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REVUE ROUMAINE DE BIOLOGIE
Splaiul Independenței 296
București 17 — România, Tel. 49.28.30

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REPUBLICII SOCIALISTE ROMÂNIA
Str. Gutenberg 3 bis—București 46
Tel. 16.40.79

REVUE ROUMAINE DE BIOLOGIE

TOME 19 1974 Nº 2

SOMMAIRE

CAMPYLASPENIS, *STYLOPTOCUMA*, *ATLANTOCUMA*, NEW GENERA OF CUMACEA FROM THE DEEP WATERS OF THE ATLANTIC

BY

MIHAI BĂCESCU and ZARUI MURADIAN

In the present paper the authors describe *Campylaspenis rowei* n.g. n.sp. and separate g. *Styloptocuma* from the *Cumella* genus with *Styloptocuma antipai* n.g. n.sp., on the basis of the material collected in the stations carried out by „Eltanin" — Beaufort, between Florida and Cape Hatteras; they also describe *Atlantocuma benguelae* n.g. n.sp., captured during the Vema 14 Expedition in S—W Africa.

In the material of the 22 stations carried out by "Eltanin", the ship of the Beaufort Research Station of the Duke University, in the Western part of the Tropical Atlantic at depths of 500 to 5,275 m, over the 1964—1967 period, we found among others some representatives of two new genera (one separated from the *Cumella* genus), whilst a third genus is given from the Vema 14 collection.

We wish to express our sincere thanks to Prof. Robert Menzies, who at that time was Director of the above mentioned research station, as well as to Dr. G. T. Rowe for having given us the opportunity to study this material. We also express our gratitude to Prof. Ewing, Director of the "Lamont Geological Observatory".

1. **Campylaspenis** n.g.

Diagnosis: cumaceans with appearance and part of their structures of *Campylaspis* type (uropoda, aberrant maxillipeds I—II—III, common to this genus; same for peraeopods I and II). A quite peculiar feature of this new genus is the presence of an exceptionally well-developed, bifid penis, loosely hanging on the last peraeonite, three times as thick as the basis of peraeopods V, among which it occurs, and nearly as large as the basis diameter of peraeopod IV; a rich ciliation borders the proper genital opening. This strange combination of *Campylaspis* characters and the presence of a very large and well-developed penis suggested us the generic name.

Campylaspenis rowei * n.sp. (Fig. 1 and 2, *A—C*; Pl. I, *A*, *B*)

Description of adult ♂. Body covered by very dense, conspicuous scales. On the lateral portions of the carapace, two strong carinae reach

* Dedicated to our friend Dr. G. T. Rowe from the Woods Hole Oceanographic Institute by whose kindness we had the possibility of studying the present material.

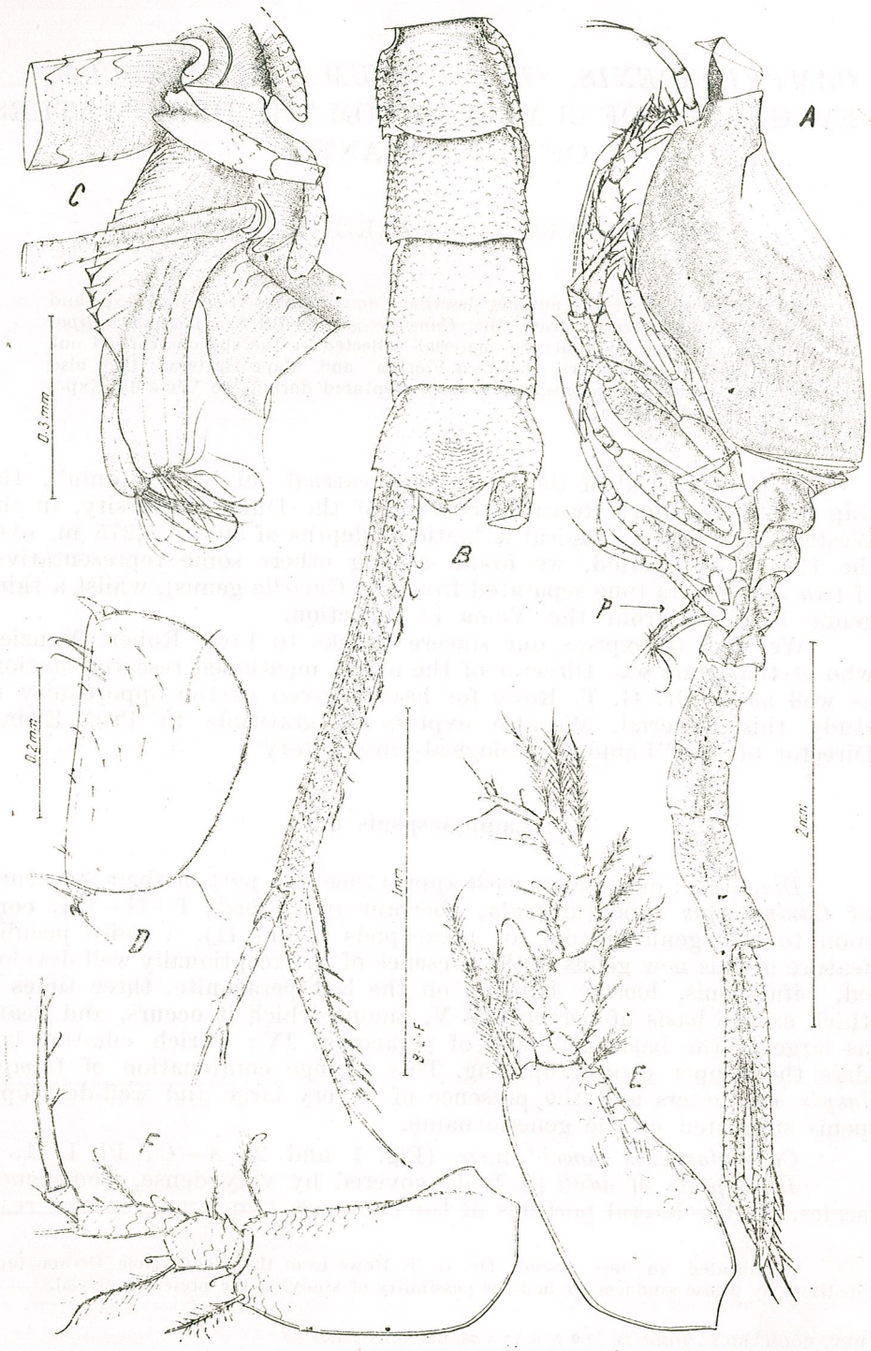

Fig. 1. — *Campylaspenis rowei* n.g.n.sp. ♂ ad. *A*, profile; *p*, penis; *B*, last 4 abdominal segments and uropoda; *C*, last 2 thoracic segments, first abdominal segment and penis; *D*, maxilliped I; *E*, peraeopod I; *F*, peraeopod II (Original).

to its posterior part, while two less apparent ones are situated both anteriorly and posteriorly as against the former. The inferior edge of the carapace is smooth. Antennae of *Campylaspis* type. A_2 flagellum broken, but the latero-abdominal notch (Fig. 1 *A*) suggests that it reaches at least up to the last abdominal segment. We point out the large internal expansion of the basipodite of maxilliped III, same as in peraeopod I (Fig. 1 *E*). Exopodites present in maxilliped III and in the first 4 pairs of peraeopods. Among the last peraeopods the common basis of a copulatory organ is fixed (Fig. 1 *A*, *C* and *p*. Fig. 1A *A*, 9 see arrow); this latter is severed in two cylindrical penes. Along the penis, unexpectedly well-developed in the Cumacean group, the ejaculatory duct may be noticed. Basis of uropod (Fig. 1 *B*), longer than the last 3 abdominal segments, is lengthwisely edged. Uropodal endopodite single-jointed, much longer than exopodite.

Colour: white, in alcohol. *Length*: 6.16 mm.

Material: One adult ♂ specimen from Beaufort E 45/8 XI 1966, St. 6234, 33°39′0″ N and 75°39′0″ W, South of Cape Hatteras, 3,045 m depth.

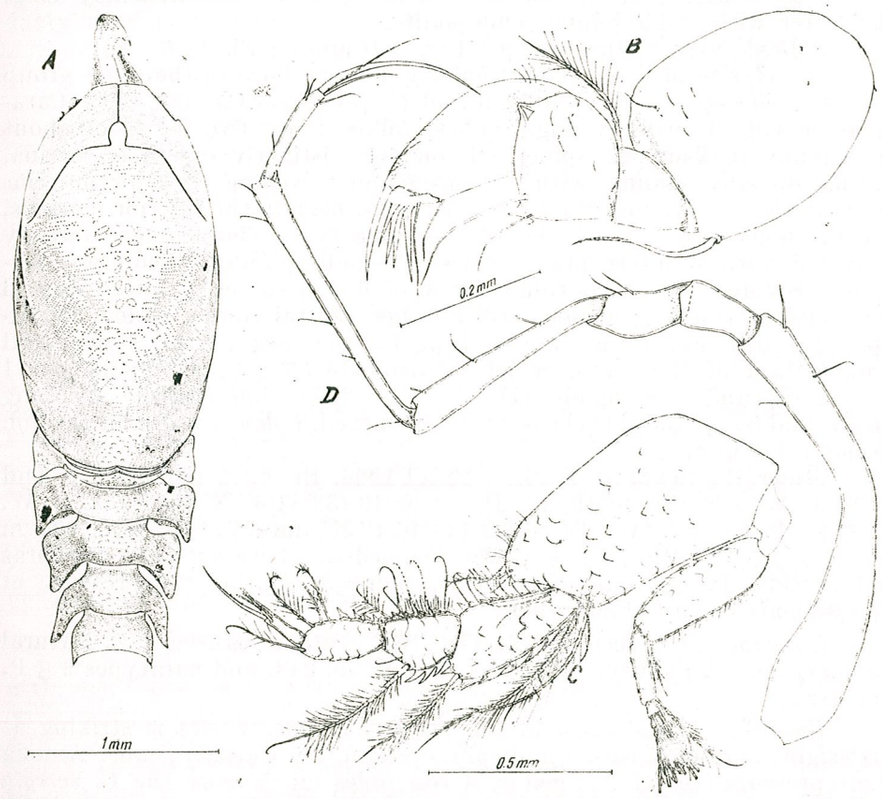

Fig. 2. — *Campylaspenis rowei* n.g.n.sp. ♂ ad. *A*, carapace and free thoracic segments in dorsal view; *B*, maxilliped II; *C*, maxilliped III. *Styloptocuma antipai* n.g.n.sp. ♀ ov. *D*, peraeopod I (Original).

Holotype ♂ recorded at the "Gr. Antipa" Museum of Natural History, no. 241.

Remarks. This campylaspoid cumacean is readily distinguished from all other known Cumacea by the presence of the above mentioned copulatory organ in males. We mention that this is the second case of a proper penis to be found in Cumacea, and also that it is better expressed than in the case of *Archaeocuma* [1]. We find now this ancestral type in one of the representatives of another family.

2. **Styloptocuma** n.g.

Diagnosis. Very elongated Cumaceans, richly provided with spines at least on the carapace. Long, pseudorostrum, suddenly curved considerably overrun in length by an aberrant ocular, styliform lobe, without any traces of visual elements, which accounts for the name of the genus. Antennule with a very long basal segment exceeding the whole length of the appendix. Peraeopods, as well as uropods are extremely long, the latter with single-jointed endopodite.

Styloptocuma antipai * n.sp. (Fig. 2 *D* and 3; Pl. I, *C—F*)

Description of ♀ ov. A particularly delicate form of the same group with *Cumella egregia* Hansen 1920 and *C. gracillima* Calman 1905. Carapace dorsally bearing 2 huge spines, followed by two series of about 10 minute hook-shaped spines, all on the distinctly expressed carina. Carina dorsally ending with a conspicuous swelling. Inferior margins of carapace, smooth; on the lateral portions, near to the inferior margins, a continuous series of spinules of the same type. Thoracomers dorsally provided with uninterrupted girdles of small spinules. Pleonites elongated provided with a continuous row of dorsal spines, 2 latero-ventral rows and in young specimens still another ventral row is added. Maxilliped III different from *Cumella* type by the lack of the dorso-distal prolongation of the basis, which is truncate (Fig. 3, *H*). Peraeopod I (Fig. 2, *D*) and peraeopods III—V (Fig. 3, *J*) characterized by long carpus and basis, and fine claws, strongly curved. *Colour*: white, in alcohol. *Length*: 4.8 mm.

Material: Beaufort E 45/9—10 XI 1966, St. 6241 (33°13′0″ N and 76°13′5″ W, 1,000 m depth, 1 ♀ P), St. 6249 (33°31′6″ N and 76°02′2″ W, 1,090 m depth, 1 ♀ ov.), St. 6252 (33°46′4″ N and 75°55′5″ W, 1,000 m depth, 3 ♂♂ P). All the stations were carried out between Cape Hatteras and Florida. In the Cumacean association we mention the presence of *Campylaspis vitrea* Calman 1906.

Holotype ♀ ov. recorded at the "Gr. Antipa" Museum of Natural History, no. 242; allotype ♂ P, recorded as no. 243, and paratypes 2 ♂ P, no. 244.

Remarks. The shape and the beauty of this species is striking at first sight. Carapace, carina and spines remind the *Cumella* genus. Among the representatives of this genus, it resembles much more the *C. egregia*

* We dedicate this species to the memory of the great Romanian oceanographer Grigore Antipa, the organizer of the Natural History Museum from Bucharest, bearing his name.

than the *C. gracillima*. These 3 species, by their styliform eyelobe, their high gracility and the continuous series of comma-shaped spinules on the margins of the carapace, as well as by the truncate, dorsally not produced distal portion of the basis of maxilliped III, confer on this group of species, the status of separate genus, g. *Styloptocuma*, as they are distinctly different from all Nannastacidae. Besides, the depths these species were found at (1,000, 2,500 and 360 up to 579 respectively) and hence the total lack of visual elements in both sexes makes it mandatory for us to take out these species from the *Cumella* genus. Here is their differentiation key:

1. Body deprived of any series of spines, except for the inferior margin of carapace . . . . . *Styloptocuma gracillima* (Calman) ♀ ad., 2.75 mm

2. Series of transversal spines on peraeonites and longitudinal ones on the ridge of the carapace, pleonites and uropods . . . . . 3(4)

3. On the ridge of the carapace an uninterrupted row of small spines; propodus of peraeopod I somewhat shorter than the carpus; dactylus of peraeopod II equal to the carpus . . . . . *Styloptocuma egregia* (Hansen) ♂ P, 4 mm

4. On the ridge of the carapace, 2 large anterior spines followed by one double row of small comma-shaped spines; propodus of peraeopod I by one third longer than carpus, while the dactylus of peraeopod II nearly twice as long as carpus . . . . . *Styloptocuma antipai* n.sp. ♀ ov., 4.6 mm

3. **Atlantocuma** n.g.

Diagnosis. Cumaceans having the appearance of *Iphinoe*, with 5 free thoracomers, the first one almost fused to carapace; the detachment from the cephalotorax is easy only at the thoracomer II level. Mandible navicula-shaped. Without telson, without pleopods (♂, ♀) with uropodal endopodite single-jointed. Four exopodites in ♂, and only two in ♀. Maxilliped I provided on the inner edge of carpus with widened dentate phanera of *Cyclaspis* type (Fig. 4 *F*). Pediform maxilliped II and III, the latter of the IInd type (according to Zimmer), Lampropid type, with long basis distally not widened. Antenna (♀) 3-jointed, rather of Leuconid type. No branchial elements characterizing the *Cumella* genus.

Atlantocuma benguelae n.sp. (Fig. 4; Pl. I, *G* and *H*)

Description of ♀ ad. and ♂ P. Tegument calcareous, breakable, glabrous. Carapace elongate, extending the thoracomers (as in *Iphinoe*), with sharp rostrum, penlike. Inferior edge smooth, straight; frontal lobe large, ocular lobe small and sharp, without any lenses. Thoracomer I, twice as short as the next one, although distinctly visible, fused to carapace as in *Iphinoe*, *Cyclaspis*. Pleonites growing in length, the fifth being as long as the first two together and about 2.5 as long as the pleotelson. A_1 alike in both sexes (Fig. 4 C), strongly geniculated between

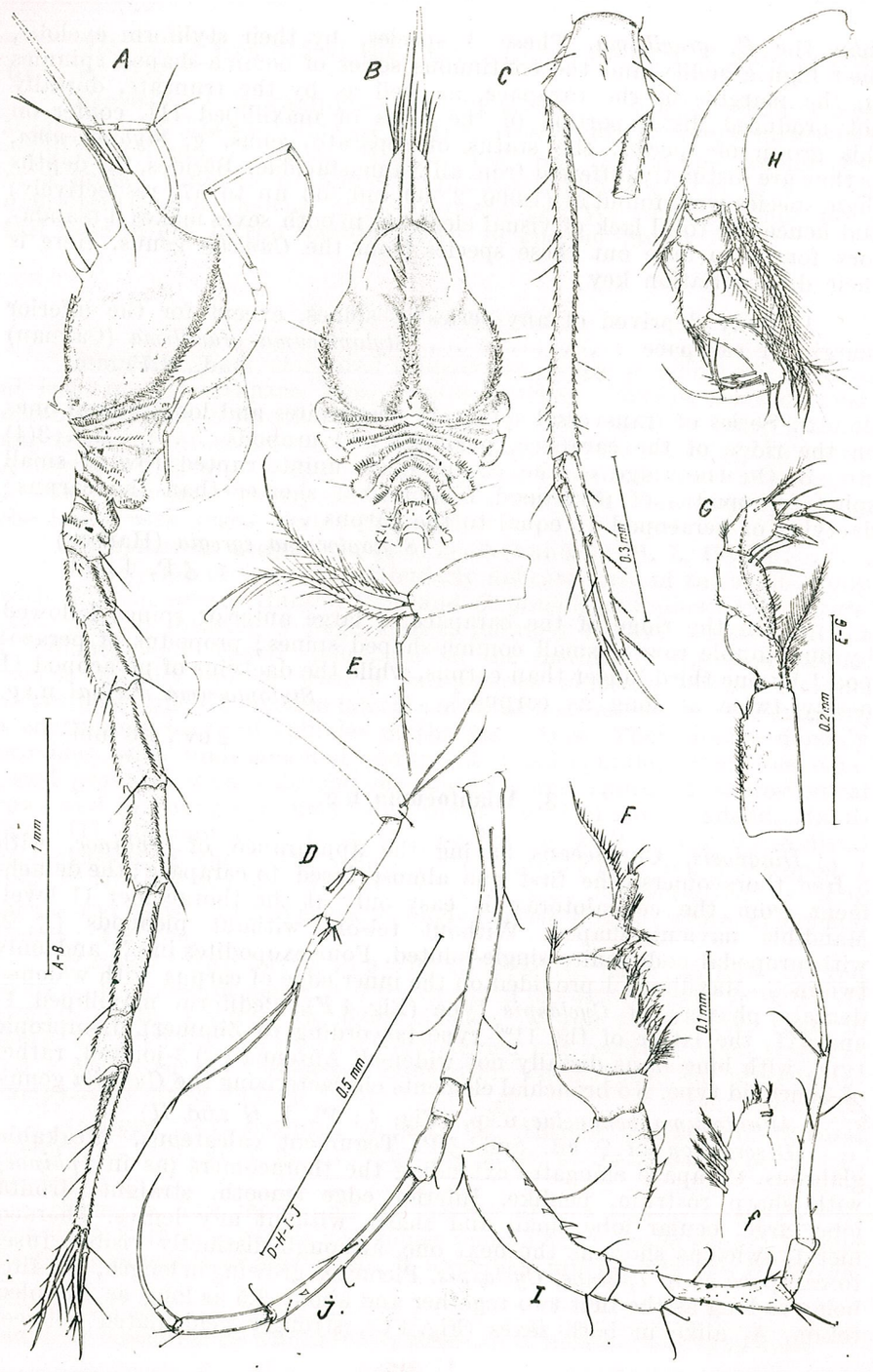

Fig. 3. — *Styloplocuma antipai* n.g.n.sp. ♀ ov. *A*, profile; *B*, cephalotorax, in dorsal view; *C*, pleotelson and uropoda; *D*, antennule; *E*, antenna; *F*, maxilliped I; *f*, its endopodite; *G*, maxilliped II; *H*, maxilliped III; *I*, peraeopod II; *J*, peraeopod IV (Original).

Fig. 4. — *Atlantocuma benguelae* n.g.n.sp. ♀ ad. (*A*, *D*, *F* and I), ♂ P (*B*, *C*, *E*, *G*, *H*, *J*—*L*). *A*, ♀ ad., profile; *B*, ♂ P, cephalothorax profile; *C*, antennule; *D*, antenna ♀; *E*, mandible; *F*, maxilliped I; *G*, maxilliped II; *H*, maxilliped III; *I*, peraeopod I; *J*, peraeopod II; *j*, its distal joints, magnified; *K*, peraeopod V; *L*, rami of uropoda (original).

first and second basal segments. A_2 in females, well-developed, the proximal segment very strong, situated in a sharp angle as against the next two segments of which the distal one bears a few sensitive hairs. A_2 in ♂ with a still undeveloped flagellum. Mandible (Fig. 4 *E*) of Bodotriid type, with an inner insertion for muscles and with a cylindrical strong pars molaris, ending in a chitinous narrow masticatory face, without hairs or rasp. Maxilla I with palp provided with 2 flagelli; no peculiarities resembling the maxilla II. Maxilliped III (Fig. 4, *H*) rather of type II ([5], p. 53), that is with carpus and propodus slightly widening. Peraeopod I (Fig. 4, *I*) just like maxilliped III has a basis longer than the remaining joints, without distal setae. Uropods have a prismatic, edged basis as in g. *Bodotria* and is twice as long as rami; endopodite single-jointed as long as the exopodite (Fig. 4, *L*). *Length* (♀ ad., ♂ P) 6—6.5 mm.

Material. 6 ♀♀ with small marsupial lamellae and 4 preadult males in the 53 Lamont Station (Vema 14), 36°34′ S and 14°08′ W, 4,893 m depth, 4 IV 1958, South of Cape Town, about the place where the Benguela Stream originates, hence the specific name.

Holotype ♀ ad. recorded at the "Gr. Antipa" Museum of Natural History, no. 245; allotype ♂ P, no. 246 and 5 paratypes, ♀ and ♂, ditto no. 246 a.

Remarks. The genus presents some of the Bodotriidae characters: first thoracomer fused to the carapace — in addition, general appearance of *Iphinoe* — shape of mandible, the 2 expodites in ♀ (uncommon to this family too), appearance of the peraeopods, especially of peraeopod II. However, the lack of pleopods makes its classification among the Bodotriidae uncertain.

As it results from the recent paper of Jones and Sanders [4], as well as from the study of the material available to us, we may assume that the oceanic abysses are still a source of many novelties in the Cumacean field, leading even to new genera and, perhaps, to new families.

Anyhow, the finding of a second Cumacean with a well-developed copulatory organ in ♂, may be a matter of further reflection on the phylogeny of this group.

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Received November 30, 1973

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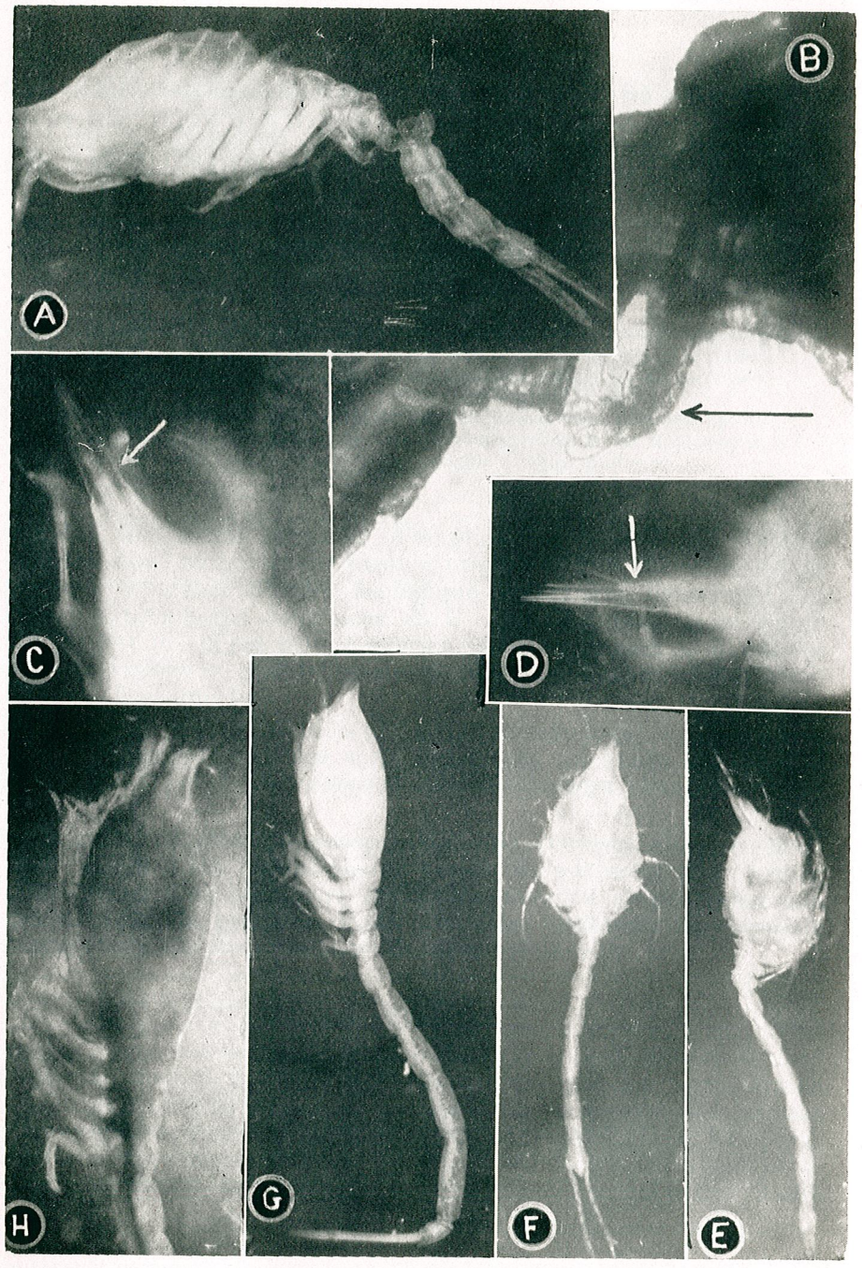

PLATE I. — *A*, *Campylaspenis rowei* n.g.n.sp., ♂ ad.; *B*, part of abdominal and thoracic segments, also penis (magnified); *C*, *Styloptocuma antipai* n.g.n.sp. ♀ ov., pseudorostrum, ocular lobe and frontal lobe; *D*, anterior portion of carapace; *E* and *F*, ensemble-profil and dorsal view; *G*, *Atlantocuma benguelae*, ♂P; *H*, cephalothorax magnified.

SUR UNE GRÉGARINE NOUVELLE À SYZYGIES MULTIPLES, *URADIOPHORA RAMOSA* N.SP., PARASITE D'UN AMPHIPODE PONTOCASPIEN DE ROUMANIE

PAR

DOÏNA BĂLCESCU-CODREANU

The trophic stadia of a new species of Gregarinida *Uradiophora ramosa* n.sp. are described from a Pontocaspian relict Amphipod, *Pontogammarus robustoides*, in the Danube Delta. The new species is characterised mainly by an increased number of repeatedly branched syzygies with a quite peculiar shape.
Gregarines having such a type of ramified associations were recorded up to now only in families parasitising marine crustacea. This is the first species living on a fresh-water host, whose marine origine is thus confirmed.

Sur les Grégarines parasites d'amphipodes relictes du système ponto-caspien, nous ne possédons jusqu'à présent que de brèves mentions à propos de recherches d'ordre écologique concernant l'ensemble des parasites chez *Gammarus balcanicus* et *G. kischineffensis* de la haute vallée du Dniestr [6] [7] et chez *Pontogammarus robustoides* et *P. crassus* provenant du Dniepr et graduellement acclimatés dans les bassins avoisinant la Mer Baltique [4].

En l'absence de toute caractérisation morphologique ou taxonomique, les Grégarines signalées sont rapportées au genre *Hirmocystis* chez *Gammarus* [7] et à *Heliospora* et *Cephaloidophora* chez *Pontogammarus* [4].

Dans un lot de *Pontogammarus robustoides* (Grimm. 1894) A. Martinov 1924, ramassé sur les racines de roseau du « Ghiolul Roşu » dans le Delta du Danube en novembre 1972, nous avons constaté, selon une fréquence de 14%, la présence d'une Grégarine filiforme, remarquable par ses syzygies multiples que nous étudions ci-après *.

RÉSULTATS

L'intestin moyen semblait presque obstrué par un considérable peloton de longs sporadins vermiformes présentant des mouvements permanents d'enroulement. Dans une goutte de liquide physiologique, les sporadins s'écartent, apparaissant très rarement solitaires, généralement associés en syzygies fronto-caudales, dont la plupart ont l'aspect d'une chaîne

* Nous prions Mme Virginia Marinescu d'agréer nos vifs remerciements pour son obligeance pendant nos campagnes de travail à la Station Hydrobiologique de Sulina, dans le Delta du Danube.

différemment ramifiée d'après le nombre, le mode de succession des satellites, leur forme et leur contenu cellulaire.

Les sporadins isolés sont rubanés, mesurant 350 μ—450 μ de longueur et 20—30 μ de largeur, à l'extrémité antérieure légèrement aiguë, alors qu'ils sont tronqués à l'extrémité postérieure. Leur cytoplasme granulaire renferme vers la moitié de sa longueur un noyau généralement ovalaire d'un diamètre de 12—18 μ (Pl. II, fig. 1).

Les syzygies typiques à deux partenaires de dimensions à peu près équivalentes, sans différences morphologiques entre eux (pl. II, fig. 2), sont peu fréquentes, et tout aussi rares celles de trois individus disposés dans le sens fronto-caudal, ayant chacun une longueur d'environ 350 μ, avec tendance à s'entortiller (Pl. II, fig. 3). Mais la caractéristique essentielle de cette espèce est la fréquence élevée de syzygies multiples. En effet, les satellites ont une forte tendance à s'associer en chaîne et, du fait qu'ils s'attachent par deux en arrière d'un primite, il en résulte des ramifications dichotomiques à différents niveaux. Il se produit en même temps une différenciation morphologique et cytologique entre le primite et ses nombreux satellites (Pl. II, fig. 4).

Les primites, plus trapus, de 200—300 μ de longueur et 25—30 μ de largeur, offrent un cytoplasme clair, à grosses granulations uniformément réparties, et dans leur moitié antérieure, un noyau arrondi-ovalaire avec un gros karyosome excentrique (Pl. III, fig. 5—6). Leur extrémité antérieure a la forme d'un rostre à cause du protomérite conique réduit, tandis que le bord postérieur tronqué porte deux satellites divergents de 1er ordre (Pl. III, fig. 6—7).

Au bout distal de ces derniers, on observe un ou deux satellites de 2^{e} ordre (Pl. III, fig. 5 et Pl. IV, fig. 8), qui à leur tour peuvent être pourvus de satellites de 3^{e} ordre, néanmoins plus rares (Pl. IV, fig. 9—10). Contrairement au primite, d'allure plus rigide, les satellites s'infléchissent en tous sens, ce qui empêche de déchiffrer immédiatement leur mode d'assemblage (Pl. IV, fig. 11). Au fur et à mesure qu'ils s'éloignent du primite, les satellites deviennent plus nettement filiformes, d'une longueur de 400—450 μ et d'une largeur de 18—23 μ. Leur cytoplasme est aussi plus dense et plus finement granulaire que dans le primite (Pl. I, *j*—*n*).

A l'aide des colorations vitales, on met en évidence une réaction cytoplasmique différentielle entre le primite et ses satellites, cette sexualisation cytoplasmique s'accompagnant chez cette espèce également de certaines différences morphologiques, mais à un degré moindre que chez les *Enterocystidae* des Ephémères [2]. Au violet de crésyl par exemple, le cytoplasme du primite, considéré comme femelle, montre de grosses granulations incolores au sein d'une pâle teinte bleuâtre, cependant que les satellites, probablement mâles, ont un cytoplasme à granulations très fines, fortement colorées en violet.

DISCUSSION ET CONCLUSIONS

Parmi les Grégarines, il y a tout d'abord des associations où les satellites primaires portent un ou plusieurs satellites secondaires, comme chez des *Gregarina* (*G. polymorpha*, Nelson et Smith, 1926, *G. aussoniae*,

Ghidini et Moriggi, 1941 et *G. soroniae*, Geus, 1969), tandis que chez d'autres *Gregarina* (*G. socialis*, d'après Léger, 1899, *G. blattarum*, d'après Sprague, 1941, *G. rostrata*, Théodoridès et Jolivet, 1959, *G. cuneata*, Geus, 1969) et *Cephaloidophora* (*C. synurellae* Bălcescu, 1972) on peut observer des satellites pygmés directement attachés au primite (Pl. I, *a*—*d*).

On connaît en outre des enchaînements linéaires de 3—5 sporadins à disposition fronto-caudale chez plusieurs espèces de *Hirmocystis* (Léger, 1899 et Geus, 1969 chez *H. polymorpha*; Watson, 1922 chez *H. harpali*; Henry, 1933 chez *H. termitis* et Geus, 1969 chez *H. socialis*, *H. gryllotalpae*, *H. mycetocharae* et *H. minima*) (Pl. I, *e*, *f*), chez *Uradiophora* (*U. cuenoti*, Mercier, 1912) et incidemment dans les genres *Cephaloidophora* et *Porospora* (Pl. I, *i*). Concernant ce type d'association multiple, Grassé (1953, p. 574) fait remarquer la taille décroissante des partenaires qui s'éloignent du primite et leur séparation par couples avant l'enkystement.

Enfin, les sporadins en file peuvent présenter des ramifications latérales par l'accolement de satellites supplémentaires. Parmi les *Uradiophoridae*, Grassé (1953, p. 574, fig. 442 C) signale une *Uradiophora sp.* d'aspect ramifié (Pl. I, *g*); dans les *Cephaloidophoridae*, Ball (1938, 1959) décrit le *Carcinoecetes hesperus* d'après son aptitude à former des syzygies ramifiées (Pl. I, *h*); chez les *Porosporidae*, les *Nematopsis*, exemple *N. legeri*, s'agencent en séries simples ou ramifiées de trophozoïtes (Grassé, 1953, p. 640, fig. 497—1; R. Kudo, 1966, p. 664, fig. 243 g).

En somme, les Grégarines à syzygies multiples ramifiées se rencontrent surtout dans certaines familles parasites des Crustacés marins, telles les Cephaloïdophoridées, Uradiophoridées, Porosporidées. Dans le présent travail, nous faisons connaître pour la première fois un cas pareil chez un malacostracé d'eau, douce, l'amphipode *Pontogammarus robustoides*.

Les caractères morphologiques et cytologiques si particuliers de la syzygie multiple ramifiée étudiée ici, et son isolement écologique, nous amènent à en faire une espèce nouvelle que nous rattachons au genre *Uradiophora* sous le nom d'*Uradiophora ramosa* n.sp.

Il est à observer que l'hôte, *Pontogammarus robustoides*, est en fait un relicte ponto-caspien et, tout en étant retiré actuellement dans les eaux douces du Delta du Danube, il a une lointaine, mais incontestable origine marine. Il y a là une preuve de l'ancienneté et de la spécificité du parasitisme des Grégarines, car notre *Uradiophora ramosa* n.sp. a de toute évidence perpétué dans son milieu aquatique secondairement adouci l'aptitude à produire des syzygies multiples, et même d'une complexité accrue.

Vu que le sexe des sporadins semble déterminé avant leur réunion en syzygies et que cet accolement a lieu à des stades variés selon les genres de Grégarines, ceci suggère un processus d'attraction due à la libération de substances sexuelles diffusibles à partir du primite. Ces substances, comparables aux gamones d'autres organismes, devraient être particulièrement actives chez les espèces aboutissant à la constitution de syzygies multiples, comme *U. ramosa n.sp.*

D'autre part, les liens unissant les partenaires successifs d'une telle chaîne font supposer l'existence d'une gamme de valences sexuelles rappelant des phénomènes de sexualité relative.

Un moyen de vérifier ces interprétations serait de pouvoir saisir le comportement des syzygies multiples lors de la formation des gamontokystes, précédant la différenciation des gamètes.

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Reçu le 27 décembre 1972

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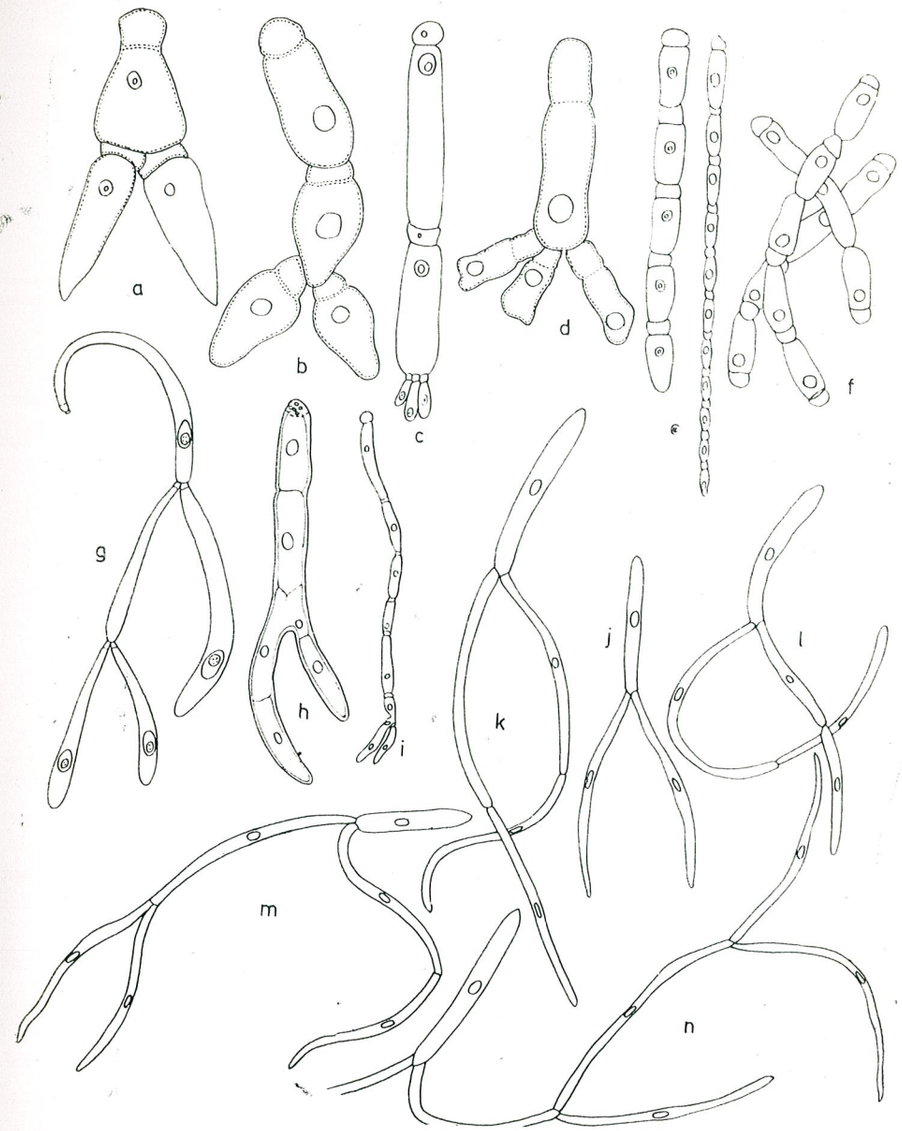


PLANCHE I Divers types de syzygies multiples.
a, *Gregarina aussoniae* d'après Ghidini et Morrigi, 1941 ; *b*, *Gregarina soroniae* d'après Geus, 1969 ; *c*, *Gregarina socialis* d'après Léger, 1899 ; *d*, *Gregarina cuneata* d'après Geus, 1969 ; *e*, *Hirmocystis polymorpha* d'après Geus, 1969 et Léger, 1899 ; *f*, *Hirmocystis socialis* d'après Geus, 1969 ; *g*, *Uradiophora sp.* d'après Grassé, 1953 ; *h*, *Carcinoecetes hesperus* d'après Ball, 1959 ; *i*, *Porospora gigantea* d'après Léger et Duboscq, 1909 ; *j*–*n*, *Uradiophora ramosa* n.sp.

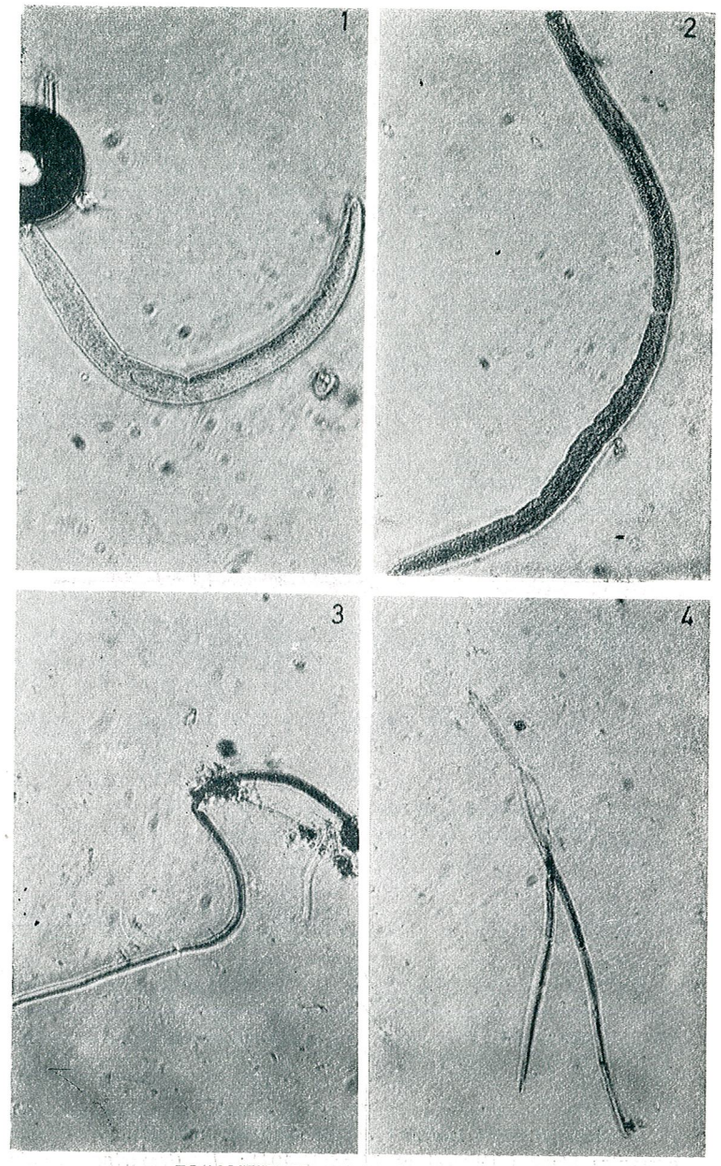


PLANCHE II *Uradiophora ramosa* n.sp.
fig. 1, sporadin isolé ; fig. 2, syzygie à deux partenaires ; fig. 3, syzygie à trois partenaires ; fig. 4, syzygie ramifiée avec des satellites de deux ordres.

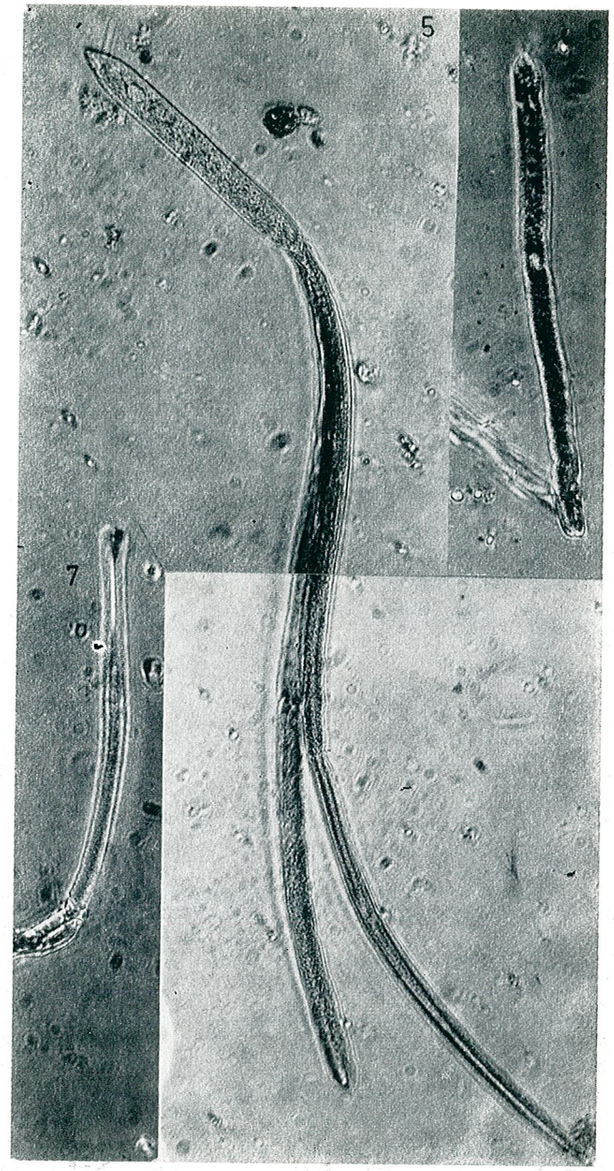


PLANCHE III *Uradiophora ramosa* n.sp.
fig. 5, syzygie multiple avec des partenaires différenciés ; fig. 6, le primite ; fig. 7, jonction au primite des deux satellites de 1er ordre.

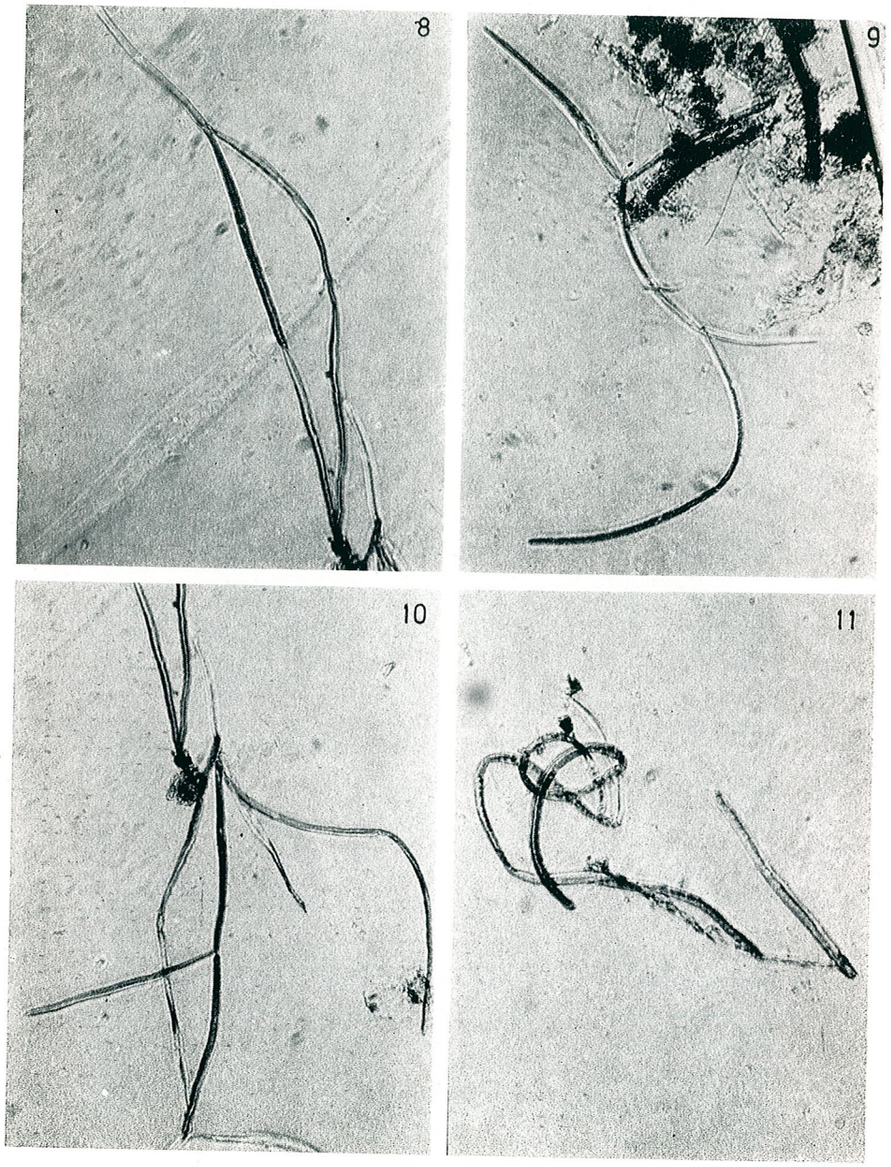


PLANCHE IV *Uradiophora ramosa* n.sp.
fig. 8—11, associations multiples variablement ramifiées. (Photos en contraste de phase)

CONTRIBUCIÓN AL ESTUDIO DE LOS AMFIPODOS (GAMMARIDEA) LITORALES DE CUBA

M. ORTIZ TOUZET

Until now only one paper was published about Cuban marine amphipode [10]. The present paper, the second one in the above mentioned field, is the starting point of a series of investigations on Gammaridean amphipods to be carried in the Cuba's west littoral waters.
Here the author points out 8 species recorded for the first time in Cuba, as well as a map with the distribution of the genera and species be found in this area.
Several zoogeographical considerations complete the text of this paper.

El presente trabajo, lo he confeccionado basándome en la colección efectuada por los doctores O. Gómez, del Instituto de Oceanología de la Academia de Ciencias de Cuba, y M. T. Gomoiu del Instituto Rumano de Investigaciones Marinas, en aguas de la plataforma occidental cubana, en el año 1969.

Las localidades de colecta fueron: El Golfo de Batabanó, y la Bahía de Cienfuegos en la costa Sur, y La Ortigosa, La Habana, y Varadero en la costa Norte.

Durante la elaboración del mismo he recibido del Prof. M. Băcescu, quien me propusiera, e iniciara en el estudio de este grupo, numerosas indicaciones y sugerencias, así como en el Museo de Historia Natural de Bucarest, que el dirige, la oportunidad de revisar su biblioteca.

Además pude consultar en la Universidad de Iaşi, gracias a la amabilidad del Prof. S. Cărăuşu, su magnífica biblioteca.

Tambien debo agradecer a los doctores G. Müller, y M. T. Gomoiu del IRCM (Secc. I. Borcea), y a D. Dancău del Instituto de Espeleología de Bucarest, la bibliografía facilitada, y sus numerosas indicaciones. A todos ellos, mi más sincero reconocimiento.

A pesar de que los amfipodos son sumamente abundantes en aguas cubanas, solamente se ha publicado hasta el presente un solo trabajo, referente a dicho grupo en nuestros mares [10], donde reportó un total de 11 especies de amfipodos (Gammaridea). El mismo autor en el año 1933, reportó tambien 3 especies para Cuba.

Sin embargo, con anterioridad a los dos trabajos citados, solo conocemos los reportes de T. R. R. Stebbing, 1897, donde al describir la especie *Lysianassa cubensis*, señaló para la misma como localidad típica, El Golfo de México, y el Mar Caribe (Cuba), y T. R. R. Stebbing [11], donde se citó *Corophium acherusicum* A. Costa, para aguas cubanas, especie que con posterioridad vuelve a ser reportada para la misma localidad [6].

Resultados de mi trabajo

Durante mis investigaciones he encontrado representantes de 13 familias, 15 géneros, y 10 especies.

De los 15 géneros encontrados, 11 son reportados en este trabajo por primera vez para Cuba. Además 8 de las 10 especies son nuevas para aguas cubanas. (Ver tabla presentada).

Queremos tambien destacar que contamos con numerosos ejemplares de la Fam. Amphithoidae incompletos, los que por este motivo no se han identificado con absoluta seguridad, pero que creemos pertenecen a los géneros *Amphithoe*, y *Cymadusa*.

De la Fam. Bateidae, tenemos varios ejemplares sin identificar por no haber podido consultar C. R. Shoemaker [8] y no incluidos en el presente trabajo, pero posiblemente pertenecientes al Género *Carinobatea*.

En lo que a distribución se refiere, vemos que la especie mediolitoral mejor distribuida en el occidente de Cuba, resultó ser *Parhyale inyacka*, mientras que en la zona infralitoral la especie más frecuente fué *Leucothoe spinicarpa*, especie que además constituye el amfipodo espongicola por excelencia en los mares cubanos. (Ver el mapa presentado).

Algunas consideraciones zoogeográficas

Desde el punto de vista zoogeográfico, la aparición de un representante de la Fam. Acanthonotozomatidae en aguas cubanas, resulta ser sin lugar a dudas el hecho más sobresaliente, si se tiene en cuenta que esta familia es primariamente antiboreal-antártica, conociendose hasta el momento solo dos géneros con representantes en mares tropicales, que son : *Panoploeopsis* Kunkel (una sola especie de aguas litorales de las islas Bermudas), e *Iphimedia* Ratke (distribuido por el Pacífico tropical, y la Antártica), según J. L. Barnard [4].

Monoculodes carinatus es una especie con distribución típica en las aguas del Atlántico norte-oriental, según A. Schellenberg [7]. Su aparición en aguas cubanas constituye sin lugar a dudas otro hecho de interés.

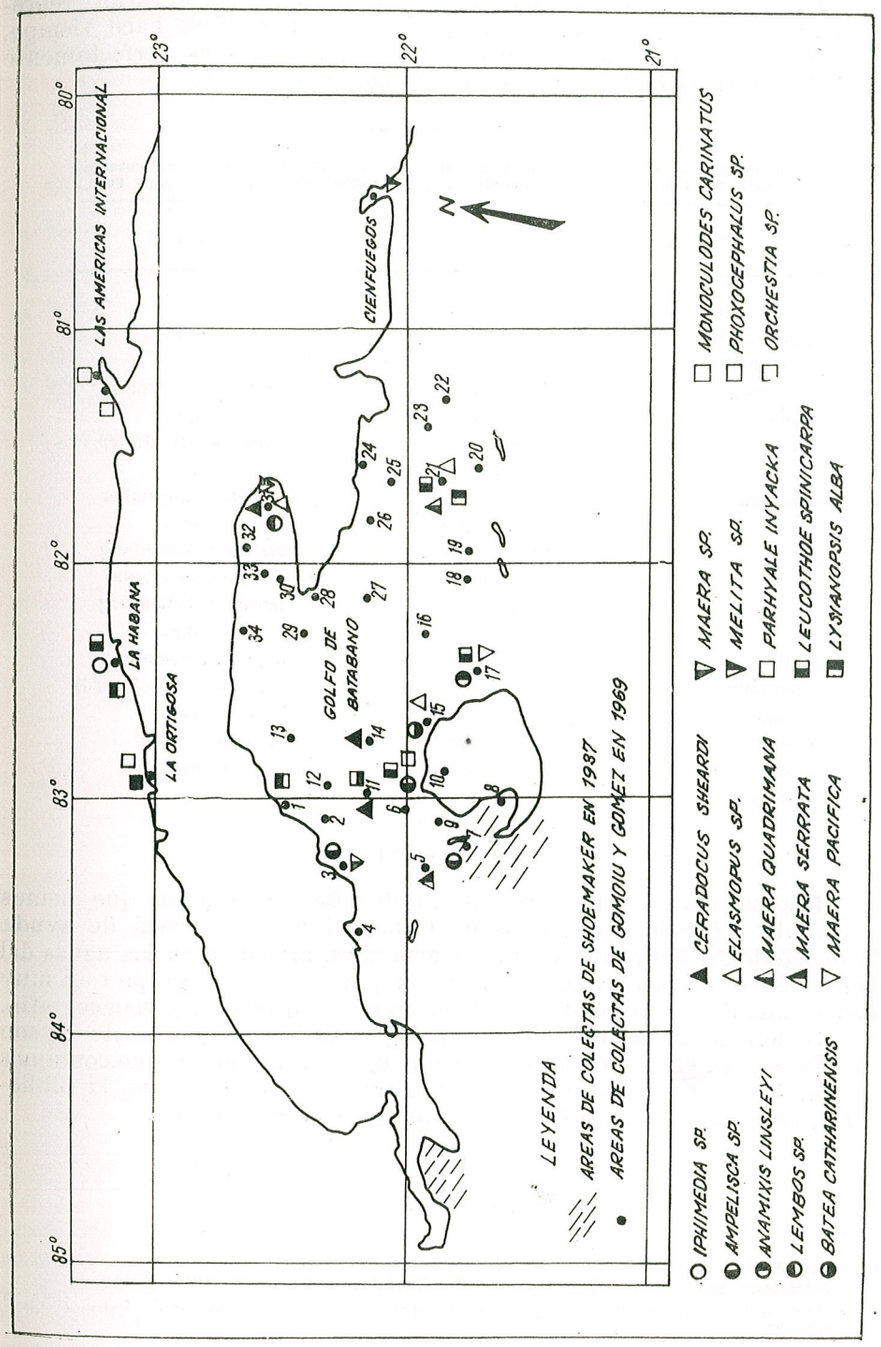
Hemos encontrado además varias especies, que hasta el momento solo se han encontrado en California, Hawaii, la Micronesia, y la Polinesia, que son *Anamixis linsleyi*, *Maera pacifica*, *M. serrata*, y *M. quadrimana* [2] [3] [5], las que al aparecer en aguas cubanas, nos hacen pensar en la posibilidad de un transporte a travéz del canal de Panamá, debido a corrientes, o a la existencia de un transporte pasivo, formando parte de la epibiosis de las embarcaciones que circulan a travéz del mismo.

Parhyale inyacka se conoce como una especia típica del Mediterráneo Americano, según K. Stephensen [12].

Sobre el género *Ampelisca*, se han reportado numerosas especies para el Caribe [1].

Batea catharinensis, tambien ha sido con anterioridad señalada para el Mar Caribe [4].

Lysianopsis alba, ha sido reportada para la Florida, y Puerto Rico [9].



Los restantes géneros encontrados durante mi trabajo, están representados en aguas del Caribe, o del Golfo de México desde hace tiempo, por pertenecer a familias cosmopolitas, o tropicales, y es perfectamente admisible la aparición de estos en aguas cubanas.

Tabla de resultados

Especies de amfípodos (Gammaridea) identificados en la plataforma occidental cubana (+ indica los géneros, o especies reportados en este trabajo por primera vez para Cuba.)

Familias	Géneros	Especies
Acanthonotozomátidae	+*Iphimedia*	sp.
Ampeliscidae	+*Ampelisca*	spp.
Amphithoidae	?	?
Anamixidae	+*Anamixis*	*linsleyi* J. Barnard
Aoridae	+*Lembos*	spp.
Bateidae	+*Batea*	*catharinensis* Müller
	?	?
Gammaridae	*Ceradocus*	*sheardi* Schoemaker
	Elasmopus	spp.
	+*Maera*	*pacifica* Schellenberg
	+*Maera*	*quadrimana* (Dana)
	+*Maera*	*serrata* Schellenberg
	+*Melita*	spp.
Hyalidae	+*Parhyale*	*inyacka* (Barnard)
Leucothoidae	*Leucothoe*	*spinicarpa* (Abildgard)
Lysianassidae	+*Lysianopsis*	*alba* Holmes
Oedicerotidae	+*Monoculodes*	*carinatus* (Bate)
Phoxocephalidae	+*Phoxocephalus*	sp.
Talitridae	+*Orchestia*	sp.

CONCLUSIONES

Presentamos los primeros resultados de un estudio, que hemos comenzado sobre los amfípodos de Cuba. El mismo servirá de ayuda al conocimiento del grupo en líneas generales, así como en las aguas del Mediterráneo Americano específicamente, donde dicho grupo está muy poco estudiado, tanto su sistemática, como su ecología, y zoogeografía.

Se han consultado más de 60 trabajos, de los cuales más de 15 son especificos para el área estudiada, lo que unido al hecho de que contamos con numerosas especies, que no se ajustan a las descritas en la bibliografía, creemos muy factible la aparición de especies nuevas.

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Recibido el 30 de Noviembre 1973

Universidad de La Habana
Centro de Investigaciones Marinas

TROIS ESPÈCES DE TRACHYSPHYRINAE (HYM., ICHNEUM.) NOUVELLES POUR LA SCIENCE

PAR

VICTOR CIOCHIA

Dans le présent travail on décrit pour la première fois trois espèces de Trachysphyroidae, à savoir : *Buathra spinipes* sp.n., *Gelis constantineanui* sp.n., dédié à l'ichneumonologue M. I. Constantineanu, collecté dans la réserve de dunes d'Agigea (Roumanie) et *Gelis napocai* sp.n., dédié à la ville de Cluj, où notre collègue C. Nagy a collecté cette espèce.

FAMILLE DES ICHNEUMONIDAE HALIDAY, 1901
SECTION DE TRACHYSPHYROIDAE
CONSTANTINEANU ET CIOCHIA, 1970.

A. Sous-famille des **Trachysphyrinae** Constantineanu, 1966.

I. Tribu des *Trachysphyrini* Constantineanu et Ciochia, 1961.

Le genre *Buathra* Cameron, 1903.

1. **Buathra spinipes** sp.n., ♀♂.

♀. La tête vue d'en face semble être triangulaire, les yeux étant proéminents, les joues longues. L'épistoma est évidente. Les mandibules sont de couleur noire, pileuses, avec deux dents inégales. Le front est très excavé, mat, avec des ornementations. Le côté situé vers les antennes est luisant avec une petite fossette devant chaque antenne. Les antennes sont filiformes, les derniers articles étant légèrement élargis.

Les fosses parapsidales sont prononcées, divisant le mésonote en trois champs évidents. Elles surpassent beaucoup la moitié du mésonote. La fosse située devant le scutellum est pourvue de côtes longitudinales. Le thorax est ponctué et couvert de poils fins. Le segment intermédiaire est pourvu d'une côte transversale postérieure évidente. Les côtes latéro-basales sont à peine esquissées. Dans la région de la surface surmédiane on peut apercevoir une petite proéminence qui accentue l'existence de celle-ci. Au reste, le segment intermédiaire est sans ornementations évidentes, ayant de fines rugosités. L'espace abrupt du segment intermédiaire est pourvu d'une fine ornementation. Le premier segment abdominal est allongé, s'élargissant évidemment dans la zone du post-pétiole. Regardé latéralement on s'aperçoit de la présence d'un angle entre le pétiole et le post-pétiole. Le post-pétiole est dépourvu d'ornementations. La couleur de l'abdomen est noire. La nervation des

REV. ROUM. BIOL., TOME 19, N° 2, p. 89–94, BUCAREST, 1974

ailes est brune foncée. La nervure cubitale s'étend jusqu'au bout de l'aile fine et de couleur brune jaunâtre. Le nervel est situé devant la fourche, étant brisé sous sa moitié. La nervure du nervel s'étend jusqu'au bout de l'aile postérieure. Les tibias et les tarses sont pourvus d'épines rares mais évidentes, brunes-rousses. La gaine de la tarière est de couleur noire, pileuse, ayant le côté distal de couleur plus claire. L'ovipositeur est brun-roux, ayant sur la partie inférieure, située vers le bout, dix petites dents.

La longueur du corps = 10,3—13 mm; la longueur de l'ovipositeur = 6,3—8 mm; la longueur de l'aile inférieure = 8,5—10,5 mm.

♂. Il est semblable à la femelle. Noir, le front très excavé. Dans la partie postérolatérale de la base des antennes on remarque l'excavation caractéristique du genre *Buathra*. Entre les deux excavations l'espace est luisant et pourvu de striures transversales. Le visage, les joues et les mandibules sont pileux. Les orbites internes sont blanches jaunâtres. Les mandibules ont, comme chez la femelle, une tâche blanche jaunâtre au milieu. Les dents sont rondes, la dent supérieure étant plus courte. L'aspect de la tête est semblable à celui de la femelle. Le vertex est étroit, avec la partie postérieure très excavée. La ligne occipitale est entière. Les antennes sont noires, sétiformes, avec le flagelle formé de 41 articles.

L'armature buccale : les palpes labiaux ont le premier article basal noir, le reste des articles étant jaunes-bruns, de forme allongée. Le premier article est court, le second article élargi, étant pourvu à la partie intérieure de poils plus longs que les poils du reste des articles. L'article distal est allongé et évidemment plus mince que le reste des articles. Les palpes maxillaires sont formés de 4 articles. L'article distal a la forme d'une faux à bout ovale. Les autres articles sont courts et larges, bruns foncés (fig. 1). Le labrum, distal, est plus allongé.

Le thorax est brun, avec de petits points, pileux. Les épomies sont présentes, ayant la forme d'un S. La fosse de la partie antérieure du scutellum est pourvue de striures longitudinales. Sur le segment intermédiaire seulement la côte postérieure transversale est évidente. Les stigmes respiratoires sont ovales allongées. Le spéculum est présent.

Les pattes sont de couleur brune jaunâtre-rousse. Les coxes et les trochanters sont noirs. Les trochanters antérieurs ont sur la partie ventrale une tâche allongée brune-rousse. Les tibias et les tarses sont pourvus d'épines courtes, rares mais évidentes, de couleur brune. Les tarses postérieurs sont pourvus d'un anneau blanc. Le bout des tibias postérieurs ont dans la partie externe 5 épines grandes et brunes.

Les ailes sont hyalines, avec la nervation brune foncée. Le nervulus est situé en arrière de la fourche. Le ramel est évident. La ptérostigme est brune foncée. Le nervel est brisé sous la moitié (fig. 2). Les tégules sont noires. Le rétinaculum est pourvu de 9 crochets, le basal étant formé d'une seule épine avec un crochet.

L'abdomen est noir, allongé, finement pileux, alutacé. Le premier segment abdominal est évidemment plus long que le second. Le troisième est plus court que le second. La partie distale des segments 2 et 3, tout comme les tâches latérales du segment 2, sont finement brunes. La zone des stigmes respiratoires du premier segment est proéminente.

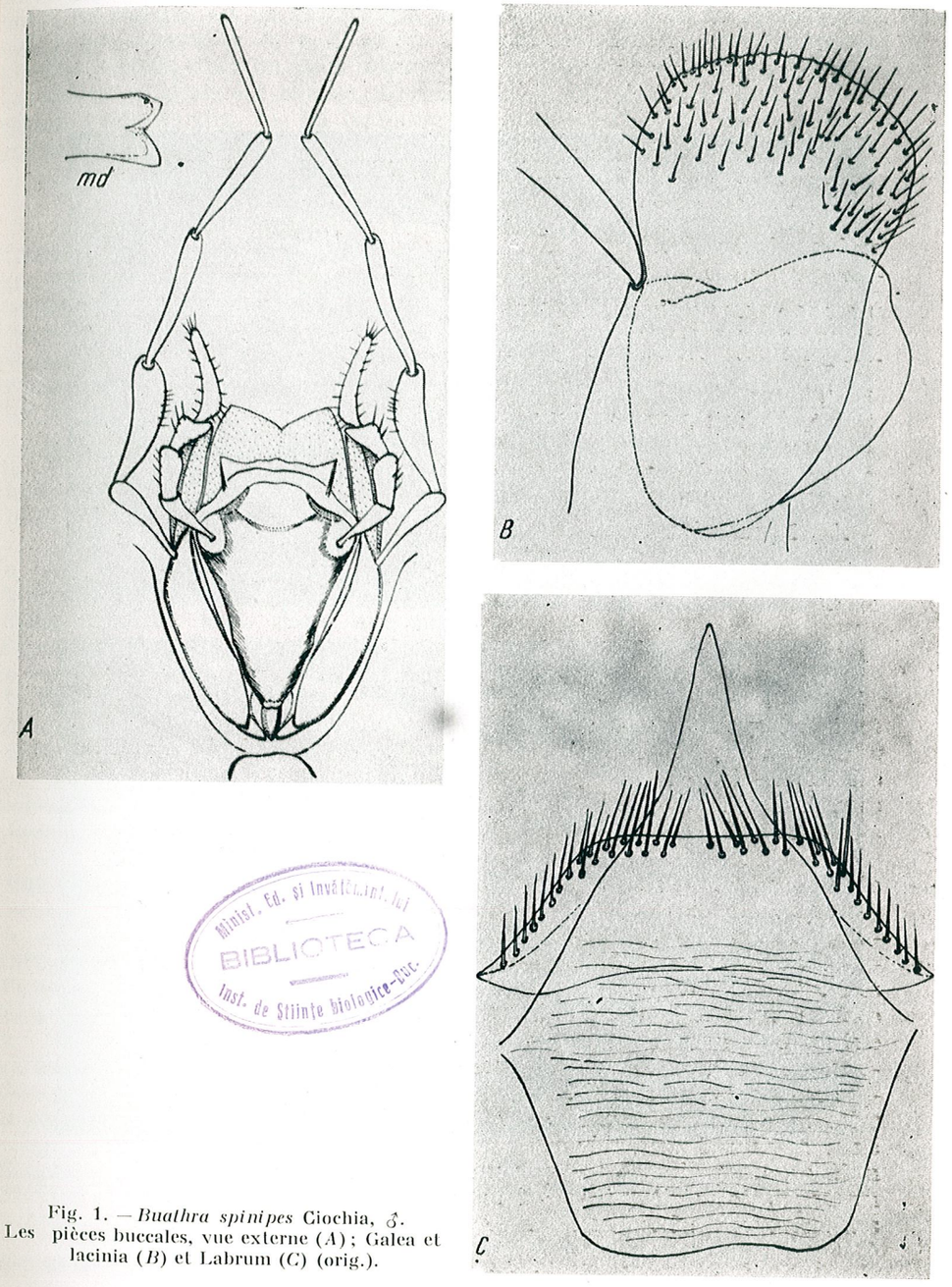

Fig. 1. — *Buathra spinipes* Ciochia, ♂. Les pièces buccales, vue externe (*A*); Galea et lacinia (*B*) et Labrum (*C*) (orig.).

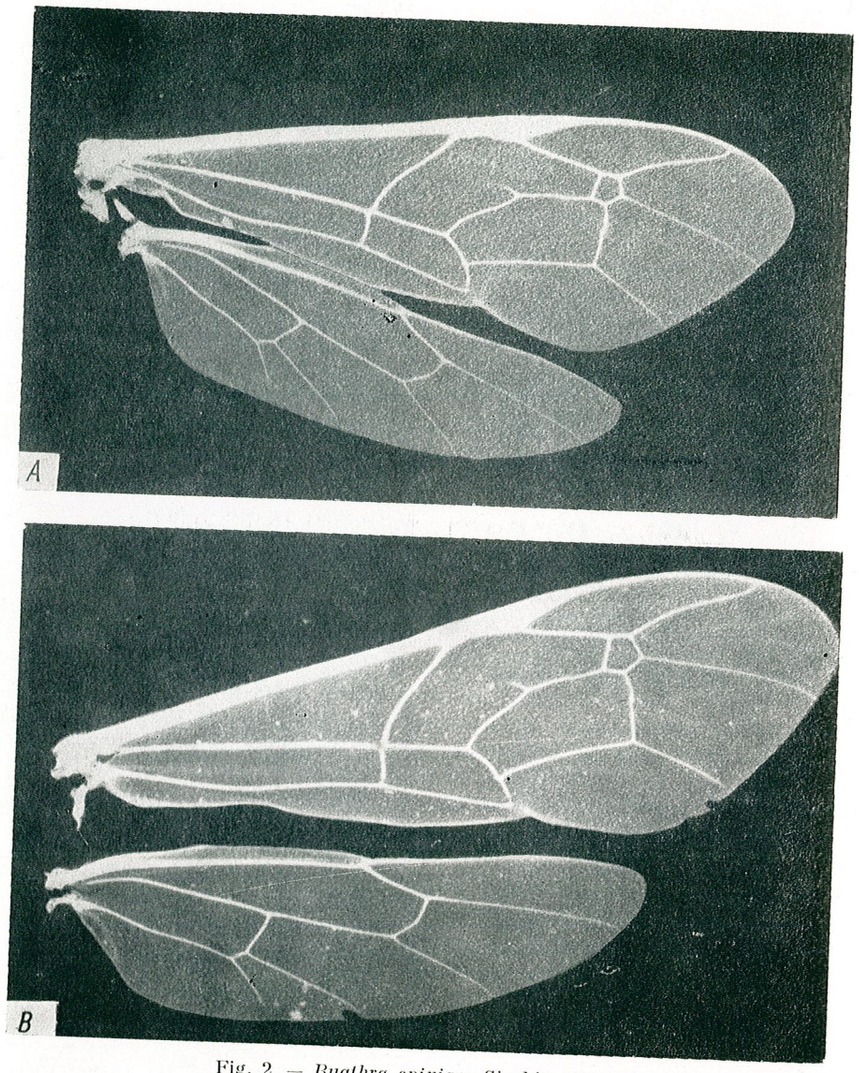

Fig. 2. — *Buathra spinipes* Ciochia, ♀♂.
Ailes droites de la femelle (*A*) et du mâle (*B*) (orig.).

Les valves génitales externes dépassent évidemment le bout de l'abdomen, elles sont noires et pileuses. Aedeagus est un peu courbé vers le bout. Le couspis est excavé vers la partie inférieure. Vers la zone terminale il est pourvu de 11 poils longs. Le digitus ne fait pas corps commun avec la volsela, mais on peut observer les endroits d'articulation (fig. 3). La plaque génitale est pileuse, pentagonale, ayant à la base une prolongation évidente (fig. 4). La longueur du corps = 11—12,8 mm ; la longueur de l'aile antérieure 8,6—9,5 mm.

OPL est un peu plus grand que OOL *

Note : Cette espèce ressemble à *Buanthra laborator* (Thunbg.)

Holotype : 1 ♀, collecté le 7.VIII.1965, dans la réserve du Dealul Cetății (commune Hărman, dép. Brașov), à une altitude de 700 m, dans une forêt de feuilleuses (*Quercus petraea* (Matt.) Liebl. *Carpinus betula* L., *Tilia parvifolia* Ehrh.,, etc.), à approximativement 10 m de la lisière d'une clairière, pendant qu'il volait au-dessus des feuilles mortes tombées à terre.

Allotype : 1 ♂, collecté le 25.VII.1965, sur le mont Postăvarul (dép. Brașov), pendant qu'il volait dans la clairière d'une forêt de feuilleuses.

Paratypes : 1 ♀, le 3.VIII.1965, dans la réserve de « Tîmpa » (Brașov), pendant qu'il volait au-dessus des feuilles de *Rubus sulcatus* Vest. dans une forêt de *Fagus silvatica* L. ; 1 ♀, le 8.VIII.1965, sur le mont Gogorița (Brașov) à une altitude de 800 m pendant qu'il volait dans une clairière ensoleillée d'une forêt composée d'un mélange (*Carpinus betula* L., *Fagus sylvatica* L., *Pinus sylvestris* L., *Larix decidua* var. *polonica* (Racib.) O.—S.—L., *Picea excelsa* (Lam.) Link., *Corylus avellana* L., etc.) ; 1 ♀ le 21.VIII.1967, sur le ruisseau Durbav (Sînpetru, dép. Brașov), sur des fleurs de *Daucus carota* L. ; 1 ♀, le 28.VII.1965, dans la réserve « Tîmpa » ; 1 ♂, le 25.VII. 1965, sur le mont Postăvarul (Brașov), d'une clairière située sous la ligne du téléférique ; 6 ♂♂, le 28.VII.1965, collectés dans la réserve « Tîmpa » (Brașov), pendant qu'ils survolaient des buissons couverts de *Clematis vitalba* L. ; 1 ♂, le 10.VII.1966, dans la région du ruisseau Durbav pendant qu'il volait parmi des graminées spontanées (Triaj, Brașov) ; 2 ♂♂, le 3.VIII.1965, réserve « Tîmpa » (Brașov), pendant qu'il volait

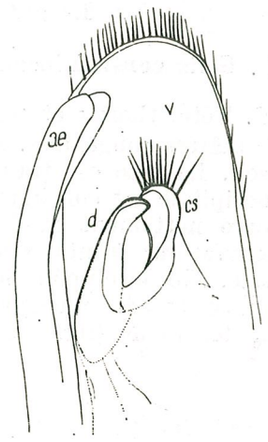

Fig. 3. — *Buathra spinipes* Ciochia, ♂.
La partie distale de l'appareil génital, vue latérale ; v, valve gauche ; cs, cuspis ; ae, aedeagus et d, digitus. (orig.).

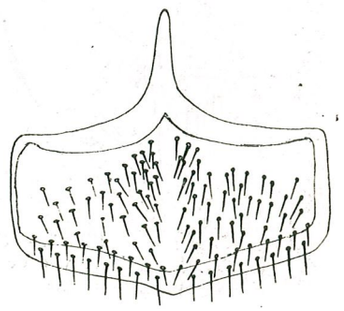

Fig. 4. — *Buathra spinipes* Ciochia, ♂.
La plaque sous-génitale (orig.).

* OOL = la distance entre les yeux composés et les ocelles postérieurs ; OPL = la distance entre les ocelles postérieurs.

au-dessus du feuillage couvrant la terre dans une forêt de *Fagus sylvatica* L. et *Carpinus betula* L.

L'holotype, l'allotype ♂ et les paratypes ♀♀ et ♂♂ se trouvent dans la collection de Victor Ciochia.

B. Sous-famille des **Gelinae** Vierek, 1918.

Tribu des *Gelini* Townes, 1951.

Le genre *Gelis* Thunberg, 1827.

2. **Gelis constantineanui** sp.n., ♀.

♀. Tête, thorax et segments abdominaux 4 et 5 noirs. Les pattes sont jaunâtres rougeâtres. Le bout des ailes est clair, ressemblant à un bourgeon. La tête est transversale, excavée en sa partie occipitale. La ligne occipitale est continue. Le front est long, l'excavation des antennes se trouve nettement au-dessous de la moitié de la tête, vue de face. Les excavations frontales sont visibles. Le front a une pilosité courte et éparse. Sur le clypéus les poils sont très longs. Sur l'épistoma et le clypéus se trouve une proéminence. La face est alutace. La bouche est sous-terminale (fig. 5 *A* et *B*). Le scape est brun noirâtre. Les trois

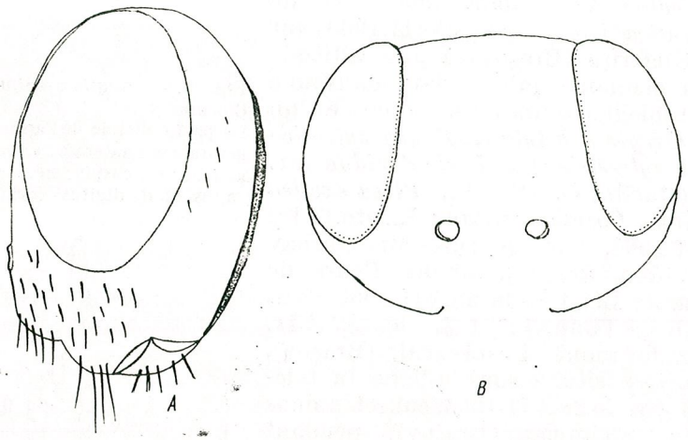

Fig. 5. — *Gelis constantineanui* Ciochia, ♀.
Tête vue de profil (*A*) et de face (*B*). (orig.).

premiers articles du funicule sont bruns jaunâtres, le reste du 5ème article étant brun noirâtre. Le 1er article est plus long que le 2ème. Le 5ème est égal au 4ème. Le funicule est formé de 20 articles. Les palpes maxillaires sont bruns noirâtres.

Le thorax est court, noir et pileux. Le scutellum est distinct. La carène postérieure transversale partage le segment médian en deux zones distinctes : une zone dorsale concave et une zone escarpée qui représente de façon évidente l'aréa postmédia qui est excavée. La carène latérale a des dents distinctes. La carène pleurale est indiquée. Les stigmes sont petites, ovales, arrondies. Les pattes sont brunes rougeâtres. Les tarses sont noirâtres.

Le premier segment abdominal est bien élargi. La partie abdominale terminale du premier segment abdominal, la partie basale du 2ème et du 3ème segments sont brunes rougeâtres, le reste des segments sont noirs. La partie terminale des segments 5—7 a une tache transversale blanchâtre. La gaine de la tarière est pileuse et noirâtre. L'abdomen est oblong-ovale (allongé), alutace. Les segments 2, 3 et 4 sont rectangulaires, mais le 5ème est trapézoïdal. La pilosité est évidente sur les côtes latérales des segments 4—7.

Longueur du corps = 3,50 mm ; longueur de la tarière = 0,95 mm ; OPL presque égal à OOL.

Holotype : 1 ♀, le 18.IV.1970, réserve de dunes d'Agigea (Dobrogea, Roumanie) à 9 m d'altitude, sur un chemin dallé de béton.

Paratype : 3 ♀♀, le 22.IV et 24.IV.1970 au même endroit que celui d'où nous avons collecté l'holotype, réserve de dunes d'Agigea.

L'holotype et le paratype se trouvent dans la collection de Victor Ciochia.

3. **Gelis napocai** sp.n., ♀.

♀. Tête noire plus large que le thorax. Le vertex est très large. Les yeux sont bruns-noirs. La tête est pileuse. Les fossettes frontales sont présentes. Les mandibules sont brunes noirâtres. L'epistoma et le clipéus ont une protubérance. Les antennes sont brunes à la base, mais vers la partie distale la couleur fonce jusqu'à ce qu'elle devienne noire. Elles sont filiformes avec 20 articles. Le 1er article du funicule est plus petit que le 2ème. Le scape est noir, cylindrique, un peu excavé. La ligne occipitale est continue. Le thorax est rouge et pileux. Le scutellum est absent. La place d'articulation des ailes antérieures est présente sur les côtes latérales du mésonotum. Les stigmas sont petites et rondes avec la marge noirâtre. Le segment intermédiaire est escarpé dans sa partie postérieure. La carène postérieure transversale ainsi que la carène pleurale sont visibles. Les hanches sont rougeâtres. Les fémurs sont noirâtres. Les tibias antérieurs ainsi que la partie intermédiaire des tibias intermédiaires sont bruns rougeâtres. Les tibias postérieurs et les tarses sont noirâtres. Les éperons sont bruns rougeâtres. L'abdomen est ovoïdal, pileux. Le 1er segment est rouge. Le 6ème segment est brun rougeâtre à sa partie distale, le reste des segments sont marrons noirâtres, couverts de poils denses et fins. Les segments sont densément ponctués. Le dernier tergite a la forme d'une lame de socle d'une charrue. La gaine et la tarière sont noires et pileuses. La tarière est brune jaunâtre. Le 3ème tergite abdominal est rectangulaire.

Longueur du corps = 4,5 mm ; longueur de la tarière = 0,3 mm ; OPL plus large que OOL avec 1/3.

Holotype : 1 ♀, le 10.V.1963, aux abords de la ville de Cluj (Roumanie), leg. C. Nagy.

Note : Le nom de cette espèce est dédié à la ville de Cluj, nommée autrefois Napoca.

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Reçu le 30 novembre 1973

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Braşov, str. Fundăturii 2

THE SPECIES OF *SCHISTURA* (= HOMATULA) FROM THE UPPER YANGTZE DRAINAGE (PISCES, COBITIDAE)

BY

PETRU BĂNĂRESCU and TEODOR T. NALBANT

Homatula Nichols, 1925 cannot be separated from *Schistura* Mc Clelland, 1839. It includes only two species in the upper Yangtze drainage : *variegata* (Sauvage & Dabry) and *potanini* Günther. *Nemachilus berezowskii* and *N. oxygnathus* are shown to be synonyms of *Sch. variegata*. Both *variegata* and *potanini* may includes several subspecies. The six nominal species from souther East Asia may be synonym or represent two or three sibling species.

The subgenus *Homatula* (type : *Nemachilus potanini* Günther) was proposed by Nichols [4] [5] for seven nominal species of Noemacheiline loaches from China ; to the same belong two species from North Vietnam described by Rendahl [6]. *Homatula* differs from all other generic groups [1] of Noemacheilinae from East Asia through the following characters : depressed head ; convex edge of dorsal fin ; scaled body ; a dorsal creast on caudal peduncle ; a toothlike prolongation (*processus dentiformis*) on the praemaxillaries ; no sexual dimorphism ; lips slightly furrowed ; short intestine ; vertical stripes on the body. The *processus dentiformis* and the absence of sexual dimorphism are the most important among these characters. But these characters occur also in the speciose group of striped South Asian Noemacheilini for which the subgeneric (actually generic) name *Schistura* Mc Cleelland, 1839, has been reactualized by Bănărescu & Nalbant in 1964 [1]. The East Asian Homatulae are especially close to the Indian *Sch. rupecula*, type-species of *Schistura*. We consider therefore *Homatula* a synonym of *Schistura*.

This paper deals only with the *Schistura* from the upper Yangtze ; those from southern East Asia (six nominal species) will be dealt with in another paper. Nichols [5] lists three species from the upper Yangtze- *berezowskii*, *oxygnatha*, *potanini* — ascribing variegata to "*Barbatula*" s. str. *together* with *toni* (actually an *Orthrias*) and with the High Asian species group.

The 47 specimens examined, including all type specimens of the four nominal species, belong to following collections : American Museum of Natural History (AMNH), British Museum, Natural History (BMNH), Field Museum of Natural History, Chicago (FMNH), The Institute of Biological Sciences, Bucharest (ISBB, formerly IBTS), The National Museum of Natural History, Paris (MNHN), United States National Museum, Washington (USNM), Zoologitchestii Inst. Akademii Nauk (ZIAN)

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1. **Schistura variegata** (Sauvage & Dabry, 1874) (Pl. I, figs 1—4)

Synonyms : *Nemachilus variegatus* Sauv. & Dabry ; *N. berezowskii* Günther, 1897 ; *N. oxygnathus* Regan, 1908.

Specimens examined : Syntypes of *N. variег.*, MNHN 7934, "China" (no locality, surely upper Yangtze), two specimens, 97.5 and 105.0 mm ; the first named is here declared lectotype ; it retained the Catalogue number holotype of *N. berez.*, ZIAN 10990, Hui-hisne, southern Kansu (probably Yangtze drainage), 122.0 mm ; syntypes of *N. oxygn.*, BMNH 1908 2.27—24, Yunnan-fu, two spec., 114.0 and 89.5 mm, the f declared lectotype ; FMNH 43840, Chung Chiang Miao, Sz 71.2 mm ; the other specimens, from Szechwan and Yunn table I.

D 3/ (7) 8 (9) ; A 2/5

Body very low and elongate ; caudal peduncle low ; e ed, truncate or quite slightly emarginate ; Many narrow reaching to the ventral side ; they are hardly distinct ar marked posteriorly ; 8—14 behind dorsal fin. The main bo are shown in table 1.

The comparison of the above mentioned specimen conspecificity. The type of *berezowskii* (and only specime is unique in having 7 branched dorsal rays ; the syntype have 9, all other specimens (including the other from Y There are also differences in body proportions (Table 1), the tiated population being that from Kuanshien, Szechw (USNM 91648 & ISBB 1078) in which both body depth a show higher values than all other available populations. The that several subspecies may be recognized in the future, cimens will be available, in which case *berezowskii* and become right names for the subspecies from southern Kansu (not whole Yunnan) ; the population from Kuanshien may re subspecies. One difficulty is that there are no data on the syntypes.

2. **Schistura potanini** (Günther, 1896) (Pl. I, fig. 5 ; Pl. II, figs 6—9)

Specimens examined : types of *N. potanini*, ZIAN 1 (probably upper Yangtze), two species, 95.0 mm (holotype (paratype) ; the other specimens, all from Szechwan and listed in table 2.

D3/(7)8;A2/5

This species differs from S. variegata mainly in ha and deeper body, a much shorter and deeper caudal pe fin slightly emarginate, more rarely truncate. Origin of that of dorsal ; praedorsal distance sligthly longer than against much shorter in *variegata*). The body proportions table 2. One remarks rather strong differences between po of which (those concerning snout length and eye diameter) may partially

Table 1

Body proportions in *Schistura variegata* (Sauvage & Dabry)

		Syntypes of *N. var.*	Type *N. berez.*	Syntypes *N. oxygnath.*	USNM 89275 Tsao-Heo, Szechwan	USNM 86869 Kiating, Szechwan	BMNH 1914, 1.28 : 46—50 Wating Chou Yunnan	USNM 91648 & ISBB 1078 ; Kuan-shien, Szechwan near Tibet
Standard length (mm)		97.5—105	122.0	89.5—115.0	87.8—100.0	117—130	80.0—89.5	64.0—106.0
in % of standard length	maximum depth	9.1—9.25	10.1	10.0—11.2	8.9—9.0	8.9—12.8	11.3—12.9	11.1—13.6 (M = 12.3)
	caudal pedunc	21.4—22.6	23.8	19.3—21.4	22.5—23.0	21.6—23.4	19.2—21.2	19.2—21.9 (M = 20.29)
	least depth	7.4— 7.8	8.3	8.7	8.0—8.2	7.5— 9.4	9.0—10.1	8.8—10.7 (M = 9.66)
	praedorsal distance	44.7—45.1	42.7	42.5—44.0	43.0—44.5	41.7—44.4	44.1—45.0	44.5—48.5 (M = 46.60)
	praeventral distance	44.7—45.6	43.8	45.5	45.0—46.7	41.7—46.8	46.0—49.0	44.5—50.0 (M = 47.15)
	P—V distance	26.6—29.5	28.3	27.2—28.8	27.2—30.6	25.4—29.0	30.4—32.2	26.8—33.6 (M = 29.50)
	V-a distance	25.7—27.2	25.6	23.8—25.2	23.4—24.6	22.3—24.6	22.3—25.6	23.4—26.9 (M = 24.19)
	head length	17.1—17.4	16.8	17.2—19.2	19.2—19.5	15.7—18.5	—	18.1—21.1 (M = 19.90)
	snout length	6.2—6.5	6.95	7.3—7.8	7.8—7.9	6.6—7.2	—	6.6—8.3 (M = 7.9)
	eye diameter	2.9—3.2	2.2	3.0—3.6	2.5—2.6	2.7—2.8	—	2.9—3.3 (M = 3.16)
in % of head	snout	35.0—38.0	41.5	38.6	39.6—40.0	38.4—41.5	—	30.0—41.7 (M = 42.00)
	eye diameter	16.4—16.6	13.2	15.8—17.7	12.8—15.6	15.5—16.9	—	14.9—19.5 (M = 16.15)
eye in % of interorbital width		70.0—72.2	60.2	81.0—93.0	58.0—60.5	66.0—70.5	—	63.0—83.0 (M = 72.20)

Table 2

Body proportions in *Schistura potanini* (Günther)

		Type & Paratype ZIAN 10005	MNHN 13—163 (1) Pin-Fa (Szechwan ?)	USNM 87610 (2) Szechwan	USNM 87428 (2) Suifu—Szechwan	USNM 130126 Kuan-shien Szechwan	BMNH 1924.1.28 : 36—45 (6) Yunnan	AMNH 15285 (2) Szechwan	AMNH 10547 (1) Szechwan
Standard length (mm)		77.0—95.0	62.5	63.5—67.5	86.0—90.2	55.0	63.5—73.0	57.0—99.0	74.8
in % of standard length	body maximum depth	14.7—16.8	14.4	18.9—19.0	15.1—15.5	15.4	14.7—16.4	13.6—14.4	15.4
	least depth	11.6—13.2	11.4	11.9—13.1	12.2—13.9	10.9	11.7—13.6	12.6—14.4	12.1
	caudal peduncle	14.3—14.7	14.9	14.7—15.5	13.8—14.5	16.4	16.2—16.7	15.0—16.4	12.3
	praedorsal distance	50.5—53.0	53.0	50.5	51.0—52.5	52.5	48.0—52.0	51.5—52.5	50.5
	praeventral distance	55.0—57.0	53.0	53.6—54.0	54.6—56.5	50.3	54.0—56.0	51.5—53.5	53.5
	P—V distance	32.8—34.6	29.6	32.8—33.2	29.4—33.8	27.2	31.0—35.0	26.8—30.2	30.8
	V—A distance	20.4—22.1	25.4	20.6—21.6	22.1—22.4	20.0	19.8—23.6	22.8—23.2	24.0
	head length	23.4—23.8	23.2	23.8—25.2	25.6	24.6	23.1—25.2	22.5—22.8	22.7
	snout length	9.5—10.5	8.5	8.9—9.4	11.2	9.7	9.4—10.6	8.0—10.3	8.4
	eye diameter	3.3—3.4	3.7	4.3—4.6	3.3—3.6	4.0	3.5—4.2	3.2—4.2	2.7
in % of head	snout leng.	40.5—44.5	36.9	37.6	43.0—44.0	40.5	39.5—43.9	34.7—44.5	37.2
	eye diameter	13.9—14.2	15.9	18.1	13.7—13.9	16.4	14.4—16.9	14.6—18.4	11.7
eye diameter in % of interorbital width		71.0—75.5	56.0	64.0—69.0	51.5—56.5	64.5	48.0—67.0	51.5—63.0	39.0

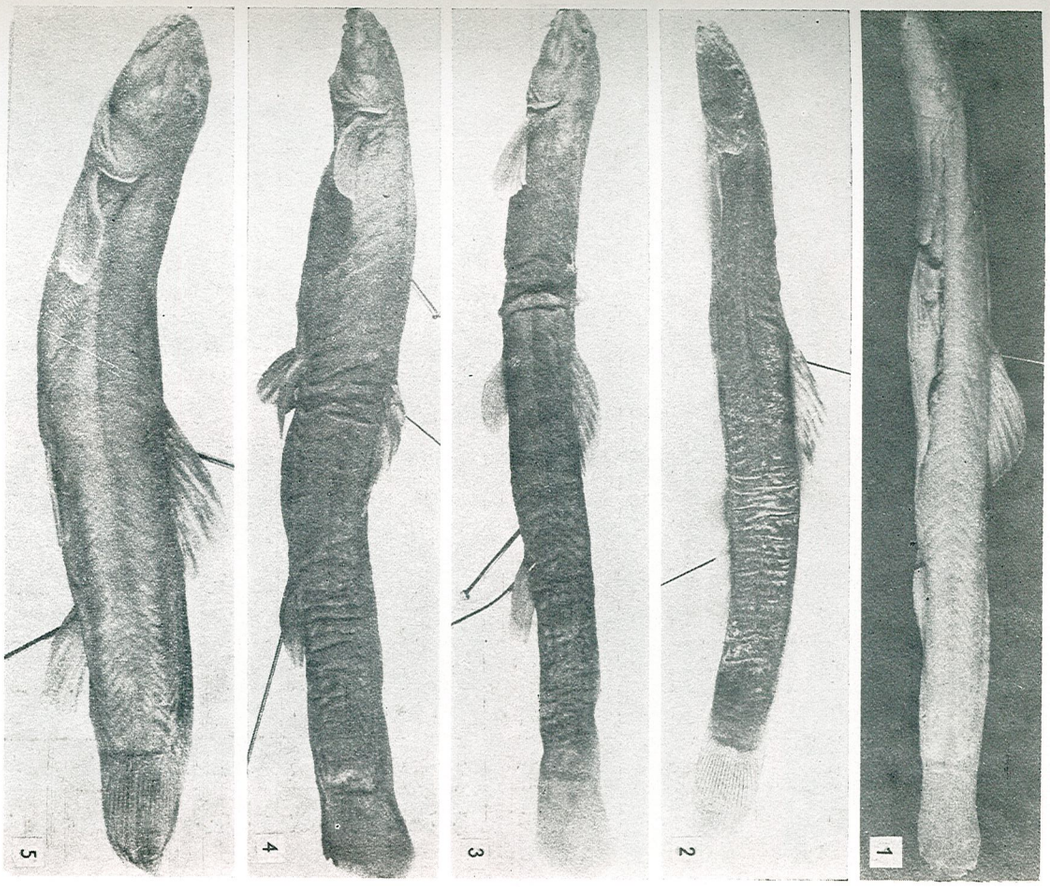
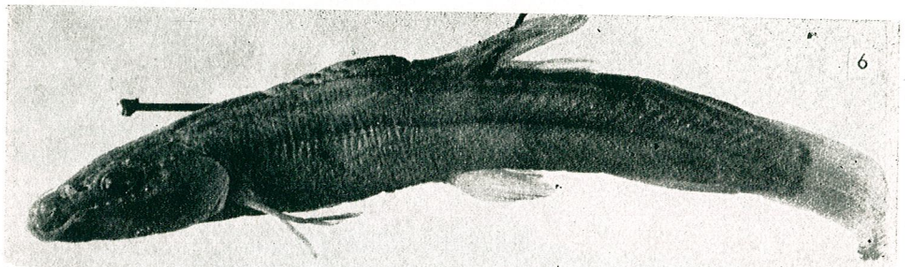
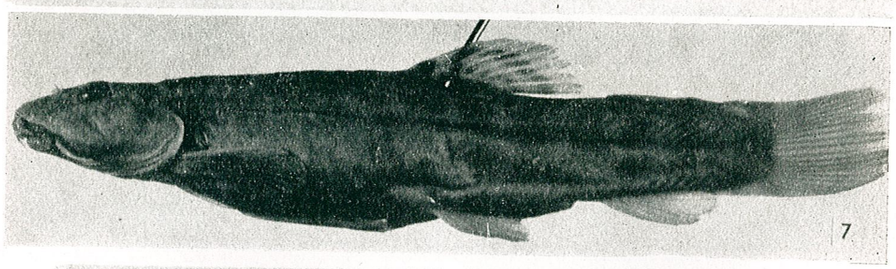


PLATE I. — Fig. 1. — *Schistura variegata* : paralectotype of *Nemachilus variegatus*, M.N.H.N. Fig. 2. — *Schistura variegata* : paralectotype of *Nemachilus oxygnathus* B.M.N.H. 1908. 2.27 : 24. Fig. 3. — *Schistura variegata*, Tsao-Heo, Szechwan, U.S.N.M. 89275. Fig. 4. — *Schistura variegata*, deep bodied form, Szechwan, U.S.N.M. 91618. Fig. 5. — *Schistura potanini*, Suifu, Szechwan, U.S.N.M. 87128.





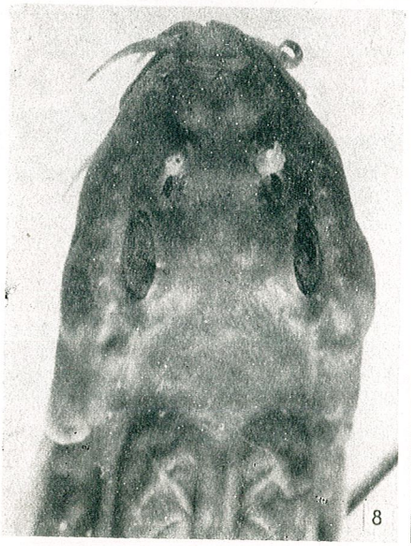


PLATE II. — Fig. 6. — *Schistura potanini*, Kuan-shien near Tibet, U.S.N.M. 130126. Fig. 7. — *Schistura potanini*, deep bodied form, Szechwan, U.S.N.M. 87610. Fig. 8. — *Schistura potanini*, dorsal view of head, U.S.N.M. 87610. Fig. 9. — *Schistura potanini*, dorsal view of head, U.S.N.M. 130126.

be due to allometry. More significant are the differences in caudal peduncle length (highest values in Yunnan specimens) and especially in body depth (by far the highest values occur in USNM 87610 specimens, figure 8). These differences may allow in the future the recognision of several subspecies.

★

The following six nominal species were described from southern East Asia: *Homaloptera fasciolata* Nichols & Pope, 1927 from Hainan Isl., *H. hingi* Here, 1934, "*Barbatula*" *incerta* Nichols. 1931, *Nem. humilis* Lin 1932 from Hsikiang drainage (the first two from Kwantung, the last named from Kweichow), *N. pellegrini* Rendahl, 1944 and *N. chapaensis* Rendahl, 1934 from North Vietnam. These six "species" are very close to each other, the only morphological differences between them which may actually have taxonomical significance are the shape and size of scales. These forms probably are conspecific or represent a few (less than six !) sibling species.

Schistura is a southern genus in the fishfauna of the upper Yangtze. It is one of the rather many tropical fish genera occurring in the upper Yangtze, as well as in the Hsikiang drainage and/or North Vietnam and Hainan island which are absent from the lower Yangtze and the Hsikiang drainage system.

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Received January 19, 1974

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THE REACTION TO INSECTICIDES OF THE PERIGANGLION SHEATH IN *BOTHYNODERES PUNCTIVENTRIS* GERM.

BY

MARIA TEODORESCU, VIORICA TRANDABURU and ADRIANA VACARU

The perineurium of *Bothynoderes punctiventris* was studied at the end of the diapause (period I) and during the egg-laying period (period II). The changes occurring in the perineurium after testing with Heptachlor and Fosfotox in sublethal and lethal doses were investigated. While in period I the perineurial epithelium shows a tendency to thickening in the male control, in period II its stratification appears in both sexes; the latter modification was, howerer, more accentuated in the male. During the first period, the treatment with sublethal Heptachlor doses induce the perineurium thickening in both sexes; the lethal dose produces the flattening and degeneration of the epithelium especially in the male. During period II, in both sexes, flattening and degeneration of the epithelium are more accentuated after a lethal Heptachlor dose. In both periods the perineurium displays a single layer under the influence of a sublethal Fosfotox dose; after a lethal dose the flattening and degeneration of the cells were recorded.

Earlier investigations showed that, among the various types of neuroglia, the perineurial epithelium is specialized in the selective control of exchanges between the neurons and haemolymph [2], [3], [6], [9], [12] [15]. [16]. Cytochemical data revealed the wealth of enzymes and —SH groups in the perineurial cells [14] and even the changes of the concentration of some enzymes (cytocromoxydase) during certain period of the year [14].

The dynamics of functional processes which proceed in the perineurium was revealed by the morphologic alteration of the glial sheath during the stages of insect biological cycle.

Based on the finding that the perineurium reacts spontaneously and demonstratively to the slightest variations of the haemolymph, we chose it as an adequate morphologic index for assessing the degree of toxicity of insecticides applied in the control of *Bothynoderes punctiventris*. The sensitivity of this weevil to certain insecticides has already been tested [4], establishing the lethal and sublethal doses of the 98% HCH gamma izomer and of DDT and HCH mixture (5+3).

MATERIAL AND TECHNIQUE

The investigations were carried out on the brain of adult *Bothynoderes* males and females, by applying the conventional histologic techniques. The perineurium was studied in normal insectes and in those exposed to organophosphoric (Fosfotox) and to organochloride

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(Heptachlor) insecticides in two stages of the insects biologic cycle: the end of the diapau (period I) and the egg-laying period (period II). The sublethal dose was of 0.025 μg f both insecticides and the lethal dose of 8 μg Fosfotox and 1.6 μg Heptachlor. The insec cides in the form of active substance dissolved in acetone were applied on the prothoraci mesothoracic articulation 2 μg amount by the topical method, using Agla microsyring

RESULTS

Clinical aspects

Insecticides alter the motility of insects; 6—12 hours after the application the insects gait is uncertain, the movement of their appendice incoordinate, they tremble and tend to lie down on one side or on thei back (clonic phase or Knock down phase). Their effort to return to a norma position fails and progressive paralysis (pseric phase) followed by deat sets in. The material was fixed in the final stage of paralysis.

Morphologic aspects

The reaction of the perineurium was investigated at the level o junction between the cerebroid ganglia and the optic lobes, at th and of the diapause during the egg-laying period. Control insects wer compared with those exposed to sublethal and lethal doses of Fosfoto and Heptachlor. The following morphologic parameters were studied stratifications of the epithelium, size of the cells, degenerative processes vacuolisation, pycnosis, etc. The perineurium changes, in both control and treated insects, were clearly revealed by comparison with the histo logic images.

1. *Control insects at the end of diapause.* The only perceptible diffe rence consisted in thickening of the perineurium in the control male (Pl. I, figs 1 and 2).

2. *Control insects during the egg-laying period.* The perineurium o male became hypertrophic and stratified (Pl. I, fig. 3). In females, th perineurium exhibited rarer cells of smaller sizes; stratifications wa less conspicuous (Pl. I, fig. 4).

3. *Treated insects at the end of diapause*

A. Sublethal doses of Heptachlor stimulated the growth in height of the perineurium, especially in the male, in which hypertrophy of th nuclei also appeared (Pl. I, figs 5/1, 6/2, 5/6). The lethal Heptachlo dose caused the flattening of the cells in both sexes (Pl. I, figs 7/5) In the females the cells were rarer, of smaller dimensions, with reduce nuclei volum (Pl. I, figs 6/8). The degenerative pycnosis phenomen were more evident in the males (Pl. I, figs 7/8). The alterations becam more evident when the effects of the lethal Heptachlor dose were com pared not only to those of the sublethal dose but also to the control insect of both sexes (Pl. I, figs 7/1, 8/2).

B. Fosfotox in sublethal doses reduced the height of the cells stratification disappeared, the cells became rarer and the nuclei smalle

(Pl. I, figs 10/2, 9/1). The lethal Fosfotox dose had a stronger effect: the flattening of perineurial epithelium, spacing of the cells, shrinking of the nuclei and the degenerative phenomena were frequently and more accentuated (Pl. I, figs 1/9/11,2/10/12).

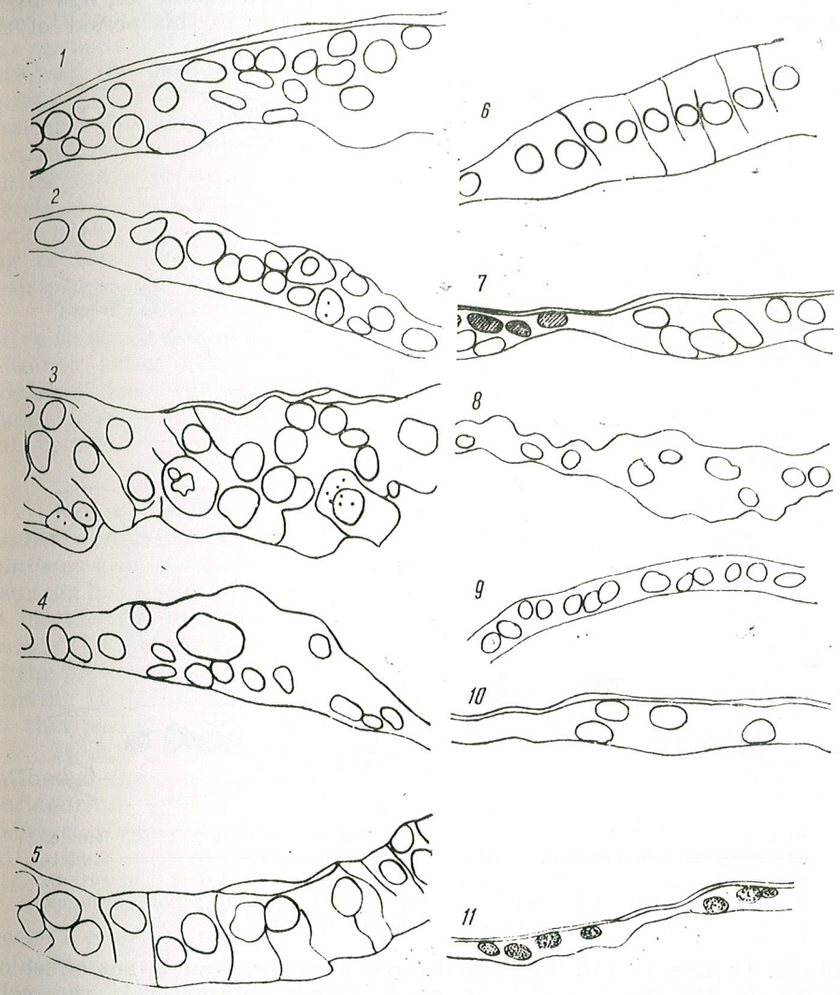

PLATE I. — Perineurial epithelium. End of the diapause (1st period): *1*, male control; *2*, female control. Egg-laying period (2nd period): *3*, male control; *4*, female control. Sublethal dose (1st period). Heptachlor treatment: *5*, male; 6, female. Lethal dose (1st period). Heptachlor treatment: *7*, male; *8*, female. Sublethal dose (1st period). Fosfotox treatment: *9*, male; *10*, female. Lethal dose (1st period). Fosfotox treatment: *11*, male.

4. Treated insects in the egg-laying period

A. In the male the sublethal Heptachlor dose brought about a decrease in the height of the cells, reduced the number of cells, with

shrinking of the nuclei and pycnosis. The lethal dose flattened the e
thelium still further (Pl. II, figs 16/13); the phenomenon was very cons
cuous when the effect of the lethal dose was compared to the contr
(Pl. I and II, figs 16/3). In the female the sublethal Heptachlor d
did not modify the height of the epithelial cells (Pl. I and II, figs 14/
the sublethal dose prevalently affecting the males in this period of t

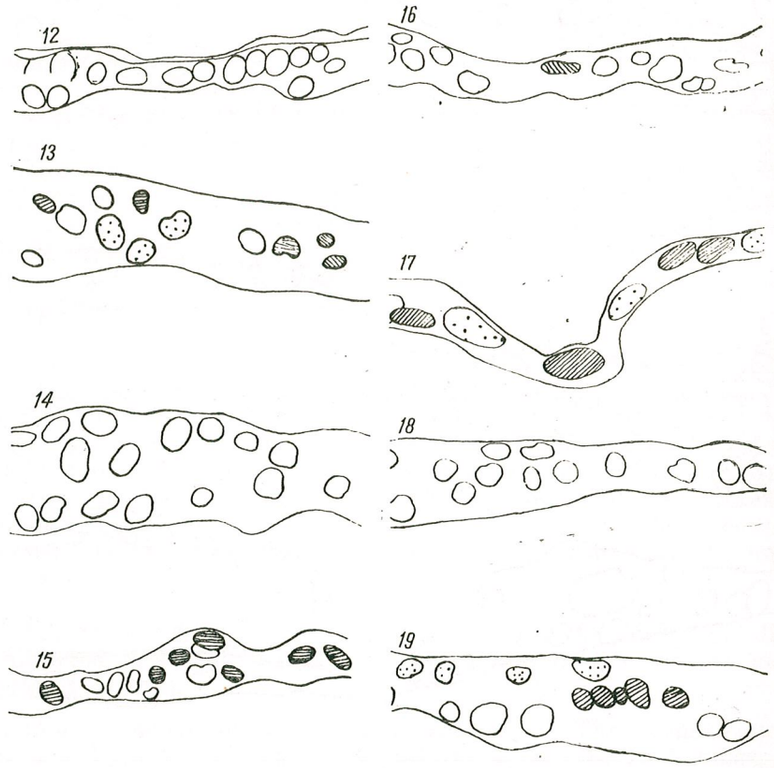

PLATE II. — Lethal dose (1st period). Fosfotox treatment: *12*, female. Sublethal dose (2nd period). Heptachlor treatment: *13*, male; *14*, female. Sublethal dose (2nd period). Fosfotox treatment: *15*, female. Lethal dose (2nd period). Heptachlor treatment: *16*, male; *17*, female. Lethal dose (2nd period) Fosfotox treatment: *18*, male; *19*, female.

year (Pl. II, figs 14/13). The lethal dose, however, had a net effect o
the female (Pl. II, figs 17/14): stratification disappeared, the cell
became endothelial and degenerations developed. These effects wer
more evident when compared to the control female (Pl. II, figs 17/4)

B. The sublethal Fosfotox dose reduced the epithelium to a singl
layer of cells, many of which were in course of degeneration. The letha
Fosfotox doses caused flattening of the perineurium in the males. Sub
lethal Fosfotox doses produced degeneration of the cells, deformit
and pycnosis of the nuclei in both sexes (Pl. I and II, figs 15/4, 18/3
19/4, 15/19).

General comments on the effect of insecticides

At the end of the diapause. Under the influence of sublethal concen
rations of Heptachlor, the perineurium in the males undergoes a rela
ive stimulation of short duration. The lethal dose no longer produces
he phenomenon; flattening and degeneration of the cells appears. Stimu
ation of the perineurium is likewise induced in the female by sublethal
loses; the lethal dose brings about spacing of the cells. Fosfotox in
ublethal and lethal doses strongly alters the perineurium in the male;
tratifications disappears, the cells are flattened and more rare, the nuclei
hrink and pycnosis sets in. In the female, neither of the Fosfotox doses
onclusively influence the perineurium. There is only a relative enlarge
ment of the cells following upon the subtlethal dose and the certain shrink
ing of the nuclei. Hence, it may be asserted that the females are more
resistant in this period of the year.

The egg-laying period. Both male and female insects proved sen
sitive to the action of sublethal and lethal doses of Heptachlor; the males,
however, being affected to the greater extent. Although after the lethal
Fosfotox dose, the perineurium of the female is not flattened, the fre
quent degradation aspects of the nuclei show that alterations of the cells
has begun.

DISCUSSION

Our morphologic analysis of the perineurium in *Bothynoderes puncti
ventris* under normal conditions and under the influence of insecticides
confirms and completes a series of earlier results, showing that the peri
neurium has a selective role in the transit of certain substances [1] [3] [8].
It has likewise been shown that the perineurium undergoes certain changes
during the metamorphosis of the insect [11]. We also observed thickening
of the perineurium in the controls, especially during the egg-laying period,
proving that the haemolymph-nervous tissue exchanges are more active
in this period.

It has been histochemically demonstrated that the factors which
influence the nervous tissue, by means of haemolymph, alter first the
perineurium [5] [12] [14]. Sharma [10] mentions degeneration of the
nervous tissue in *Poecilocerus pictus* as effect of certain insecticides.
Alterations produced by insecticides have also been described at the
ultrastructural level [7].

In addition to the above data mention should be made in *Bothy
noderes punctiventris* that the perineurium may be used as a sensitive
morphologic element to the action of insecticides applied by us. The
reaction of the perineurium is specific function of sex and dose. Increase
in the size of the cells under the influence of sublethal Heptachlor doses,
at the end of the diapause may be interpreted as a compensatory reaction;
the hypertrophy of nuclei as a criterion of an increase activity of the
perineurial cells, which are the first affected by the toxic factor from
the haemolymph. When the Heptachlor dose is increased the perineurial
cells no longer react and degenerative phenomena are recorded. During
the egg-laying period, the loss of stratification of the perineurium under
the influence of both Heptachlor doses, probably appears as a necessity
dictated by the effort of the nervous system to survive under conditions

of intoxication of the haemolymph with insecticides. The compensato tissular reaction observed after the sublethal Heptachlor dose does n appear at low Fosfotox concentrations. The males are more sensitive Fosfotox than the females.

From the above findings it results that the perineurium forms barrier in the transit of substances from the haemolymph and retai the toxins, thus shielding the nervous tissue from intoxication from so time. This proves both selective and defense role of the perineurium, the limit between the nervous tissue and the haemolymph. The perine rium may be considered as a very sensitive morphologic indication assessing the reaction of the nervous and glial tissue to the influence various normal and experimental factors.

CONCLUSIONS

1. Relative thickening of the perineurium in the control male the first period was noticed.

2. Marked stratification of the perineurial epithelium in the contro of both sexes in the second period was described.

3. In period I, the sublethal Heptachlor dose thickens the per neurium in both males and females.

4. In period I, the lethal Heptachlor dose reduces the thickne of the perineurium in both sexes, but especially in the males.

5. In period II, the cells are flattened and altered in both mal and females, especially after a lethal Heptachlor dose.

6. In periods I and II the perineurium is reduced to a single laye after using a sublethal Fosfotox dose; using a lethal dose it becam flattened and degenerated.

7. In both periods the males are preponderantly affected.

8. In both periods the insecticides also produce clinical manife tations : incoordinate movements, trembling, loss of balance and progre sive paralysis.

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Received December 22, 1973

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CAROTENOIDS IN LARVAE OF *LEPTINOTARSA DECEMLINEATA* SAY DURING PUPATION

BY

MATILDA JITARIU and IONEL PETCU

The quantitative and qualitative evolution of carotenoid pigments in *Leptinotarsa decemlineata* Say during pupation was followed.
The largest form variety and biggest quantity of certain carotenoid pigments were found three days after pupation.
After five days of pupation, the total amount of carotenoids was smaller, showing their intense participation in the formation of the adult.
The higher increase of prevailing pigment — that is of the mono-hydroxy-carotene three days after pupation is being discussed.

A previous paper [5] was dealing with the quantitative and qualitative analysis of carotenoid pigments in larvae of Leptinotarsa decemlineata Say, performed at the moment of pupation and three days thereafter.

This paper sets out to complete the previous one by giving the results of the analysis carried out in five-day-old pupae of the same insect.

MATERIAL AND METHOD

Out of a large group of pupating larvae a part was selected and kept in absolute ethanol five days after pupation. The result of the analysis made on this material had been checked the following summer on fresh material. The values of each pigment as well as the values of the entire quantity of carotenoids were found slightly higher in the fresh than in the preserved material, yet maintaining the relation and metabolic variations of fresh as well as of the preserved material.

The methods applied for extracting and processing the pigments were the same as those used in the previous work [5]. The isolation and characterization of pigments were effected both with saponified extracts separated in epi- and hypophase and with raw nonsaponified extracts. After chromatographing the latter on column of activated aluminium, the isolated pigments were saponified by methanol KOH 15% for 24—48 hours at room temperature, in the dark, and in N atmosphere. At the same time, certain pigments in the early extract, still preserving their ester shape after the first saponification, were again saponified by methanol KOH 15% for 24—48 hours, at room temperature, in the dark and in N atmosphere.

The concentration of each pigment was calculated according to Bonaly's method [1], the value of $E\,^{1}/_{00}$ cm being found equal to 2,500.

The statistical calculation was made according to Student.

RESULTS

We should point out, first of all, that the stage V larvae, appart the main pigments in regard to their amount or chemical nature, there exists a trace of α-carotene, whose amount is always very small.

Among the quantitatively prevailing pigments in five-day-ol pupae, we come across the two pigments were found in larvae on th point to become pupae as well as in three-day-old pupae. We calle these pigments, Ela1 and Ela2.

I.R. spectra recording showed that after a 12-hour saponificatio by aqueous KOH 60 %, the pigment still preserved its ester shape. A fres 40-hour saponification at the room temperature, in dark, and in N atm sphere by methanol KOH 15 % of the ether-ethilic solution of the pigmen the substances being on a 1 : 2 relation, led to the release of two pi ments for Ela2 and of a pigment for Ela1. The first liberated pigmen *Ela2b*, in light-petroleum solution presented a yellow-greenish colo with an absorption maximum in visible spectrum of 475—446—425 m and a partition coefficient of 88 : 12. It was therefore a monohydroxy or β-carotene [12]. Thus, what we assumed in our previous paper [5 about the chemical nature of the pigment was firmly attested.

The second pigment, Ela 2c, presented a maximum absorption i the range of 478—449(50)—425 mμ and a partition coefficient of 1. Takin the same Rf into account as an authentic test of β-carotene, it wa to be β-carotene.

The pigment Ela1 was a monohydroxy-carotene, the more so a after a 40 hour hydrolysis by methanol KOH 15%, it released a singl pigment with a maximum absorption of 476—446—427 mμ in ligh petroleum solution. It was referred to as an isomer of the Ela 2b pigment

Since further analysis have not made so far on these pigment we would not tell with certainty which one of them is a α-cryptoxanthi and which one is an isocryptoxanthin. But we may fairly say that despit their spectral resemblance and their chromatographic behaviour toward β-carotene, the pigments Ela1 and Ela2 are not purely β-carotene, bu a mixture of the latter with monohydroxylated forms of α- or β-carotene

Apart from these two pigments which prevail quantitatively during the whole pupa stage, we have met, five days after pupation, a serie of forms which have already been present three days after pupation that is : echinenone, canthaxanthin, lutein, astaxanthin, as well as th pigment referred to as monadoxanthin, is presenting resemblances rathe with this one than with isolutein, as we thought earlier.

Relevant for the presence of monadoxanthin is the negative reac tion to epoxide of the respective pigment, in which the maximum absorp tion — almost as high as that of lutein in varying solvents (Table 1 and the partition coefficient equal to 21.5/78.5 — is nearer to tha of monadoxanthine, (27/73) than to that of lutein (39/61).

Table 1

The absorption) maximum in visible light of Lutein and our pigment (Monadoxanthin) in different solvents

Light Petroleum	Lutein	474—445 ~ 425 mμ
	Monadoxanthin	473—443 ~ 423 mμ
Benzene	Lutein	489—459 ~ 435 mμ
	Monadoxanthin	488—457 ~ 437 mμ
Ethanol	Lutein	476—447 ~ 429 mμ
	Monadoxanthin	477—447 ~ 426 mμ

In contrast with the three-day-old pupae, in which a lot of epoxides — though in very small amounts — may be found, the five-day-old pupae do not contain so many epoxide forms. In return, apart from keto-carotenoids with a maximum absorption of 455 mμ and the other of 453 mμ they have a monoketo-carotenoid with a maximum absorption of 450 mμ. But because of the small amount, work was hardly possible.

DISCUSSIONS

The first thing to be pointed out is the presence in the larva body prior to pupation, of oxidated forms in quite noticeable amounts compared to hydrocarbons that are like trace (α- and β-carotene).

On pursuing the evolution of monohydroxylated pigments (Ela1 and Ela2) of echinenone, canthaxanthin, lutein and monadoxanthin along the period of pupation, we obtained results showing an intense metabolism of all these forms — in other words, we perceived their effective participation in the process of adult formation.

The large amount of one of the two monohydroxy-carotene forms (Ela2b) — likely to be an izocryptoxanthin — already present in the larva on the point of pupation, and its quantitative predominance during the time of pupation, should be regarded as a reserve out of which further forms more oxidated may arise.

Izocryptoxanthin has already been revealed in certain Crustacea as a natural pigment [7—9] [13] and a lot of researchers [2] [4] [7] [13—14] take it as an intermediate form, very quickly metabolized in the way that β-carotene turns into astaxanthin.

As we have already mentioned in respect to the eggs of these insect during embryogenesis [6], it is again this pigment that dominates quantitatively, even if the whole of carotenoids drops sharply towards the end of embryogenesis.

During those still 84 hours of pupation (3—5 days) when strong anabolic processes in the wake of high tissular hydrolysis take place, this pigment is fully used, since it is markedly slighter 5 days than 3 days after pupation (Fig. 1).

It is worth noticing that for 3—6 days an intense mitosis occurs in the pupa, especially in "corpora allata and corpora cardiaca" followed by an abundant secretion [11]. In this time, the abdominal muscles — particularly those of the genitals — fully recover due to the formation of new myofibres of a morphological and partly biochemical structure quite different from that of the larval myofibre.

One clearly perceives that just in this period, the more oxygenated pigments are more solicited. Thus, if cathaxanthin (astaxanthin has not been worked on) stays relatively constant three days after pupation, in the period of tissue recovery, typical for adults (3—5 days) being in return hugely consumed (Fig. 2*a*). From 37.10 μg/g at three days of nymphosis, echinenone drops to 17.71 μg/g (Fig. 2*b*), while hydroxyechinenone practically does no longer exist at five days pupation. Lutein and monadoxanthin change all along pupation, reaching at the stage of imaginary moulting (7—8 days after pupation — 5 days in our case) the point of close quantitative equality (Fig. 3).

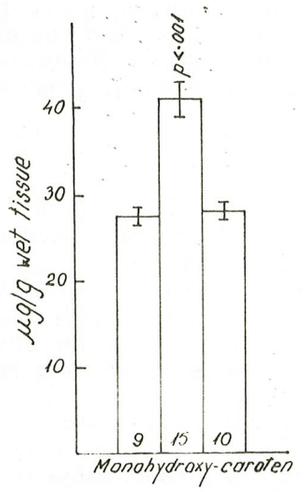

Fig. 1. — Metabolic variations of the monohydroxylated pigment Ela2, during nymphosis in *Leptino ar.a decemlineata* Say.

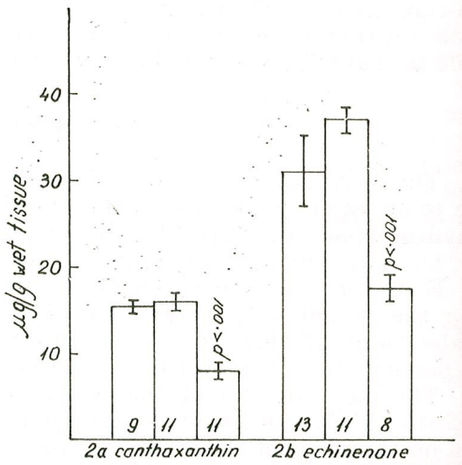

Fig. 2. — Metabolic variations of canthaxanthin (2*a*) and echinenone (2*b*) during nymphosis in *Leptinotarsa decemlineata* Say.

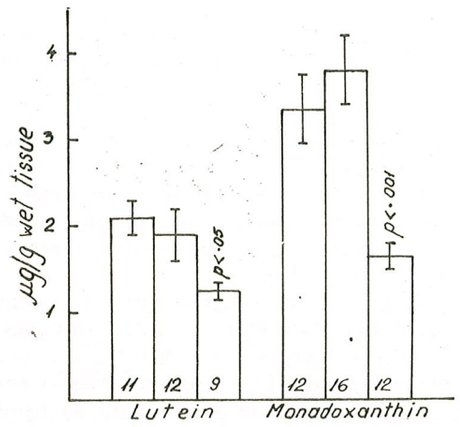

Fig. 3. — Metabolic variations of lutein and monadoxanthin during nymphosis in *Leptinotarsa decemlineata* Say.

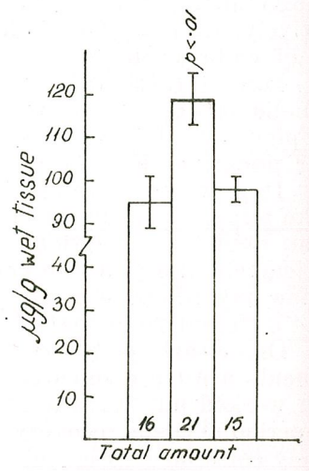

Fig. 4. — Variations of the total amount of carotenoids during nymphosis in *Leptinotarsa decemlineata* Say.

Yet, if three days after pupation the more oxygenated forms are found to be more solicited, the monohydroxy-carotene form (likely to be izocryptoxanthin) grows quantitatively at the same time (Fig. 1).

Our data concerning this fact are in agreement with those recorded by Campbell [3] who found in *Mytillus edulis*, kept for 4 months under starvation, a quantitative increase of alloxanthin, the prevailing pigment in this animal. The authoress ascribes this increase either to the formation of this pigment from others, or to the incapacity of the animal to excreting it.

In our previous paper [5] we assumed the same fact as Campbell that the addition of pigment Ela1 and Ela2 might have been provided by another pigment in an opposite way as the formation of a more oxygenated pigment from less oxygenated ones [2] [4] [7] [13]; in other words, the rise of Ela2 was due to the hydroxyechinenone, which three days after population presented an amount half as big as at the moment of pupation.

This time, by calculating the entire amount of carotenoid, we found a significant increase in the total extracts (Fig. 4). This increase was due to a larger amount of monohydroxylated pigment (Fig. 1).

Consequently, we are not in the presence of an accumulation of certain forms provided by other forms or in the presence of the incapacity of the organism to excrete the former. Quite likely, we are in the presence of a synthesis process of the latter. Since the insects have "farnezyl" the precursor of certain hormons — we should set the hypothesis of this molecule being at the same time the precursor of this carotenoid form.

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Received January 21, 1974

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RELATIONSHIPS BETWEEN GLUCOSE AND FATTY ACIDS AS ENERGY SOURCES IN THE ISOLATED AND PERFUSED FROG HEART. BIOCHEMICAL MODIFICATIONS IN THE CARDIAC MUSCLE

BY

C. DOBRESCU, I. V. DEACIUC and EUGEN A. PORA

The reciprocal effect of oleate and glucose on their consumption by the perfused heart of the spring frog (*Rana temporaria*) was investigated, in correlation with the modification of some intracellular metabolites. When added together to the perfusion fluid, the consumption of both glucose and oleate decreases to 25.7% and 53.9% respectively, as compared to the values found when the concentration of one substrate was administered alone. The modifications in intracellular metabolites allow the conclusion that glycolysis is inhibited at the level of NAD^+-depending enzymes.

In previous experiments [3] we observed that the isolated and perfused heart of the frog *Rana temporaria* differs in its behaviour concerning the utilization of carbohydrate and lipid substrates, from the rat heart [24]. In summer frog heart 0.5—1.0 mM oleic acid in the perfusion fluid that contains glucose (5.0 mM) and insulin enhances the glucose consumption, meanwhile in the mammalian heart the fatty acids inhibit glucose utilization [6] [20] [22] [24]. This fact may be put in relation with more general modifications of the energetic summer metabolism as stated by Freeman et al. [5], who showed that the animals are preparing themselves for the winter starvation by deposition of large amounts of fat [15] from free fatty acids and from glycolytically produced glycerol.

In this paper, modifications of some intracellular compounds were followed, as depending on the substrate used in the perfusion of the heart.

MATERIAL AND METHODS

Chemicals. Glucosoxidase was kindly supplied to one of us by Prof. M. F. Gulyi from the Institute of Biochemistry of Kiev, USSR. Peroxidase (type I from horse radish) was purchased from Sigma Chemical Co., St. Louis, USA. NAD*, NADP, G6P-dehydrogenase, PEP, LDH and PK, from Boehringer GmbH, Mannheim, Federal Republic of Germany. Hexokinase, from Man Research Laboratories Inc., New York. Bovine albumine (BSA) (Cohn, fraction V) and NADH, from Serva, Heilderberg, Federal Republic of Germany. All other chemicals were of the highest purity commercially available.

Animals. All the experiments were made on *Rana temporaria*, males and females, having 80—140 g body weight, during April-May. Animals were caught in a pond during April and kept in an open tank with running water; they were brought in the laboratory (18—20°C) 12—14 hours before the experiment.

Heart perfusion. Animals were immobilized by destroying the spinal cord; after opening the body, the heart was cannulated and perfused. One cannula was introduced into the venous sinus, the other in the left aorta; the right aorta was ligated. After 15 minutes of preliminary perfusion with the saline of Schüller [19] buffered with 5.2 m M Na phosphate, pH 7.4, without energetic substrate, the hearts were put into the perfusion device. Perfused

was performed during two hours, with the same saline containing energetic substrates. Oth technical details were described previously [2].

When glucose was added to the perfusion fluid, insulin was also added (10 mU per ml When oleic acid was added, this was previously complexed with BSA according to va Harken et al. [7] to give an 25 mM oleic acid solution, pH 7.4, and a molar oleate p BSA ratio of 17.4. Appropriate quantitative from this (freshly prepared) solution were add to the perfusion fluid, to obtain final concentrations of 0.5—1.0 mM, and a final volume 10 ml. Simultaneously, BSA was added to the medium, to a final concentration of 1 % the same was done in the experiments with glucose only (without oleate). Room temperatu was maintained about 26°C. In order to avoid bacterial degradation of substrates, penicill and streptomycin were added to the perfusion medium, in concentrations of 5 and 10 m per 100 ml, respectively.

Assay of metabolism. Glucose and oleate were determined from appropriate aliquo of the perfusion fluid, before perfusion and after this. For glucose determination, protei were precipitated with perchloric acid, in a final concentration of 3 %.

Glucose was determined following the procedure of Krebs et al. [11], with the exce tion that after incubating the samples with the glucoseoxidase reagent an equal volume 50 % (v/v) H_2SO_4 was added, under refrigeration [21]. Oleate was determined following t method of Novák [16].

In view of determining the intracellular compounds of the cardiac muscle, the hea was rapidly blotted on filter paper and immersed in liquid nitrogen, in porcellan morta and grinded. The powder obtained was extracted with 6 % $HClO_4$, 3 ml per each heart; t extract was brought to pH 6 with 5 M K_2CO_3, and centrifuged. The following metabolites we determined from the supernatant fluid: G6P and ATP, according to Lampreht and Trausch [12]; ADP, by the method of Hohorst et al. [8]; CP, following the procedure of Fiske an Subbarow [4]; Pi, according to Lowry et al. [14]. Proteins, precipitated with $HClO_4$ and sed mented by centrifugation, were dissolved in 1 ml NaOH 1 N, at 100°C appropriately dilute and assayed according to Lowry et al. (14).

Optical measurements were made with a VSU-1 Zeiss spectrophotometer for G6P, ATP CP, and Pi; with a FEK-N photocolorimeter for glucose, oleic acid and proteins, using a gre filter for glucose and oleate, and a red one for proteins.

Statistical processing of results was done according to Student's "t" test.

RESULTS

Reciprocal effect of glucose and oleate upon their consumption. In th presence of 0.5 mM oleate, glucose was consumed at a lower rate durin the two hours of perfusion, than when oleate was absent. 5.0 mM glucos exerted an inhibitory effect on oleate consumption, when added together Thus, we can tell that in the cardiac muscle of spring frogs, glucose an oleate compete for the cellular energetics.

Modifications of intracellular compounds, depending on the energeti substrate used in perfusion (Table 1). G6P concentration after two hour of perfusion was not significantly different depending on whether oleat was added to the glucose perfusion or not. It results that, unlike in th mammalian cardiac muscle [1] [20], in the frog one glycolysis is no inhibited by oleate at the PFK level. When oleate was used as sing substrate, cardiac G6P was significantly increased as compared to th other two perfusion variants. This shows that oleate spares the glycoge reserves, by inhibiting in these conditions glycolysis at the PFK level ATP concentration was maximal in the glucose perfusion, and minima in the oleate one. Pi concentration did not exhibit large differences That of PC was decreased in perfusions with oleate only, against th other variants.

Table 1

Glucose and oleate consumption and intracellular metabolite concentrations in isolated and perfused frog heart

Perfusion with	Metabolites (μmoles per 100 mg proteins)								G6P nmoles
	Glucose	Ol. Ac.	ATP		ADP		Pa	CP	
GLUCOSE	35±13 (9) *	—	12.95±2.05 (15) **		1.42±0.08 (15) **		12.37±0.41 (15) *	1.93±0.16 (15) **	187.5±17.9 (10) **
GLUC.+OLEATE	9.3±1.09 *	6.21±0.82 (16) *	3.41±0.23 (14) **		1.97±0.18 (13) *		11.4±0.16 (14) *	1.90±0.4 (9) *	154±12.4 (9) *
OLEATE	—	11.5±1.2 (9) *	2.21±0.46 (8) **		2.18±0.18 (10) **		11.7±0.27 (10)	0.28±0.1 (10) **	337±56 (10) **

Values are means ± standard errors; in parantheses, number of animals. Values in the somme column labeled with (*) or (**) are significantly different between them ($P < 0.01$).

DISCUSSIONS

Firstly, it is to observe that in our spring frogs, which have had no possibilities of nutrition up to the date of experimentation, the oleate did not stimulate the utilization of glucose, as it was previously found in perfused hearts of summer frogs [3]. In our present experiments, oleate inhibited glucose utilisation, a phenomenon which is normal in mammalian hearts too [20] [23] [24]. Reciprocally, significant inhibition of oleate consumption by the glucose added, also occurred. This last phenomenon was more pronounced in summer frogs [3], but never occurs in mammalian cardiac muscle [24].

The inhibition effect of the oleate on the glucose consumption is not exerted at the PFK level, as it is in mammalian heart [1] [20]. The drastical decrease of ATP concentration in glucose + oleate perfusion as compared to that with glucose only, is to be explained as a consequence of its utilization in the activitation of oleate. This decrease may be a stimulating factor of the glycolysis by liberating the PFK from the ATP inhibition, as it is shown by the diminished concentration of G6P [13] [17]. In the same time, the activation of oleate and its entrance into β oxidation must shift the NAD*-NADH system toward a more reduced state; this leads to an inhibition of the glycolysis at the level of NAD*-dependent enzymes.

The mechanism of the nearly 50% reduction of oleate utilization by the presence of glucose is not obvious. A possibility would be that oleate and glycolytical pyruvate would compete with equal chances for the CoA.

A transfer of high energy phosphate from CP to ADP is to be supposed; this would supply the ATP used in oleate activation. The great decrease of CP in oleate perfusion as compared to the other variants would be explained by this way.

In the hearts perfuse with glucose + oleate, ATP concentration is lowered as compared to the "glucose only" variant, meanwhile CP concentration is not. As the G6P concentration is also lowered, we can suppose that glycolysis is the furnisher of the ATP necessary for oleate activation, at least during the two hours of perfusion.

Finally, the results obtained in these experiments as well as those previously published [3], show that, concerning the relation of glucose and fatty acid metabolism, the isolated perfused frog heart differs from that of mammals; seasonal differences are also manifested.

The stimulation of the glucose utilization by the oleate in the isolated and perfused heart of summer frog [3] [11] [12] [25], as well as of the glycogen utilization in the ventricle tissue incubated with octanoat [5], are due to triglyceride synthesis from free fatty acids taken up but not used. This process needs the glycerol formed during the glycolysis. The excess of fatty acids would be a signal for glyceride synthesis and implicitly for the stimulation of glycolysis. This fact has a functional significance for the whole organism, since during the summer, toads [5] and frogs [3] are engaged in depositing fat as a reserve for winter starvation. Thus, we can understand the differences against the results obtained on rat heart.

Our results on summer frogs [3] are in agreement with those of Freeman et al. [5], who found that, after incubation of cardiac tissue with glucose-U-^{14}C, a great deal of ^{14}C is present in triglycerides and phospholipids.

In the cardiac muscle of spring frogs a difference is observed as compared to that of summer frogs: fatty acids do not stimulate more glucose utilization. An explanation might be that during the winter the frogs living buried in the ooze (anoxic environment), they consume only stored glycogen. Towards the spring, they lift themselves in the well oxygenated water, probably due to the rise of blood acidosis. This leads to a shift from carbohydrate to lipid utilisation. It is very probably that our experiments were done in this situation.

În conclusion, the above presented results as well as the previous ones [3] show that the perfused frog heart exhibits a different behaviour as compared to the mammalian one, as far as the relation between carbohydrates and lipids as energy sources is concerned. The situation is even more complicated by the seasonal differences [24]. It is suggested that the season may be a triggering factor for some regulation mechanisms, leading to the preferential utilization of one or of the other substrate for the energetic needs of the cardiac muscle.

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Received February 6, 1974

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EXTRACTION WITH DETERGENTS OF THE CATALASE FROM THE BOVINE HEPATIC TISSUE

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The activity of catalase preparations extracted from the bovine hepatic tissue records a maximum value in the case of the delipidization of the biological material with acetone. Ionic detergents evince a solubilization action of the enzyme bound to cytoplasmatic formations. The influence of the various deoxycholate concentrations on the solubilization of this enzyme was studied. The variation of the enzymatic reaction rate with the H_2O_2 concentration and with the pH is different in the case of the utilization of the three extraction media: water, 0.5% deoxycholate solution and 3% deoxycholate solution. The stability of the activity of an enzymatic preparation in the course of five days of preservation at 7°C decreases with the increase of the detergent concentration in extraction medium. The multiple molecular forms of the hepatic catalase were evidenced by electrophoresis in agar gel.

The catalase (EC 1.11.1.6) enzyme met with in vegetal cells [8], in the hepatic tissue of mammals [9], in erythrocytes [6], as well as in microorganisms [3], carry out a major physiological role, catalysing the decomposition of H_2O_2, a metabolite toxic for cells [7]. Intracellularly, the catalase is localized in the cytoplasm microbodies of aerobiotic cells, together with dehydrogenases which produce hydrogen peroxide, having the role of permanently regenerating the oxygen necessary to these enzymes in the metabolization of their specific substrates [13].

Though the enzyme isolated from the bovine hepatic tissue was and is the object of numerous studies [12] [14] our present knowledge regarding the catalase are sometimes contradictory, due probably to the structural particularities of the enzyme, which confers it a great sensitivity to different treatments, inherent and necessary in the isolation and purification processes.

In a series of preliminary experiments it was recorded that in total proteic extracts, catalase presents a different behaviour from that of other enzymes. Thus, by the dilution of the proteic extract in view of determining the proportionality range of the reaction rate with the proteic concentration the loss of enzymatic activity is recorded. The progress of catalytic reaction in the course of the first minutes from the initiation is non-proportional, irrespective of the adjustment of the other kinetic parameters, which determined us to perform the measuring of a single rate variable at 30 sec, 45 sec and 60 sec, in two parallel sets, the result representing the average of six determinations. By the variation of an optimum action parameter of the enzyme a sudden modification of the values of the others is recorded.

REV. ROUM. BIOL., TOME 19, No 2, p. 119–127, BUCAREST, 1974

These experimental observations determined us to perform the study of the catalase of the bovine hepatic tissue, in close connection with its solubilization procedure, a delipidization of the biological source from which it is isolated. By effecting the delipidization with acetone of the hepatic tissue we recorded the disappearance, at least partially, of the above mentioned behaviour particularities.

MATERIAL AND METHODS

Several variants for enzyme extraction were probed.

1) 1 g bovine hepatic tissue was macerated by means of a waring blendor or of a potter, in the presence of 10 ml water. The extraction was realized for one hour, after which the homogenate was submitted to a rapid treatment with 10 ml delipidization agent (chloroform, 3 : 1 mixture chloroform methanol, 3 : 1 mixture chloroform ethanol) and to a centrifugation at 10,000 r.p.m. for 15 minutes, taking into the study the aqueous phase.

2) 1 g bovine hepatic tissue was macerated by means of a potter in the presence of 15 ml delipidization agent (chloroform, a 3 : 1 chloroform methanol mixture, 3 : 1 chloroform ethanol mixture). The homogenate was centrifuged at 10,000 r.p.m., for 15 minutes and the sediment was retaken in 10 ml water. After one hour's extraction, the delipidized homogenate was again centrifuged at 10,000 r.p.m. for 15 minutes, the catalase activity and the protein concentration of the supernatant being investigated.

3) The hepatic tissue was mortared in the presence of acetone, chloroform and a 3 : 1 chloroform ethanol mixture to the obtention of a powder in the first case, or of a moist residue in the last two cases. 1 g of powder or of defatted material was subjected to extraction with 20 ml water or to an other extraction medium, for one hour, at 7°C. The homogenate is centrifuged at 10,000 r.p.m. for 15 minutes and the supernatant is examined.

Catalase activity determination was carried out by the method described by Sinha [10]. in conformity with which H_2O_2 remained undescomposed is dosed with a mixture of bichromate-glacial acetic acid (1 : 3) and the chromic acetate formed as a result of the oxido-reduction was colorimetrically dosed at 570 nm, by means of a Spekol the results being computed from a H_2O_2 titration standard curve.

The enzyme specific activity was expressed in μmoles H_2O_2/mg protein/min/20°C. The reaction mixture contains 80 μmoles substrate.

Protein concentration in samples was determined by Lowry's et al. method [5], utilizing as standard protein doubly crystallized bovine serum albumine.

Catalase isoenzymes were separated by electrophoresis on agar gel (1.25%), in Michaelis buffer, for four hours, at 5 mA/plate and 120 V. Their visualization was performed in conformity with Woodbury's et al. method [15].

RESULTS

The results of the different maceration modalities of the bovine hepatic tissue (waring blendor, potter and mortar), as well as the influence of the delipidization agent of the biological material on enzyme solubilization are rendered in table 1. As it may be seen, the maceration mode of the hepatic tissue does not affect the enzymatic activity, though Brown [1] in an older work considers that some discrepancies in the values of enzymatic activities are due to different tissue maceration procedures. For discovering a maximum enzymatic activity the delipidization of the hepatic tissue is necessary. The most efficient delipidization agent proved to be the acetone. The 3 : 1 chloroform ethanol mixture, quoted in literature by most research workers [2] as optimum catalase extraction and delipidization agent, permits the finding out of a relatively small enzymatic activity in the total proteic extracts

obtained from the bovine hepatic tissue and does not lead to a good protein solubilization, in general. The mortaring procedure of the hepatic tissue in the presence of acetone proved to be the most efficient one and was utilized for the investigations hereunder (1 g acetonic powder/20 ml extraction medium).

Table 1

Influence of maceration and defatting procedure of bovine hepatic tissue on catalase solubilization

Maceration procedure	Extraction procedure *	Protein (mg/ml)	μmoles H_2O_2/ml/min/20°C	μmoles H_2O_2/mg protein/min/20°C
Waring blendor	1 g tissue/10 ml water	4.2	16,500	3,928
	no. 1 with $CHCl_3$	2.8	19,200	6,857
	no. 1 with $CHCl_3$: C_2H_5OH (3 : 1) mixture	1.4	8,100	5,785
Potter	1 g tissue/10 ml water	5.0	17,500	3,500
	no. 1 with $CHCl_3$	3.3	21,300	6,454
	no. 2 with $CHCl_3$	1.8	13,000	7,222
	no. 2 with $CHCl_3$: CH_3OH (3 : 1) mixture	0.7	0	0
	no. 2 with $CHCl_3$: C_2H_5OH (3 : 1) mixture	0.9	0	0
Mortar	no. 3 with acetone	5.0	40,000	8,000
	no. 3 with $CHCl_3$	2.4	17,200	7,166
	no. 3 with $CHCl_3$: C_2H_5OH (3 : 1) mixture	0.75	0	0

* Extraction and defatting protocoles (nr. 1, 2 and 3) are described in Material and Methods

Further on the catalase activity was determined depending on the chemical nature of the extraction medium. From table 2 it is established that five ionic detergents — sodium deoxycholate, sodium laurylsulphate, sodium taurocholate, brije and saponine — in 0.5% concentration — extract the greatest quantities of total proteins, permitting at the same time the finding out of the highest enzymatic activities. The best results were obtained by using sodium deoxycholate and laurylsulphate. In the presence of water the slightest total protein extraction as well as of catalase, in particular, is obtained.

By the increase of the extraction time from one hour to two hours and even to 24 hours, a smaller total protein solubilization rate is recorded,

Table 2

Variation of catalase activity from the bovine hepatic tissue with chemical nature of the extraction medium

Extraction medium	Protein (mg/ml)	μmoles H_2O_2/ml/ min/20°C	μ moles H_2O_2/ mg protein/ min/20°C
deoxycholate 0.5 %	9.9	65,600	6,626
laurylsulphate 0.5 %	11.2	65,200	5,821
taurocholate 0.5 %	8.5	53,400	6,282
brij 0.5 %	9.8	62,900	6,418
saponine 0.5 %	8.2	52,800	6,429
K_2HPO_4/KH_2PO_4, 1×10^{-2}M pH 7 buffer	6.1	51,800	8,500
NaCl 0.5 %	7.2	50,700	7,041
KCl 0.5 %	7.2	50,700	7,041
sucrose 0.5 %	5.2	51,200	9,846
Control (water)	5.0	40,000	8,000

in the case of the utilization of water as extraction medium than the one observed when utilizing deoxycholate (Table 3). Although protein concentration extracted increases in time, the catalase activity does not grow significantly during the first two hours of extraction and decreases in 24 hours, therefore in subsequent experiments, enzyme extraction from the acetonic powder was always realized in the course of one hour.

Table 3

Determination of catalase optimum extraction time from acetonic powder of the bovine hepatic tissue

Extraction medium	Extraction time (hours)	Protein (mg/ml)	μmoles H_2O_2/ml/ min/20°C
water	1	5.0	36,000
	2	5.0	38,000
	24	6.2	29,000
deoxycholate 0.5 %	1	12.4	56,000
	2	14.4	56,000
	24	16.2	50,000

The influence of different deoxycholate concentrations on the catalase extraction from the bovine hepatic tissue is presented in table 4. By the increase of the detergent concentration from 0.5 % to 4 %, the quantity of total proteins extracted grows from 9.9 mg/ml to 14.8 mg/ml while the enzymatic reaction rate increases in the domain of 0.5 %—3 %

detergent, after which it decreases due either to an enzyme denaturation, or to the formation of deoxycholate myceles which involves also the enzyme macromolecules rendering them inaccessible to catalysis, a phenomenon indicated also by the stationing of total protein solubilization in the case of large concentrations of detergent (Table 4).

Table 4

Influence of the various sodium deoxycholate concentrations on the extraction of catalase from the acetonic powder of the bovine hepatic tissue

Deoxycholate concentration	Protein (mg/ml)	μmoles H_2O_2/ml/min/20°C
0.5 %	9.9	65,600
1 %	10.8	66,000
2 %	10.8	80,800
3 %	12.4	83,600
4 %	14.8	71,200
5 %	14.8	69,500

The determining of the variation of the enzymatic reaction rate depending on H_2O_2 concentration of the reaction medium was performed on preparations in which water and 0.5 % and 3 % sodium deoxycholate solutions were used, either immediately after the processing of the total proteic extracts or after 24 hours from their obtention. When the extraction medium is water, the curve which renders the variation of the reaction rate in relation with the substrate concentration has a sigmoid aspect, which is retained even after the 24 hours conservation of the proteic extract at 7°C. Immediately after the preparation of total proteic extracts, the curves of this type realized with deoxycholate in solutions of 0.5 % and 3 % have a typical hyperbolic aspect. After 24 hours, in the case of a 3 % solution of deoxycholate extraction, a Michaelis curve is obtained with a level of the domain of 96—144 μmoles H_2O_2, while in the case of solubilization with 0.5 % deoxycholate — a sigmoid curve with common points in the domain of 0—48 μmoles H_2O_2 with the characteristic one of water, when this is utilized as extraction agent (Fig. 1) is obtained.

The variations of the reaction rate with the concentration of hydrogen ions were likewise investigated, depending on the extraction medium of proteins from the acetonic powder of the bovine hepatic tissue (Fig. 2). The optimum pH of catalase action, irrespective of the employed extraction medium is 7 but the recorded curves present different aspects, particularly in slightly acid pH values.

The course of the reaction catalyzed by the enzyme isolated from the bovine hepatic tissue is presented in figure 3. It is recorded that the 80 μmoles H_2O_2 initially introduced into the reaction medium are consumed, in the case of water being utilized as extraction agent, in the course of five minutes, in the case of 0.5 % deoxycholate within four minutes, while in the case of the 5 % concentration in only three minutes.

The stability of the enzyme activity depending on the nature of the extraction agent is presented in figure 4. It can be seen that the stability of the catalase activity diminishes as the sodium deoxycholate concentration increases.

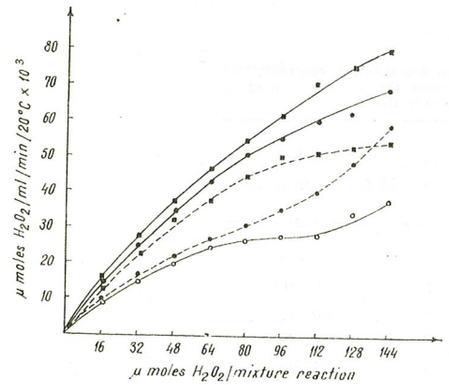

Fig. 1. — Variation of catalase activity extracted from acetonic powder of the bovine hepatic tissue with: water, deoxycholate — solutions 0.5% and 3%, depending on H_2O_2 concentration present in reaction medium. Curves represented dottedly indicate determinations effected in the same conditions, but after 24 hours preservation of enzymatic preparations at 7°C.
-o-o-extraction medium — water; -●-●- extraction medium — 0.5% deoxycholate; -■-■- extraction medium — 3% deoxycholate.

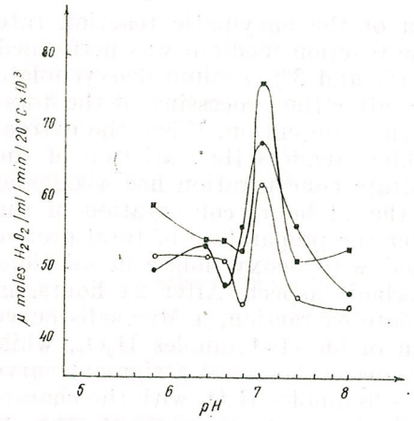

Fig. 2. — Variation curves of enzymatic reaction rate with pH in the case of the utilization of three extraction media.
-o-o-water; -●-●- 0.5% solution deoxycholate; -■-■- 3% solution deoxycholate.

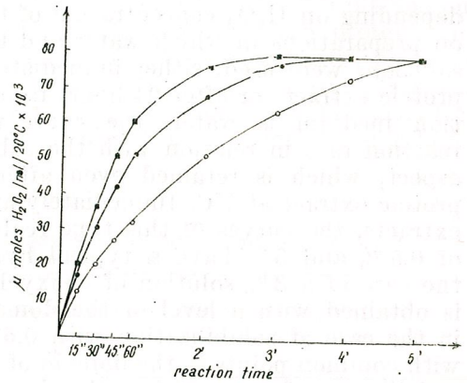

Fig. 3. — Enzymatic reaction course catalyzed by the enzyme extracted from acetonic powder of the bovine hepatic tissue with water, solution 0.5% deoxycholate and solution 3% deoxycholate, in the course of five minutes.

DISCUSSION

One of the conditions of obtaining a total proteic extract with a rich catalase activity consists in the corresponding delipidization of the hepatic tissue. The 3 : 1 chloroform ethanol mixture recommended by other research workers for the extraction of animal catalases proved to be inefficient due to its denaturating action. Even when the extrac-

tion of proteins is done in water, the simple addition of this mixture induces the precipitation by the denaturation of proteins already solubilized. Detergent solutions extract more catalase than water and the tested saline solutions, probably by having also a solubilizing action of enzyme bound to peroxisomes. Electrophoregrams of proteins and the enzymograms of total proteic extracts obtained with the three extraction media (water, 0.5% deoxycholate and 3% deoxycholate) are completely different (Fig. 5). When the extraction medium is an aqueous

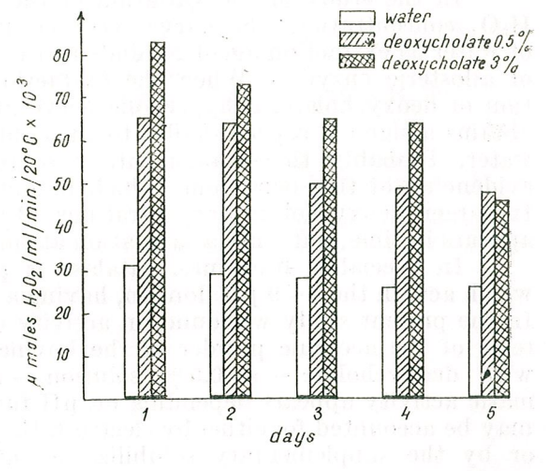

Fig. 4. — Activity stability of some catalase preparations realized by the three extraction media: water, solutions 0.5% and 3% deoxycholate in the course of five days' stationing at 7°C.

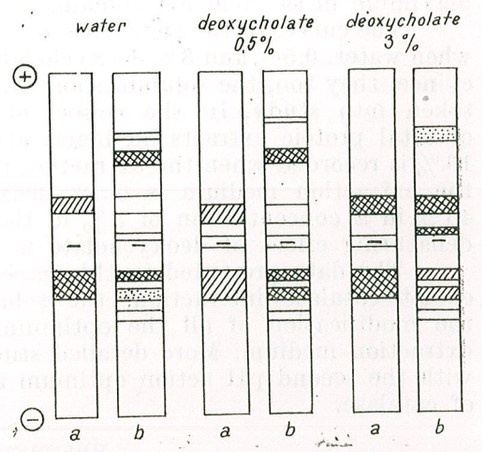

Fig. 5. — Electrophoregrams of total proteic extracts obtained from acetonic powder of the bovine hepatic tissue with water (*1*); 0.5% deoxycholate (*2*) and 3% deoxycholate (*3*) *a*, bands with catalasic activity; *b*, proteic bands.

solution of the tested detergent, a band with enzymatic activity in start appears, while the band which migrates towards the anode has an all the greater activity as the deoxycholate concentration increases. Our results are totally different from those presented by Holmes and Masters

[4], in a recent paper, on subcellular localization and electrophoretic heterogenity of catalase from the hepatic tissue of mammals. These authors showed that the enzyme from the organism of bovines is homogeneous from the electrophoretic point of view, although they record also the existence of a catalase activity in the subcellular fraction (mitochondria, lisosomes and peroxisomes). The multiplicity of bovine hepatic catalase found out by us may be due both to the completely different solubilization procedure as well as to the utilization of a more sensitive visualization method of isoenzymes.

In the study of the variation of the enzymatic reaction rate with H_2O_2 concentration, the curve recorded in the case of the utilization of water as extraction agent reminds us the one which appears in the case of allosteric enzymes. When the extraction medium is the 0.5 % solution of deoxycholate, a hyperbolic curve appears which, after 24 hours, obtains a sigmoid aspect similar to that obtained in the experiment with water. Probably the supplementary solubilized proteins hinders the evidencing of this behaviour, which becomes however manifest in time. In larger deoxycholate concentrations (3 %), the sigmoidity no longer appears in time, but only a saturation at smaller substrate concentrations.

In speciality literature, catalase is presented as an enzyme [11] which acts in the 4—9 pH domain, having a maximum activity at pH 6.8. In the present study we found an activity optimum at pH 7. When proteins of the acetonic powder of the bovine hepatic tissue are extracted with deoxycholate — a 0.5 % solution — another maximum of enzymatic activity appears depending on pH function of the 6.4 value, which may be accounted for either by electrostatic protein-detergent interactions, or by the supplementary solubilization of another molecular form of catalase with another pH optimum. When the extraction medium is water or the 3 % deoxycholate solution, the curves tend to form a second maximum in the acid pH domain.

The curves of the enzymatic reaction course during the five minutes when water, 0.5 % and 3 % deoxycholate are utilized as extraction medium evince, they too, the solubilization capacity of catalase by the detergent taken into study. In the course of five days of preservation at 7°C of total proteic extracts an inactivation of enzyme in a percentage of 16 % is recorded when the extraction medium is water and of 26 % when the extraction medium is 0.5 % deoxycholate and in a percentage of 45 % in a concentration of 3 % in the detergent, which shows that the denaturing effect of deoxycholate is accentuated in time.

The data presented in this paper attest the phenomena of deoxycholate-catalase interaction, the solubilizing action of detergent and the modification of all the optimum action parameters depending on extraction medium. More detailed studies are being made in connection with the second pH action optimum and with the two molecular forms of catalase.

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Received June 4, 1973

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BĂNĂRESCU P., 1973, *Principiile şi metodele zoologiei sistematice* (Principles and methods of Systematic Zoology), Edit. Academiei R.S. România, 219 pag., 15 fig.

The appearance of Dr. Bănărescu's book in a period when several biologists are questioning the right of existence of Systematics, claiming that it is outdated, is most welcomed. Written by a zoologist very familiar with taxonomic and phylogenetic research, the new book fills up a gap in our zoological literature, presenting a survey of the recent trends in Systematic Zoology, including also the authors views on the different theories. The basic opinions of Dr. Bănărescu are mostly in concordance with the principles asserted by Rensch, Mayr, Simpson and accepted by the overwhelming part of the zoologists.

The first chapter deals with the definition of taxonomy as a science studying the diversity of the living world and disclosing its causes. Therefore, the realm of systematic research cannot be restricted or removed by the new branches of biology, but rather enriched.

Species and speciation are largely discussed in the second chapter, the typologic and nondimensional species concepts are rejected and the biological multidimensional species concept — based on reproductive isolation and potentially interbreeding within the species — is adopted. At the subspecific level, the categories of subspecies and cline are considered as phenotypical expressions of geographical variation of the genotype. Other infraspecific concepts as natio, praenatio, physiological races etc. are not recognized. The population concept, as basic element of the species, is analyzed in detail, as well as the genetic of populations; the genetic and ecologic mechanisms of reproductive isolation are discussed. Some characteristic features of the species and their biological significance are pointed out: polymorphism and sibling species. A particularly fascinating part of the book is the record of the difficulties of applying the biological criterion of the species; many examples are shown from different groups of plants and animals, as well as the respective solutions. The author considers the parthenogenetic forms as species; in the case of isolated populations where the gene flow is interrupted, as in island populations, the decision depends of the degree of reproductive barriers and the amount of evolutive divergence. Another important section deals with the controversed problem of sympatric speciation. The author, in agreement with Mayr and most modern taxonomists, considers the possibility of sympatric speciation in biparental organisms as doubtful, but he admits contrary to Mayr a geographical speciation "by distance", without a discontinuity or interruption of the range, since remote populations are no more influenced by the gene flow. The alleged "species flocks" in ancient lakes could be accounted for by repeated colonization or former splitting of the biotope. The recent theories on the role of polyploidy in sympatric speciation are considered as possible. Alloploid hybridization and autopolyploidy may favorise sympatric speciation in plants but not in biparental animals.

An extensive chapter is devoted to the supraspecific taxa, which although representing the historic reality of common descent, are not actually real, but the result of a subjective choice and therefore more or less arbitrary delimited. The only clues of establishing the supraspecific taxa are the existing discontinuities between them and sometimes obvious morphological divergence based often on particular ecological adaptation. The author expresses his opinion about the controverse between "cladistic" and "evolutionary" phylogeny. He rejects Hennigs categorical assumption of invariable dichotomic speciation as well as his considering of the time of divergence as unique criterion of phyletic relationship; classification should consider also the degree of morphological divergence of branches and their unequal subsequent ramification. Although the paleontological record is of paramount importance in building phylogenetical trees and classifications which include fossil taxa are more consistent, the number of discovered fossils is still unsatisfactory.

In modern systematic research, serological and biochemical methods as well as cariology are of great importance. The author rejects the assumption of numerical taxonomy to avoid the phyletic research in favour of the comparison of the quantitative parameters of the characters.

Chapter four deals with the working methods in systematics, emphasizing the necessity of multilateral training of modern systematicians, who need to be familiar with the findings of ecology, genetics, physiology and chemo-taxonomy. The author points out the importance of zoological collections and of the types. Further, the Code of zoological nomenclature and its rules are presented.

REV. ROUM. BIOL., TOME 19, Nº 2, p. 129 – 131, BUCAREST, 1974

In the fifth chapter, the contemporary aims of zoological systematics are outlined: the studies of regional faunae, the world wide revisions of higher taxa and phylogenetic researches (based mainly on anatomy and embriology).

An original chapter deals with ethic in systematics, showing some negative aspects occuring in the activities of individual zoologists as well as zoological institutions, like competition in naming new taxa, not acknowledging the help of assistants, encroaching upon the zoologists working their national fauna, refusal to make available museal collections to foreign researchers, excess of criticism when appreciating the work of predecessors, etc.

The final chapter presents the contribution of the Romanian zoologists to the progress of taxonomy. The pioneering work of Racoviţă in fundamental items of modern systematics is emphasized, especially his concept of biological species, presented as early as 1912. The work on speciation of several recent romanian zoologists are mentioned, including cytotaxonomic and chemotaxonomic studies.

I feel sure that Dr. Bănărescu's book will receive a wide audience and help many young taxonomist to find the right solutions.

I. E. Fuhn

P. BĂNĂRESCU, N. BOŞCAIU. *Biogeografie. Perspectivă genetică şi istorică.* (*Biogeography. Genetical and historical survey*). Ed. Ştiinţifică, Bucharest, 1973, 302 pag., 48 fig.

Following the book "Principles of Zoogeography" (1970), this new work meets the need of a general and unitary survey of the trends in contemporary biogeography, an interdisciplinary science, where the phytogeographic aspects should balance the zoogeographic features. Although this project is rather difficult to achieve, the present book illustrates the successful cooperation of two top representatives of so different disciplines as botany and zoology, manifested by the remarquably unity of conception and fluent style.

The first chapter outlines the scope of biogeography and its relations with different disciplines: zoology, botany, paleontology, palinology, paleogeography, paleoclimatology, ecology, geobotanics, genetics, the theory of evolution, physical geography. The branches of biogeography are presented-descriptive biogeography, comparative biogeography, genetical-historical biogeography.

The authors follow a genetical-historical point of view, aiming to explain the actual distribution of plants and animals through the conditions which generated the apparition and extension of their areals. An extensive chapter deals with speciation, kinds of speciation and the biogeographic results of the most important form of speciation — the allopatric speciation. The third chapter is devoted to a new direction in biogeography-cytogeography — based on the assumption that the present distribution areas have resulted from the instable balance between the genetical systems and the environmental factors.

The areal problem is widely discussed in chapter IV; the areals of species as well as of the supraspecific taxa are considered. The methods of outlining an areal are shown; different aspects of distributions are further analyzed: size, species distribution within their areals (continuous or discontinuous), role of barriers in the extension of areals, changes in areals, biogeographical significance of migrations.

The next chapter presents the mechanisms of the genesis of the diverse florae and faunae, the analysis of floristic and faunistic elements, the criteria of establishing the geographic origin (arealographic, genetic, historic, migrative), in agreement with G. de Lattin. The island florae and faunae are extensively discussed.

Chapter VI is devoted to the paleogeographical factors of the organism's distribution, the existing theories are reviewed: the theory of continental bridges, the theory of the permanence of continents and oceans, the theory of continental drift. Although the authors prefer the latter theory, based on the recent findings of geodesy, geophysics, geology, paleontology and climatology, they reserve to the other theories some euristical possibilities. Quaternary glaciations have played obviously an important role in the modifications of the florae and faunae; these aspects are dealt with in chapter VII, which considers the evolution of quaternary climate and vegetation as well as the biogeographical consequences of the glaciations. Examples of analytical biogeography are presented in chapter VIII, i.e. the reconstruction of the evolution in time and space of the areals of mammals, primary fresh-water fishes and gymnosperms. The last chapters-IX and X-show the synthetic aspects, i.e. the different geographic regions-marine, terrestrial, fresh-water and their evolution. These biogeographical syntheses are particularly successful; the cooperation between the botanist and the zoologist is very close; for example, in the section devoted to the terrestrial biogeographic region, the reader can follow simultaneously the evolution of the floristic and faunistic elements. As it was to be expected, the holarctic region is presented most extensively.

I consider this new book as a valuable reference and working instrument for all the biologists and of real help when the biogeographical view is requested for the solution of some controversed items.

I. E. Fuhn