (T. M. L. Wigley and M. C. Brown) deals with the meteorology and geophysical detection of caves. Cave faunas (G. T. Jefferson) includes interstitial organisms plus those normally associated with the cave environment with emphasis on British species. Cave flora (B. D. Cubbon) stresses, of necessity, the detection, role and culturing of bacteria and fungi. Bats in caves (J. H. D. Hooper) is a generalized, but complete summary of all aspects of chiroptean behavior. Cave paleontology and archeology (A. J. Sutcliffe, D. Bramwell, A. King and M. Walker) is an extraordinarily complete review, not only of British sites, but many European and non-European sites as well. The computer in speleology (J. D. Wilcock) stresses information retrieval, routine processing of data, graphics and statistics with applications to cave studies. This is a complete index of caves, plus a subject index. The text is supplemented by several hundred figures, maps, charts and diagrams.

HOLSINGER, John, Roger A. BAROODY and David C. CULVER, *The Invertebrate Cave Fauna of West Virginia. West Virginia Speleological Survey,* Bull. 7: 1-82. 1976.

Approximately 188 species of invertebrate animals representing 63 families and 102 genera are recorded from West Virginia caves; 68 species, or about 36 percent of the total invertebrate fauna, are troglobites (obligatory cavernicoles). Data were collected and analyzed from 180 caves in 14 counties. The taxa represented are: flatworms (6 species), snails (9 species), clitellates (19 species), crustaceans (39 species), symphylans (1 species), millipeds (20 species), insects (56 species) and arachnids (38 species).

Three regional cave faunas are recognized: (1) the upper Potomac Basin fauna, (2) the upper Monongahela Basin fauna and (3) the upper Kanawha Basin fauna. Faunal regions are further subdivided into faunal subunits based on endemic species and local geology. The richest cave faunal subunit in terms of species diversity is the Greenbrier Valley which extends from northeastern Pocahontas County south through Greenbrier County to southern Monroe County. This area contains 35 troglobites, of which 18 are endemic.

Based on dispersal and distribution patterns, troglobitic species are assigned to one of five categories: (1) highly localized endemics, (2) subunit endemics, (3) regional endemics, (4) inter-regional endemics, (5) widely distributed species. Interstitial dispersal is offered as an explanation for the rather extensive ranges of some of the aquatic troglobites, whereas the wide ranges of some of the terrestrial troglobites are believed to result from limited surface migration through endogean habitats. The evolution of terrestrial troglobites probably resulted from a complex sequence of events during the Pleistocene that involved troglophile ancestors. A multiple origin involving both phreatobites and drainage changes is postulated for aquatic troglobites.

Abstracts

ANELLI, Franco. Nuove osservazioni sui fenomeni carsici, paracarsici e pseudocarsici. *Atti del Seminario di Speleogenesi Varenna, 5-8 ottobre 1972. Grotte d'Italia* 4: 165-192. 1975.

This paper deals with a problem of terminology first considered ten years ago. It is considered proper to give a precise definition of the term karst, parakarst and pseudokarst, referring to the different features in various lithostratigrafic complexes and therefore depending upon different alteration processes. Recalling the fundamental concepts reported by Gortani in 1933 on karst processes, the author emphasizes the importance of a clear classification of the karstic phenomena in the three terms listed above. To confirm such a classification a long list of karst features and many examples of peculiar forms are reported. These examples refer to initial and fully developed features originated both by water solution processes in water soluble rocks and in insoluble rocks having only a morphological (but not genetical) analogy with the former.

ANON. Sto rokov archeologického výskumu v jaskyniach na Slovensky. *Slovenský Kras,* 13: 3-37. 1975.

Archeological research in the caves of Slovakia started in the year 1871 when B. Majliáth excavated fossil skulls, silexes and mammoth molars from the Liskovská caves. These were presented to the National Museum in Budapest where the skull in particular created a great sensation. These remains are probably of Neolithic age. Between 1874 and 1879 investigations were undertaken in the Aksamitka cave near Haligovce and the finds sent to Budapest. These artifacts and remains were considered of paleolithic age. Burned bones from the Velká Ružínska cave, discovered in 1880, are also considered to be paleolithic. Between the two world wars extensive systematic research was undertaken with paleolithic finds from the Prepoštská cave near Bojnice and at Horná Tufna. Remains of neolithic, bronze and Hallstatt age were uncovered in the Jasovská cave (1924-1925) and of neolithic age from the Ardovská cave and Domica and Dupna holes (1932-1935). After World War II when science was widely supported by the state, considerable speleoarcheologic work was undertaken. Revised investigation of Prepostská cave near Bojnice in 1950 confirmed the Mousterian settlement which had not been previously recognized. The systematic research of C̄ertova pec near Radou verified the superposition of three paleolithic cultures and the most ancient settlement of old Mousterian. Paleolithic settlement of the Jasovská and Aksamitaka caves has also been established. Two human masks of great antiquity have been obtained from two vertical caves near Silica. Remarkable economico-social knowledge from the Neolithic age was gained from the Ardovska cave and

Domica. In Chvalovská cave were found traces of coinforging in the 15th century.

BARRA, J. A. and K. CHRISTIANSEN. Experimental study of aggregation during the development of *Pseudosinella impediens* (Collembola, Entomobryidae). *Pedobiologia,* 15: 343-347. 1975.

The general and food aggregation tendencies of the Collembola *Pseudosinella impediens* were tested under stable cave conditions of no light, high humidity and fixed temperature (12-13°C). Organisms were tested at each instar from 1 to V. It was found that there was a striking increase in general aggregation and a somewhat less striking decrease in food aggregation. It is suggested that these changes may recapitulate the evolutionary history of habitat change in this species. The study demonstrates the repeatibility of behavioural tests of this sort.

BECK, Barry, Mitchell FRAM and Juan R. CARVAJAL. The Aguas Buenas caves, Puerto Rico: geology, hydrology, and ecology with special reference to the histoplasmosis fungus. *NSS, Bull.,* 38 (1): 1-16. 1976.

The Aguas Buenas Caves drain an area of 5.6 km^2 and provide approximately 350,000 m^3 (90,000,000 gallons) of potable water annually, a major portion of the water supply of the town of Aguas Buenas. The bat population is an important part of the local ecology, helping both to control insects and to pollinate plants. Almost all the life inside the caves is dependent upon the bat guano as the base of its food supply. *Histoplasma capsulatum* is definitely present and is a serious health hazard. Although this fungus lives in the bat guano, the removal of the bats will not necessarily eliminate the fungus. The bat population is delicate and easily disturbed. It would be detrimental to destroy any of the bats or to unnecessarily disturb their roosting areas, with the exception of excluding them from those portions of the cave which are to be developed. Any chemicals used to eliminate histoplasmosis from the guano will rapidly contaminate the river which is a part of the water supply of the town of Aguas Buenas.

BOEGLI, Alfred. La Corrosione per Miscela D'arque. *Atti del seminario di speleogenesi Varenna, 5-8 ottobre, 1972. Grotte d'Italia,* 4: 333-344. 1975.

Karstic caves are due mainly to corrosion; as is pointed out by the well-known formula, the presence of CO_2 is always necessary. This kind of dissolution explains the corrosive action of water circulating within a free air surface and, under certain circumstances, of water flowing in the vadose zone. The corrosion acting in the phreatic zone is not explained with the exception of a rare instance in the upper part of the zone. Corrosion by mixing waters containing different amounts of calcium bicarbonate concerns every karstic zone, from the deepest parts of the phreatic zone to the surface. Existence of this type of corrosion is supported by many morphological examples.

BONZANO, C. Attività biospeleologica ne 1975. Boll. Interno Gruppo Speleol. Imperiese CAI, 5: 35-37. 1975.

A summary of 1975 biological collecting by Il Gruppo Speleologico Imperiese CAI. Several crustaceans *(Proasellus, Niphargus, Buddelundiella,* and *Porcellio),* an undescribed spider *(Troglohyphantes),* pseudoscorpions *(Cthonius, Roncus),* diplopods *(Crossosoma, Antroherposoma, Polydesmus, Antroverhoeffia),* carabid beetles *(Duvalius, Sphodropsis, Actenipus, Trichotichnus, Scotodipnus),* pselaphid beetles *(Glyphybythus),* catopid beetles *(Parabathysca),* a salamander *(Salamandra),* a toad *(Bufo),* and several bats *(Rhinolophus, Myotis)* are mentioned.

BOWMAN, T. E. A new genus and species of troglobitic cirolanid isopod from San Luis Potosi, Mexico. *Occ. Pap. Mus. Texas Tech. Univ.,* 27: 1-7. 1975.

Mexilana saluposi, new genus and species, is described from Cueva del Huisache. The relationships of all the cirolanid genera are discussed.

DELAMARE DEBOUTTEVILLE, C., N. COINEAU and E. SERBAN. Découverte de la famille des Parabathynellidae (Bathynellacea) en Amérique du Nord: *Texanobathynella bowmani, C. R., Acad. Sci.,* 280: 2223-2226. 1975.

A new genus and species described from Gorman Cave, San Saba County and Roaring Springs, Motley County, Texas.

DROPPA, Anton. Krasové javy Belianskej doliny vo velkej fatre. *Slovenský Kras,* 13: 107-129. 1975.

The karst terrain of Belianska dolina on the northwestern side of the Great Fatra (in the western Carpathians) contains dark-grey limestones and dolomites of the middle Triassic. Surface streams divide the terrain into a series of mountainous ridges and isolated summits. From the surface karst formations there emerge talus springs, karst springs, plus dry valleys. Nine caves are known, 25-225 m long, in this area. All these caves are of fissure-corrosion origin, enlarged by collapse and not exhibiting any secondary deposition. The lack of sinkholes and simultaneous origin of fluvial caves can be explained by the surface streams being of autochotonous origin and heavily saturated with $CaCO_3$. Belianska dolina karst is a type of incised karst with imperfectly developed surface and subterranean forms.

FORD, Derek. Castleguard cave, an alpine cave in the Canadian Rockies. *Studies in Speleology,* 2 (7-8): 299-310. 1975.

Castleguard Cave, a limestone cave with a total surveyed length of 11 kilometers, opens at an altitude of 2000 metres in the Canadian Rockies. It extends further beneath existing glaciers than any other known cave, terminating in a flat wall of glacier ice at the base of the Columbia Icefield, estimated to be 335 metres thick at that point. The author classifies the cave as "alpine" and compares it with caves in more temperate regions. He shows it to be of phreatic origin, modified in parts by vadose entrenchment and, near the mouth, by later frost shattering. Seasonal ice extends into the cave for a distance of 450 metres and there are fine stalactite formations in the interior.

FORTI, F. Studio geomorfologico dei fori di dissoluzione delle carbonatici calcaree del Carso Triestino. *Atti e Mem. Comm. Gr. "E. Boegan",* 12: 19-28. 1973.

Morphological and genetical aspects of dissolution holes in the carbonatic rocks in the Trieste Carso are described. They were produced by the disolution occurring in correspondence of joints on outcropping rock surfaces, usually compact calcium carbonates. The difference is pointed out between their karst forms brought about by frontal corrosion and the other karst micro-forms caused by ridge corrosion.

GAVRILIVIĆ, Dušan. Katastar Pecina kao osnova za speleolosko reoniranje krasa Istocne Srbije. *Naše jame,* 17: 35-44. 1975.

Of a total area of 15,704 km² in Eastern Serbia, 22% of the region is formed by limestone. In this area about 300 speleological objects have been recorded with 68% being non-active caves and 32% being active or temporarily active caves. 60% have objects of quaternary age while 32% have objects of pre-quaternary or Neogene age. The special characteristic of the Eastern Serbia karst is that there are a great number of short, tunnel shaped caves and cave systems with many levels of galleries. Karst regions can be divided into two basic types: 1. Beljanički type (dry cave galleries with great vertical and horizontal development with high density); 2. Miročki type (a great number of active cave channels with small vertical development and low density).

GELUSO, Kenneth, J. SCOTT ALTENBACH and Don E. WILSON. Bat Mortality; pesticide poisoning and migratory stress. *Science,* 194 (4261): 184-186. 1976.

Organochlorine residues in the fat of young Mexican free-tailed bats, *Tadarida* *brasiliensis* obtained from Carlsbad Caverns, reached the brain and caused symptoms of poisoning after the fat mobilization that takes place during migratory flight was simulated. These chemical body burdens were obtained naturally under free-living conditions at the maternity roost. The data obtained support the hypothesis that pesticides have contributed to recent declines in populations of this bat.

GRINDLEY, J. Fauna of the Cape Peninsula Caves. *Proc. Int. Cong. Cave Biology and Paleontology, Oudshoorn, South Africa* Aug., 1975, pages 7-11. 1976.

Sandstone caves of Table Mountain and Kalk Bay Mountains, South Africa have been visited by biologists from early 1900 onwards. The fauna collected includes the world's only cave *Peripatus* and the unique *Spelaeogrephus* which constituted a completely new order of crustacea. The tiny white isopod *Protojanira leleupi* occurs only in Kalk Bay caves. Certain genera of cave spiders have a species restricted to Cape Peninsula Caves.

HARESIMIUK, M., A. HENKIEL and K. PEKALA. Nisznany typ form krasu kopelneco ns rostoczu. *Speleologia* 8 (2): 98-108. 1975.

These tubular corrosional forms were found in the Neocene detrital limestones of the Hoztooze Hills. Their length/width ratio approaches 1:20. The straight tubular shape and closed end are the most characteristic features of these forms, which were filled with allochtonous material of solifluctional or eolian origin. Pelitic calcium carbonate covers the tube walls. The forms are called "candles" (swiece - in Polish) and show no distinct connection with bedding or jointing planes. The origin of "candles" is explained in terms of corrosional activity of rain and melt water during the Quaternary. These waters had been accumulated in small dispersions on bare limestone surfaces.

HABE, France. Problemi jamskega katastra v Jugoslaviji. *Naše jame,* 17: 11-14. 1975.

In Yugoslavia about 10,000 speleological objects have been explored. Their records are organized by the Republics. The records of SR Slovenia contain 4100 caves and is organized in duplicate by the Spleleological Association and the Institute for Karst Research of the Slovene Academy. In SR Croatia about 4400 caves are recorded; in SR Bosna and Herzegovina 351; in SR Serbia about 500; in SR Črha gora 400; in SR Macedonia 156. In all republics the records are organized by local institutions. The records in different republics have been combined and thus present a unified system of speleological records.

HABIČ, Peter. Razlike med alpskim in Dinarskim krasom. *Naše jame,* 17: 77-84. 1975.

Geological, hydrological, topographic and speleological characteristics of the Alpine and Dinaric karst differ to such a degree that they can be regarded as two distinct karst types. This is emphasized by the climatic, vegetative and pedologic differences. Both types may represent different phases of karst that have the same origin. However, the genesis of both types has not been completely studied.

HUMPHREY, Stephen R. and Thomas H. KUNTZ. Ecology of a Pleistocene relict, the western big-eared bat *(Plecotus townsendii),* in the Southern Great Plains. *J. Mammal.,* 57 (3): 470-494. 1976.

In karst regions of western Oklahoma and Kansas, *Plecotus townsendii* is locally ubiquitous but low in density. Natality is low but reproductive effort and pre-weaning survival of young are comparatively high. Movement data show no consistent pattern of dispersal that would account for a low density. *P. townsendii* uses nursery roosts that are marginal or uninhabitable for locally sympatric cave-dwelling species. Reproductive success depends on the ability of individuals in small nursery colonies to maintain a high degree of thermal exchange during pregnancy and lactation. This is facilitated by the formation of dense clusters in which metabolic heat is shared directly by body contact. In early and late winter most *P. townsendii* are absent from caves; presumably they occupy alternate roosts and forage on warm evenings. In autumn and early winter some individuals hibernate singly near cave entrances where ambient temperatures are variable but cool cave rock stabilizes body temperatures. By midwinter these sites reach subfreezing temperatures and many bats move deep into caves, where they may form clusters.

KANE, Thomas C. and Thomas L. POULSON. Foraging by cave beetles, spatial and temporal heterogeneity of prey. *Ecology* 57: 793-800. 1976.

This study assesses the effect of temporal and spatial heterogeneity of preferred prey on the foraging strategies of two carabid cave beetles. *Neaphaenops tellkampfii* and *Pseudanophthalmus menetriesii. Neaphaenops tellkampfii* prefers the eggs and nymphs of the common cave cricket *(Hadenoecus subterraneus)* which are patchy in time but not in space. Cave cricket eggs are laid in sandy habitats which support few alternate prey. As a result *N. tellkampfii* is forced to switch diet, habitat, and foraging mode in the fall when cricket eggs and nymphs become rare.

Pseudanophthalmus menetriesii preys on collembola that are confined to habitats with organic matter. These items are patchily distributed but continuously available. In sand or mud habitats collembola are rare and *P. mene-*

triesii appears to incur higher foraging costs in these habitats than in litter areas. As a result *P. menetriesii* shows restricted habitat preference and tracks its prey between various organic patches.

LEWIS, Jerry. The invertebrate fauna of Mystery Cave, Perry County, Missouri. *Missouri Speleology,* 14 (4): 1-19. 1974.

During the spring of 1972 collecting commenced for the purpose of conducting a survey of the invertebrate fauna of Mystery Cave. Most collections were made in the North Upper Passage of this 20 km cave system. At the present, 71 invertebrate species are known from the cave including 7 troglobites: 2 species of collembolans and 1 species each of planarian, isopod, amphipod, pseudoscorpion, dipluran and gastropod. Collection records for several other species are also given for other caves in the area surrounding Mystery Cave. The Schiner system has been used for the ecological classification of each species. Abundance and distribution within the cave has been presented as well as epigen distribution of nontroglobites.

MARTINI, J. E. J. The Fluorite deposits in the Dolomite Series of the Marico District, Transvaal, South Africa. *Economic Geol.* 71: 625-635. 1976.

The upper part of the Precambrian Dolomite series is mineralized with fluorite and minor lead-zinc. The deposits show similarities to the Mississippi Valley type but were subsequently metamorphosed in the contact aureole of the Bushveld Igneous Complex. The ore locations are mostly controlled by paleoporosity of the dolomite such as vuggy horizons and paleokarst. On a larger scale the fluorite district is localized on a paleorelief, including paleocaves, which acted as an ore trap. Several genetic hypothesis are proposed: the most attractive one is precipitation from solutions which derived fluorine from the Dolomite Series and/or Pretoria Series during diagenesis.

MAUCCI, Walter. L'ipotesi dell "erosione inversa" come contributo allo studio della speleogenesi. *Atti del Seminario di Speleogenesi Varenna 5-8 ottobre 1972. Grotte d'Italia:* 4: 235-285. 1975.

This is a speleogenetic hypothesis based on research on the karst areas near Trieste but applicable to other karst terrains. Speleogenesis is an ongoing, not fossil, phenomenon. The percolating waters of meteoric origin in vadous conditions suffice to explain the generic of caves. Speleogenesis originates underground, not from the surface. The embryonic stage from which most karst caves evolve is a vertical spindleshaped hollow. But in caves acting as sinkholes a reverse origin results by the retroversion of the course of the river flowing into the cave.

PERRATT, B. B. and E. K. TRATMAN. The hydrology of the Coolagh River catchment and its caves, Co. Clare, Ireland. *Proc. Speleological Soc.*, 14 (1): 83-105. 1975.

This paper is based on three main ideas. First that the Coolagh River Valley was formed by ice action during the last glaciation. Second that during the retreat stages of the ice caves were developed and swallet retreat took place. Third that its form differs from that of the Aille, which was cut in the Clare Shales, because the Coolagh River Valley was cut largely in the underlying Carboniferous Limestone and not in the shale.

PETERSON, Gilbert M. Pollen Analysis and the Origin of Cave Sediments in the Central Kentucky Karst. *N.S.S. Bull.* 38 (3): 53-58. 1976.

Pollen analyses of cave and surface sediments in and near the Central Kentucky Karst show that pollen spectra from cave sediments do not reflect regional pollen spectra, but, instead, are similar to local pollen spectra from the points where sediments enter the cave. Pollen spectra from passages draining the wooded Mammoth Cave Plateau are characterized by high percentages of arboreal pollen, while spectra from passages subject to river flooding show low percentages of arboreal pollen. Thus, pollen analysis is a possible means to distinguish cave sediments from different source areas. Pollen is presently transported into cave passages for distances of at least one-half mile, although no pollen is preserved in older cave sediments.

PIERCE, T. G. Observations on the fauna and flora of Ingleborough Cavern, Yorkshire, *Trans. British Cave Res. Assoc.*, 2 (3): 107-115. 1975.

The progressive increase in shade and relative humidity across the threshold of Ingleborough Cavern is reflected in the composition of the flora of this zone. The fauna of the cave proper enjoys a relatively constant environment in which food is scarce. The few animal species present are mainly detritus feeders, including the earthworm *Allolobophora chlorotica* and *A. rosae* which are rarely seen at the soil surface outside caves and which produce casts underground. Deep with the permanently dark zone these species work at the superficial layers of sediment deposits, which are covered with their casts in many places.

PIRES-FERREIRA, Jane WHEELER: Edgardo PIRES-FERREIRA, Peter KAULICKE. Preceramic animal utilization in the Central Peruvian Andes. *Science,* 194 (4264): 483-490. 1976.

The analysis of animal bones recovered from preceramic period deposits at Uchcumachay Cave and other sites in the Puna of Junin has documented the development of an economy involving primary carnelid utilization beginning about 5500 B.C. and culminating with the appearance of domestic forms between 2,500 and 1,750 B.C. A model that can be used to explain this process in both the Puna of Junin and the Central Andes is presented.

PREMRU, Urŏs. Geoloska Zgradba Julijskih in Savinjskih Alp. *Naše jame,* 17: 67-75. 1975.

A short description is given of the stratigraphy, paleogeography and tectonic structure of the Julian and Savinja Alps, including their northern extension into the Karavanke Mountains and their southern extension into the nearby foothills. The main lithologic members range from Devonian to Holocene. The main phases of the paleogeographic development in individual geologic ages and the recent structure of the Slovenian sections of the Alps are described.

PULPÁN, Jan and Eva ZD'ÁRKOVÁ. Využití Dobsinské ledové jeskyně pro unchovani populací roztoče dravého - *Cheyletus eruditus* (Schrank). *Slovenský Kras,* 13: 173-179. 1975.

The main prerequisite from the viewpoint of successful biological control of stored product mites *Cheyletus eruditus* is the availability of a large number to withstand any calamitous impact. One means of providing an appropriate supply is the cold-preservation of these populations. They can survive at temperatures ranging from —1.7° C to +2.4° C with relative humidities of 80-90% from five to seven months and still retain their ability to reproduce upon transfer to favorable conditions. Naturally occuring populations may be used for preventive or repressive biological control, eliminating the necessity of mass-rearing these mites in the laboratory. The ice cave of Dobsiná serves as an excellent experiment area for this purpose due to the constant conditions necessary and in particular the high relative humidity.

REINBOTH, F. and F. GOEBEL. Bemerkungen zum Excentriesproblem. *Die Höhle,* 26 (4): 123-130. 1975.

New observations on these cave excentrics indicates that the aerosol theory of formation is untenable. The theory of capillary action suffices to explain the cross section of these excentrics.

ROQUES, Henri. Chimica dei carbonati eo idrogeologia carsica. *Atti del Seminario di Speleogenesi Varenna, 5-8 ottobre 1972. Grotte d'Italia* 4: 37-71. Bologna, 1975.

The karstic phenomenon is considerably influenced by the chemical reactions taking place in the limestone area. The chemistry of carbonates is therefore predominant in any chemical study of karst. The karsts waters play also a fundamental role in the equilibrium of the above reactions. Influence of sulfate, chlorid and magnesium are taken into account as well as that of temperature. Concluding is a discussion of the equilibria in function of these different parameters.

RUTHERFORD, John M. and Robert H. HANDLEY. The Greenbrier Caverns. *N.S.S. Bull.* 38 (3): 41-52. 1976.

The Greenbrier Caverns is the largest-known American cave east of the Central Kentucky Karst. Modern-day exploration of the cave began in the very late forties, when a small group from Charleston, West Virginia headed by Bob Handley became interested in "Organ-Hedricks" Cave. One discovery followed another in rapid succession. By 1958, the Greenbrier Caverns was known to have seven interconnected entrances and, with recent reports of over 44 miles of mapped passages and two more entrances, the system is surely among the 10 largest caves known in the world. The cave has a long history, dating back to the days of President Jefferson. Two distinctly different types of saltpeter workings are present in the cave. The more recent and extensive of the two is of Civil War age, or older. Collection of biological specimens from the system began in the thirties and a diverse fauna is known today. The cave is the type locality for four of the 14 troglobitic species known to occur in it. Bone collections from three different areas of the cave include (extinct) Pleistocene nine-banded armadillo, mastodon, Jefferson's ground sloth, and peccary. Geologically, the cave is very complex and the effects on cavern development of a variety of geologic factors can be seen. Joint control, bedding plane control, tilted and folded strata, numerous faults and thrust planes, and the contact between the Greenbrier limestone and the underlying Maccrady shale are among the geologic factors illustrated. The hydrology of the cave is correspondingly complex and consists of two (or three) parallel drainage lines developed along a structurally complex syncline.

SCHULTZ, George A. *Miktoniscus halophilus* Blake, *M. Medcofi* (Van Name) and *M. morganensis,* n. comb., reconsidered with notes on New World species of the genus (Crustacea, Isopoda, Trichoniscidae) *Amer. Midl. Nat.* 95 (1): 28-44. 1976.

Species of the trichoniscidae genus, *Miktoniscus* Kesselyak, from the New World are reviewed. Two of them, *M. halophilus* and *M. Medcofi* are redescribed and are the principal species in Eastern United States. *M. Medcofi* ranges from West of the Appalachian Mountains to the Mississippi Valley and frequently is present in caves. A new name, *M. Morganesis* n. comb., is proposed for specimens called *M. Medcofi* from a cave in Morgan County, Alabama.

SNOW, D. W. Oilbirds, cave-living birds of South America. *Studies in Speleology,* 2 (7-8): 257-264. 1975.

The oil bird, *Steatornis caripensis,* of the New World tropics is one of the few species of birds that are absolutely dependent on caves. Discovered by von Humboldt in 1799, it was studied by anatomists in the 19th century, who demonstrated that it is almost certainly a specialised offshoot of the nightjar stock, but its natural history remained little known for 150 years. In 1953 D. R. Griffin showed that it uses echolocation for orientation and navigation within its caves and in 1957-61 a general study was made of its natural history in Trinidad. Oilbirds are nocturnal spending all day and nesting deep inside caves. They are entirely frugivorous, ranging widely over the forest at night and plucking fruits from the trees in flight. The fruit is brought back to the caves, where the outer flesh is digested and the seed regurgitated intact. The masses of regurgitated seeds accumulate to form deep banks of decaying matter which supports a rich invertebrate fauna. There is good evidence that the olfactory sense is used in locating trees with ripe fruit. The tempo of breeding is slow. Young remain in the nest for 3 or 4 months and become extremely fat. Today the destruction of the forest is the greatest threat to the oilbird's survival.

SUSTERSIC, France. Principi vodenja jamskega katastra JZS. *Nase jame,* 17: 15-27. 1975.

The first part of this article is devoted to the development of the cave recording system which has existed since 1689 when the first preserved cave plan was published. In 1970 a general reorganization was undertaken. The basic intent is to assure the identification of all speleological objects and to record all exploration in these sites. The identity mark of a cave is its cadastral number and this number is systematized according to the Gauss-Krüger coordinates of the entrances. For this purpose the JZS printed new forms and published instructions for the proper recording of cave information. In 1975 a catalogue of 5000 known caves in the territory of Slovenia will appear. This catalogue will contain all the basic data of these caves.

SZYMCZAKOWSKI, Waclaw. Formes cavernicoles d'*Adelopsis brunneus* Jeann. du Venezuela et de l'ile de Trinidad (Coleoptera: Catopidae). *Bol. Soc. Venezolana Espel.,* 6 (11): 13-24. 1975.

The author describes four new sub-species of *Adelopsis brunneus,* three from Venezuelan caves and another from a cave in Trinidad. He has confirmed that each of these caves lodges a different population, resembly *Adelopsis linaresis* from the Guacharo cave. Comparing all these populations with the *A. brunneus* type, described from Colombia by Jeannel in 1936, the author reaches the conclusion of considering all of them, in conjunction with *Adelopsis linaresis*, as endemic micro-breeds of each cave and attributes to them sub-species status. As normal in coleopterans from the tropical zone, the forms of *A. brunneus* are not troglobitic and do not present any morphological adaptation to subterranean life. They are normally pigmented, winged and macro-ophthalmed. It would be of great interest to carry out a statistical analysis of the morphology of larger samples of the populations in their respective caves and the eventual epigean populations.

TUTTLE, Merlin D. Population ecology of the gray bat *(Myotis grisescens)*: philopatry, timing and patterns of movement, weight loss during migration, and seasonal adaptive strategies. *Univ. Kansas, Mus. Nat. Hist., Occ. Pap.* 54: 1-38. 1976.

An intensive handling and recovery study of *M. grisescens* resulted in 19,691 recoveries at 120 locations. Included were many multiple recaptures and roundtrip recoveries between materning and hibernating caves. Gray bats demonstrated strong loyalty to a summer home range, often including six or more caves, as well as to their wintering site. Adult females emerged from hibernation first, in early April, followed by yearlings of both sexes and lastly by adult males. Once at the summer home range, adult females congregated in one preferred maternity site to rear young, while adult males and yearlings clustered in smaller groups, usually in caves other than maternity caves. After the fledging of young, sex and age segregation weakened, and individuals were more evenly dispersed through the home range. Fall migration took place in approximately the same order as spring emergence, with adult females leaving in early September and juveniles remaining behind with the last males to leave, usually by mid-October.

TUTTLE, Merlin D. Population ecology of the gray bat *(Myotis grisescens)*; Factors influencing growth and survival of newly volant young. *Ecology*, 57: 587-595. 1976.

Growth success and survival of newly volant *Myotis grisescens* were observed in colonies traveling a variety of distances between the roost cave and foraging area (0.0-6.6 km). Foraging occured primarily over large rivers and resevoirs. Quality of the foraging area, climatic conditions, and cave temperatures were thought to be some of the potential factors influencing growth and survival, most noticeably when distances traveled were minimal; however, when distance became excessive relative to other variables, it was found to be a highly significant factor.

Int. J. Speleol. 9 (1977), pp. 87-88.

7th International Speleological Congress, Sheffield, 10th—17th September, 1977

by

Gordon T. WARWICK

International speleological congresses have now become established scientific events of considerable importance, affording speleologists and scientists in allied fields an opportunity to meet and discuss common and unusual problems. The 6th such meeting held in Olomouc in 1973 set a very high standard which it is hoped at least to try and equal.

The main meetings at Sheffield will be held under one roof in Ranmoor House, a Hall of Residence belonging to the University of Sheffield. Most of the delegates will also be housed in the same building and there is overflow space in an adjoining hall. Also there are hotels in the close vicinity and it is hoped to arrange a camp at no great distance for the less affluent. It is also to be hoped that we shall see more of the ordinary caver who is also interested in certain scientific and technical aspects of his sport. This dual interest is reflected in the financial support provided both by the Royal Society of London and the Sports Council.

It is hoped to pre-publish the papers which should be kept short and suitable for delivery in 20 minutes or less. In line with this policy a limit of 1200 words has been put on each paper plus a 100 word summary. The dead-line for receipt of papers was 30th January 1977. Those who returned the first circular and offered papers should consider their titles to be acceptable and forward their main paper(s) with their final booking form. The programme will be organized in 10 main sections dealing with Geology/Mineralogy; Karst Morphology; Speleogenesis; Hydrogeology, Cave Chemistry/Physics; Cave Biology, Cave Archaeology/Palaeontology; Documentation: Techniques/Equipment; Conservation/Tourism. In addition it is hoped to organize 10 special seminars on Karst Typology; Speleochronology; Karst Surface Processes; Marine Karst; Cave Climates; Cave Development Processes; Applied Hydrogeology; Archaeological Sequences in Caves; Cave Equipment and Techniques; Recreation and Tourism in Karst. Papers should wherever possible be designed to fit in with this wide range of themes. Many of these topics are designed to fit in with special commissions and study groups. There will also be space and time set aside for meetings of the commissions for business matters.

During the evenings of the main meeting there will be a social programme including receptions given by the City and University of Sheffield as well as slide and film shows. Each afternoon there will be excursions to local caves and also more general tours for wives and delegates who wish to take a day off!

Before the meeting there will be long excursions in the Yorkshire Dales, the Mendips and South Wales and afterwards to Yorkshire and to Ireland. A new departure will be caving camps in the Yorkshire Dales, the Mendips, South Wales and Fermanagh, Northern Ireland, both before and after the main meeting. These will be based upon caving club huts and cavers must bring their own equipment and lighting.

For those who have not as yet received a copy of any of the circulars, they should write immediately for the second circular to the following address:

7th International Speleological Congress,
c/o B.E.C. Travel Limited,
63, Dunkeld Road,
Eccleshall,
SHEFFIELD, S11 9HN,
ENGLAND.

5 The Editors reserve the right to refuse any manuscript submitted, whether on invitation or otherwise, and to make suggestions and modifications before publication. Submitted papers should be in a final form ready for publication. All corrections in the proofs other than printing errors are at the author's expense.

6 Bibliographical references should be listed in alphabetical order at the end of the paper.

7 References to periodicals should include the following elements: name(s) and initial(s) of author(s); year of publication; title of the paper; title of the periodical; volume number (Arabic numerals); first and last page number. For periodicals the recognized abbreviations laid down in the "World List of Scientific Periodicals 1900-'60" (London, 1965) and "World Medical Periodicals" (World Medical Assocation, New York, 1961) should be used.
Example: HALBERG, F., 1963. Periodicity analysis. A potential tool for biometeorology. Int. J. Biometeor., 7 : 167-191.

8 References to books should include: name(s) and initial(s) of author(s); year of publication; exact title; name(s) of publisher; town of publication; page number (where pages are specifically cited).
Example: SOLLBERGER, A., 1965. Biological Rhythm Research. Elsevier Publ. Co. Amsterdam.

9 References should be cited in the text in parentheses by the name(s) of author(s) followed by the year of publication, e.g. "(Jones, 1961)" except when the author's name is part of the sentence, e.g. "Jones (1961) has shown that..." If there are more than two authors, it is in order to put "et al." behind the first name, e.g. "Smith et al., 1961".

10 Each table should be typed on a separate sheet of paper. Tables should be numbered consecutively in Arabic numerals, e.g. "Table 1, Table 2, etc.", and attached to the end of the text. Should a table not be an original, the exact reference should be quoted. Tables should be supplied with headings and kept as simple as possible and should be referred to in the text as "Table 2", etc.

11 Figures (including photographic prints, line drawings in black Indian ink on strong white or transparent paper, and maps) should be numbered consecutively in Arabic numerals, e.g. "Fig. 1, etc." and attached to the text behind the tables. Graphs and diagrams should be large enough to permit reduction to a size of 10 x 10 cm (4 x 4 inches).
Legends for figures should be listed consecutively on a separate sheet of paper. Photographs can be easily reproduced in offset print but should be unmounted, glossy prints, permitting reduction to a size of 10 x 10 cm without effecting legibility.
Authors will be asked to contribute to the cost of excessive illustrations and elaborate tables. The cost of reproducing coloured plates must be met in full. Estimates may be obtained in advance.

12 For further instructions in preparing manuscripts for publication, authors should use the "Style Manual for Biological Journals" (Amer. Inst. Biol. Sci., Washington, D.C. 1964).

13 Articles accepted by the Editor-in-Chief will become the property of Swets & Zeitlinger B.V., Amsterdam. No article or any part thereof may be reproduced in whatever form, without the written permission of the Publisher.

14 The Editors and the Publisher of the International Journal of Speleology are not responsible for the scientific content and statements of the authors of accepted papers.

15 Reprints may be ordered when proof is returned.

CONTENTS