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MYSIDIUM ILIFFEI N.SP. ET *AMATHIMYSIS SÂRBUI* N.SP., MYSIDACÉS NOUVEAUX DANS LE SECTEUR DES ANTILLES

MIHAI BĂCESCU

On présente *Mysidium iliffei* n. sp. et *Amathimysis sârbui* n.sp., mysidacés nouveaux dans le secteur des Antilles.

INTRODUCTION

Le dr T. Iliffe nous a envoyé quatre échantillons de mysidacés récoltés dans les eaux de la Jamaïque à Sa Rba et M. Sârbu nous en a remis un seul échantillon de Bahamas. Nous en remercions ces deux spécialistes en plongée pour leurs captures.

I. LA JAMAÏQUE

Voilà les stations :

St. A. notée „10.034 Sa Rba, 30.VI.1990 *Mysidium columbiae*, Zimmer 1915 ; 8♂,5♀ ;

— St. B, „10. 034, Sa Rba, 30.VI.90. *Mysidium cubanense* : 32 ♀ 14 ♂ ad.et quatre juv. (2—3 mm) ; l'extrémité de l'exopode atteint la base du telson ;

— St. C, 90.012, Sa Rba, 14.VI.90. *Mysidium cubanense* ♂ ♀ ♀ = 5 mm, 1 ♀ = 4 mm ;

— St.D, 90.026, Sa Rba, 26.VI.1990. *Mysidium cubanense* (des dizaines) et *M. iliffei* : 3 ♂ juv. et 5♀ (ovigères).

Mysidium iliffei n. sp.
(Fig. 1 A—J)

Diagnose. Espèce de petite taille (4—4,2 mm) apparentée à *M. columbiae* Zimmer 1915. Telson aux bords parallèles ±droits, le retrécissement très faible dans son tiers distal ; 35 lamines sur l'apex ; celles-là et surtout celles latéro-terminales sont pointues ; aucune gélifiée.

Matériel 3 ♂ j et plusieurs femelles, St. D, 90.026 Sa Rba, 26.VI.1990.

Description ♂j, ♀. Le front de la carapace arrondi ; l'œil globuleux ; sa partie cornéale rouge occupe 2/3 du tout (Fig. 1C) ; son lobe ♂ est couvert de longues soies plumeuses sur toute sa longueur.

Antennule de type commun au genre (Fig. 11), l'antenne (Fig. 1B) à l'exopodite plus court que chez la *M. columbiae*, ne dépassant pas l'apex de l'A_1 ; son article apical porte 6 soies plumeuses. Rien de particulier pour les pièces bucales.

Péréiopodes avec 2 et 3 articles tarsaux (Fig. 1 F et E) ; la base de leur exopodite allongée.

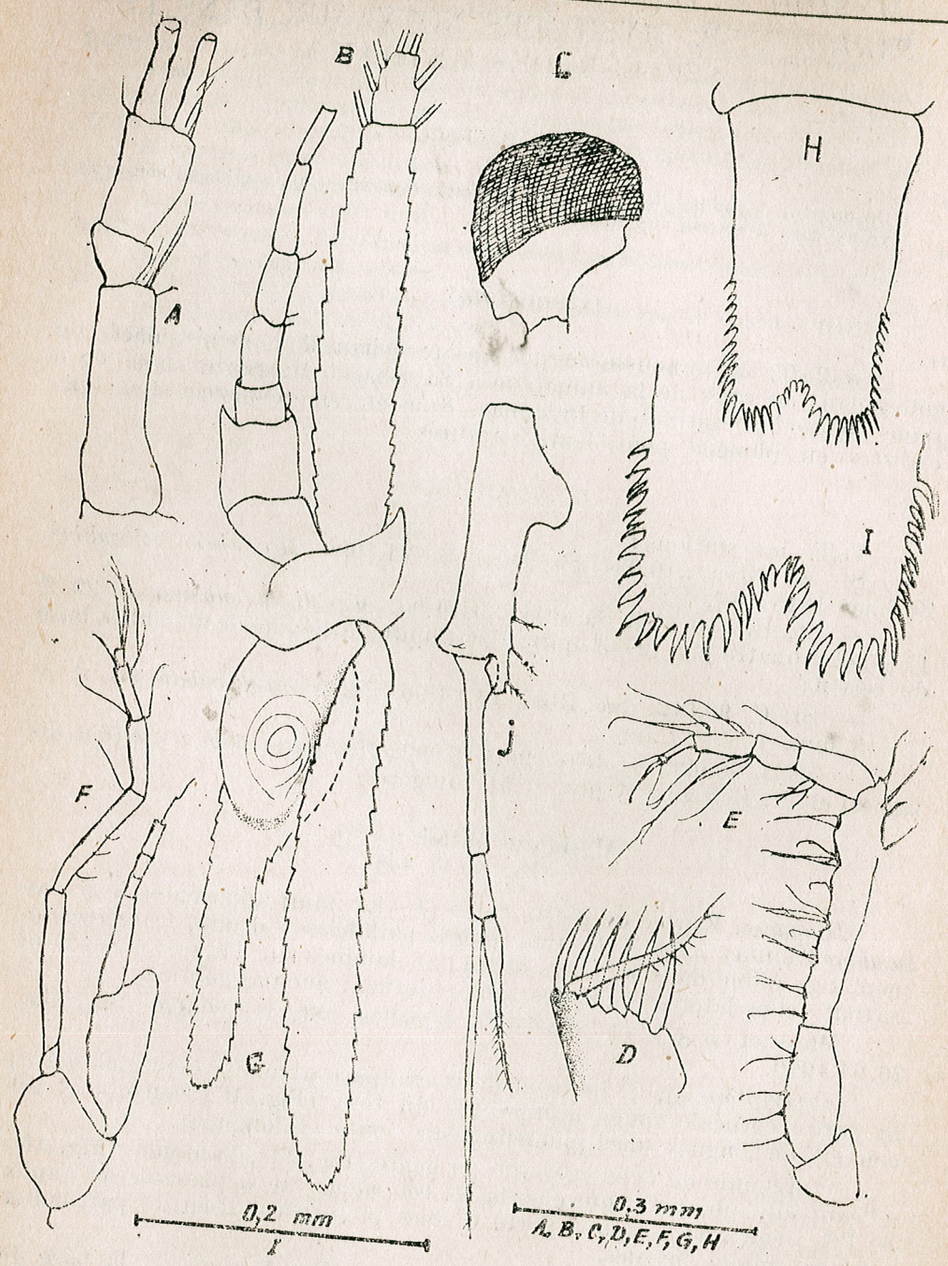

Fig. 1. — *Mysidium illifei* ♀ A, antennule ; B, antenne ; C, l'œil ; D, l'article apical du palpe mandibulaire ; E, péréiopode I ; F, le dernier péréiopode ; G, uropodes ; H, telson ; I, sa partie apicale, grossie ; J, pléopode IV ♂ de *Mysidium columbiae*.

Le IV^e^ pléopode ♂ dépasse à peine le milieu du V^e^ pléonite.

Le telson aux bords latéraux ± droits, marquant un faible retrécissement dans sa partie distale (Fig. 1 H).

Sa partie apicale est armée de cca 35 lamines, donc sans articulations, pas une gélifiée et de cca 11 latéro-terminales de chaque côté ; toutes ces lamines sont pointues ; les plus longues occupent le double-apex du telson et leur taille diminue tant vers le fond du sinus que vers celles latérales. L'émargination du bout du telson représente à peine 1/7 de sa longueur (Fig. 1H).

Les uropodes (Fig. 1G). L'exopode ne dépasse pas beaucoup la longueur de l'endopode (22 : 18), comme chez les autres espèces du genre.

Ecologie. M. iliffei, récoltée en compagnie de *M. cubanense*, au plancton, contribue aux aggrégations caractéristiques du genre. Les ♀ ont en moyenne 5—6 œufs.

Observations. M. iliffei est assez proche de *M. columbiae* ; elle en diffère par :

— le petit rétrécisement du telson dans sa partie distale ;

— il est armé de 35 longues lamines pointues, toutes sans gelifications, par rapport à 43—52 (cca une dizaine capuchonée), comme chez l'espèce apparentée ;

— taille plus petite : 4,2 par rapport à 7,3 ;

— l'article basal du IV^e^ pléopode ♂ n'a pas la dilatation si caractéristique de *M. columbiae* (flèche, Fig. 1J).

Derivatio nominis : espèce dédicacée au D^r^ T. Iliffe, qui l'a récoltée.

Holotype ♀, collection carcinologique du Musée « Grigore Antipa » n^o^ 748 ; allotype ♀, *ibid*, n^o^ 749.

Courtes remarques sur les autres espèces de Mysidium de cette collection.

M. columbiae est plus fine et plus longue que *M. cubanense* ; le plus grand ♂ examiné = 6,8 mm ; sur le matériel frais on le reconnaît facilement d'après ses yeux rouges—claires. La *M. cubanense* est la plus répandue et nombreuse (trouvée dans 3 des quatre stations même à la Jamaïque).

Chez certaines *M. cubanense* l'apex du telson est ± tronqué et orné de cca 40 lamines plus courtes que celles latérales ; les petits exemplaires en ont l'apex arrondi ; chez les adultes, le IV^e^ pléopode ♂ atteint l'apex du telson.

La présence d'au moins une espèce de *Mysidium* dans les récoltes mysidologiques, faites partout dans l'espace caribbean, prouve que cet espace est aussi le centre génétique de ce genre, tout comme pour le cumacé *Cumella*.

La seule espèce trouvée dans les eaux californiennes, *M. columbiae*, tout comme la seule espèce d'*Amathimysis* (voir plus loin). *A. trigibba* Murano, soulève un problème zoogéographique importante, qui nous fait penser à l'époque où les deux Amériques étaient séparées.

Le diverticule de la hampe du IV^e^ pléople ♂ de *M. columbiae* de la Jamaïque est plus développé que celui figuré par Brattegard 1969.

II. BAHAMAS

Un seule petite collection faite en plongée par les D^r^ T. Iliffe et S. Sârbu dans une grotte de l'île Abaco-Bahamas le 11.XII.1990.

Nous y avons trouvé 15 expl. *Mysidium cubanense*, 1 expl. d'*Anchialina tipica* ♀ et un seul exemplaire d'*Amathimysis* ♀, que nous décrivons comme.

Amathimysis sârbui n. sp.
(Fig. 2 A—H)

Diagnose. A. sârbui a l'écaille antennaire rhombique, deux fois seulement plus longue que large, ayant la suture, qui sépare son long lobe distal, au niveau de l'épine extérieure.

Les uropodes courts, l'exopodite plus court et plus large que l'endopodite. Péréiopode I massif, les autres grêles, avec la typique articulation oblique du genre entre les articles du „tars". Les pléopodes ♀ en forme de plaques vaguement triangulaires, légèrement échancrés et ayant une soie à leur angle extéro-supérieur.

Description (♀). Tégument ivorin, tacheté de points noirs surtout pour les pièces bucales et les premiers 2 thoracopodes. Cephalothorax beaucoup plus large que le pléon.

Antennule massive (Fig. 2 A), ayant un gonflement médian à la base du fort flagelle; l'œil le dépasse un peu en diamètre. *Antenne* (Fig. 2 B) avec un gros endopodite et une écaille losangique, deux fois seulement plus longue que large, finissant avec un long lobe apical, à suture évidente et portant 9 longues soies.

Le palpe de la mandibule a une forte garniture d'épines-soies sur le bord intérieur de l'article terminal, oval et 12 longues soies sur le fort article médian.

Maxillipède I avec une courte griffe dactylaire et sur le bout interne du propodite, presque discoïdal, 6 courtes épines et 3 longues soies. Le II^e^ maxillipède commun, lui aussi, au genre (Fig. 2 C).

Premier péréiopode massif, avec une articulation tarsale oblique, le carpus large et le méros avec une caractéristique dilatation circulaire (la flèche, Fig. 2 D); les autres péréiopodes sont plus fins;, leurs exopodites ont de fortes basis qui égalent en longueur les huits articles de leur flagelle.

Pléon avec les somites ± égaux en longueur; même le 5^e^ ne dépasse pas le dernier; celui-ci présente un prolongement dorsal triangulaire qui couvre la base du telson; ses bords intéro-postérieurs ont la forme discoïdale (la flèche, Fig. 2 G). De forts cromatophores noirs marquent le milieu du bord postérieur de chaque pléonite (Fig. 2 E, la flèche).

Le telson légèrement cordiforme, typique au genre, porte deux longues soies-épines apicales et deux petites sous-apicales (Fig. 2 H).

Les pléopodes de la ♀ *A. sarbui* sont bien caractéristiques à notre espèce: ils ont la forme d'une plaque vaguement triangulaire, légèrement échancrée, pourvue d'une soie simple à son angle latéro-dorsal (Fig. 2E); le 5^e^ est semblable aux autres, pas comme chez la *A. trigibba* Murano 1987 (Fig. 1 A, p. 183, p.ex).

Les uropodes courts et larges, l'endopodite, égalant en longueur les deux derniers pléonites, est un peu plus étroit et plus long que l'exopodite (Fig. 2 F)

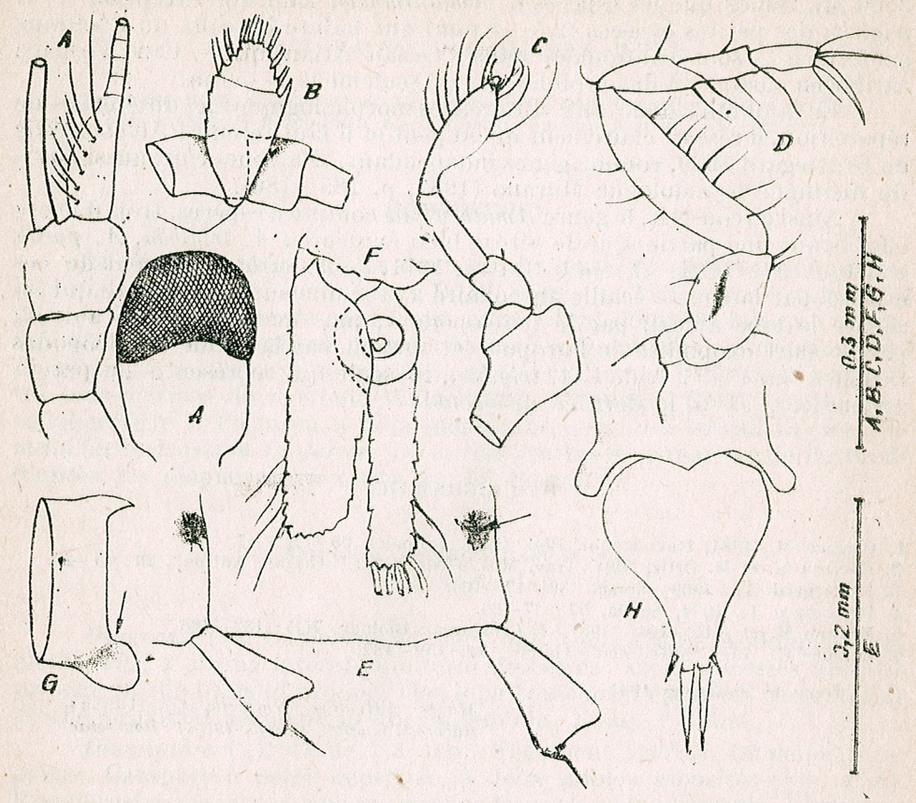

Fig. 2. — *Amathimysis illifei* n.sp. ♀ A, antennule et oeil; B, antenne et son exopodite; C, maxillipède II; D, péréiopode I; la flèche: l'expansion du meros; E, les pléopodes des plémères IV et V; fléche = les chromatophores noirs; F, les uropodes; G, extrémité du dernier pléonite, de profil; la flèche: l'élargisement discoïdal de son extrémité postéro-inférieure; H, telson.

Holotype ♀, abîmée et disséquée, fait partie de la collection de crustacés du Musée d'Histoire-Naturelle, « Grigore Antipa », sous le n^o^. 247.

Derivatio nominis: espèce dédicacée au D^r^ Sârbu S., un des plongeurs qui l'ont collectée dans la grotte sous-marine de Bahamas mentionnée plus haut.

Observations. En 1984, en décrivant l'éspèce *brasilliana*, je l'ai placée dans le genre *Katerythrops*, sous-genre *Amathimysis*. Depuis j'ai analysé d'autres espèces de ces Erythropini et surtout la répartition zoogéographique et bathyale de toutes les espèces apparentées à ces deux taxons:

Or, la répartition des espèces de *Katerythrops* est fort différente de celle d'*Amathimysis*. Toutes les espèces du premier genre (sauf une, *K. resimora* de Cape Town) sont des espèces plus grandes (5—12mm); cantonnées dans l'Océan Indien, y habitant les grandes profondeurs (800—

3500 m), tandis que les espèces d '*Amathimysis*, sauf une exception — *A. trigibba* des petites espèces (2,5—4 mm) qui habite les eaux du Pacifique californien — sont cantonnées dans l'Océan Atlantique — dans l'espace caribbéen surtout, à des profondeurs de seulement 1—50 m.

En ajoutant donc aux différences morphologiques les différences de répartition, il ressort clairement qu'on peut et il faut retenir l'*Amathimysis* de Brattegard 1969, comme genre indépendant. D'ailleurs c'est aussi l'avis du distingué mysidologue Murano (1987, p. 185—186).

Ainsi circonscrit, le genre *Amathimysis* compte 8 espèces, trois d'entre elles ayant une partie apicale sétose bien évidente ; *A. trigibba*, *A. polita* Brattg. 1971 et *A. cherados* Brattg. 1971, or *A. sarbui* diffèrent de ces espèces par la courte écaille antennaire ayant une suture évidente qui lui sépare le lobe apical, par le péréiopode I, plus massif que les autres ; par le court exopodite de l'uropode et surtout par la forme de pléopodes femelles. La Fig. 1 A de l' *A. trigibba*, la seule qui représente de pareils appendices, en a le dernier différent.

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NOUVELLES ESPÈCES DE *CUMELLA* DES GROTTES SOUS-MARINES DE BERMUDE

MIHAI BĂCESCU et THOMAS ILIFFE

On présente la description et les diagnoses de trois nouvelles espèces de *Cumella* : *Cumella bermudensis* n.sp., *Cumella spinosa* n.sp. et *C. sterreri* n.sp.

INTRODUCTION

Il s'agit de trois espèces dont les diagnoses et quelques figures caractéristiques ont été envoyées au Dr W. Sterrer en 1984 pour être incluses dans le volume *Bermuda cave symposium*. Etant donné le fait que ce travail n'a pas été imprimé, nous donnons à présent leur description plus détaillée. Le matériel a été capturé par le Dr Thomas Iliffe dans les grottes sous-marines du système Walsingham, Bermuda. Entre temps, mon collaborateur I. Petrescu a déjà mentionné dans son travail (2) les *Cumella bermudensis* et *C. sterreri* des autres grottes sous-marines de Bermude d'après les diagnoses envoyées au Dr Sterrer.

Cumella bermudensis n. sp.
(Fig. 1 A—G)

Diagnosis (♀). Cumacean of the coralicolous group. Abdominal segments with 2 strong lateral spiniform processes ; such processes are also present on the basis of uropod. One long transluced épine on the exterior of the proximal segment of the antennula. Long. 1.8 mm.

Description (♀). Taille 1,8 mm. Tégument ivorine, faiblement rugueux. Carapace à peine déprimée, à deux faibles gibosités (Fig. 1 A). Pseudorostre incourvé ; siphons courts ; les yeux non apparents. L'antenne est armée d'une longue pseudoépine à l'extérieur de l'article proximal (Fig. 1 C, la flèche). L'encoche antennaire réduite.

Les thoracomères ont de longues soies simples, gluantes (Fig. 1 B) ; des soies plus courtes sur les côtes des pléonites, armées de deux épines, sauf le premier qui n'en a qu'une. Les soies sont tellement gluantes et couvertes de vase, qu'on les nettoie difficilement.

Les pléons des mâles de cette espèce ont une espèce d'auricules sur la partie inférolatérale des IVe segments. Pour le maxillipède III, voir la Fig. 1 F. Le premier péréiopode est armé d'une forte griffe (Fig. 1E) ; le IIe prp. chez le dactyle égale en longueur le carpopodite (fig. 1 F).

Le pédoncule de l'uropode, deux fois plus court que l'endopodite, porte 3 épines (Fig. 1 G) ; il est orné de 2 soies flagellées terminales (Fig. 1 H) dont l'apicale dépasse un peu en longueur l'endopodite. L'exopodite, plus court et plus riche en longues soies simples que l'endopodite, finit par une très longue soie non flagellée, qui atteint le bout de la soie apicale de l'endopodite.

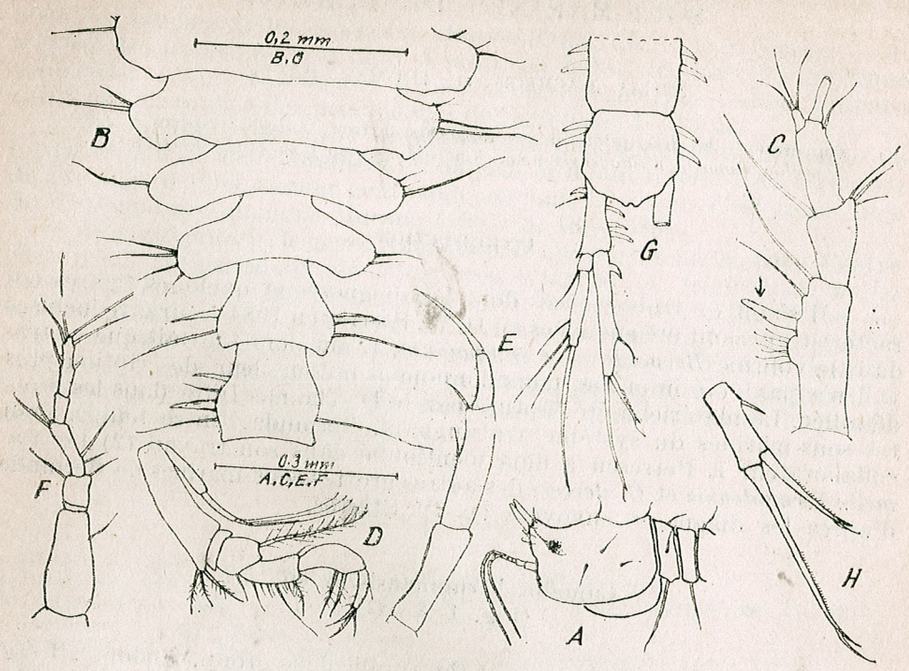

Fig. 1. — *Cumella bermudensis* n.sp. (♀)
A. Carapace de profil ; B. les trois derniéres thoracomères du cephalothorax ; C. antennule ; D. maxillipède III ; E. extrémité du premier péréiopode ; F. péréiopode II ; G. complexe caudal ; H. les soies apicales de l'endopodite de l'uropode.

OBSERVATIONS

C. bermudensis fait partie du groupe « scabra », type *C. agglutinanta* Băcescu 1971, *C. coralicola* Băcescu 1971, etc. ; elle est assez proche de *C. spinosa* par l'armure des pléonites et par la morphologie du complexe caudal.

Matériel 2 ♀, Walsingham Cave (III) 5.VII.79.

Derivatio nominis : d'après le pays d'origine.

Holotype ♀. Musée « Gr. Antipa » n° 415 ; paratypes (2 juv.) *ibid.*, 415 b.

Cumella spinosa n. sp.
(Fig. 2 A—G)

Diagnosis (♀). Cumaceans from the *C. pilosa* Băcescu 1971 group, characterized by a rich series of phanerae and a pseudorostrum strongly raised and weakly obturned, with enormous siphons. Anténnula with an internal dactyloid process on the second segment.

Description (♀), tégument à de rares poils et longues soies surtout sur les côtes des pléonites ou entre elles, une épine hyalinoïde. Carapace

légèrement aplatie avec un pseudorostre relativement long et obliquement dirigé en haut, comme chez le *C. tripuncta* (Fig. 2 A), avec d'énormes siphons ; deux taches légèrement colorées indiquent les yeux.

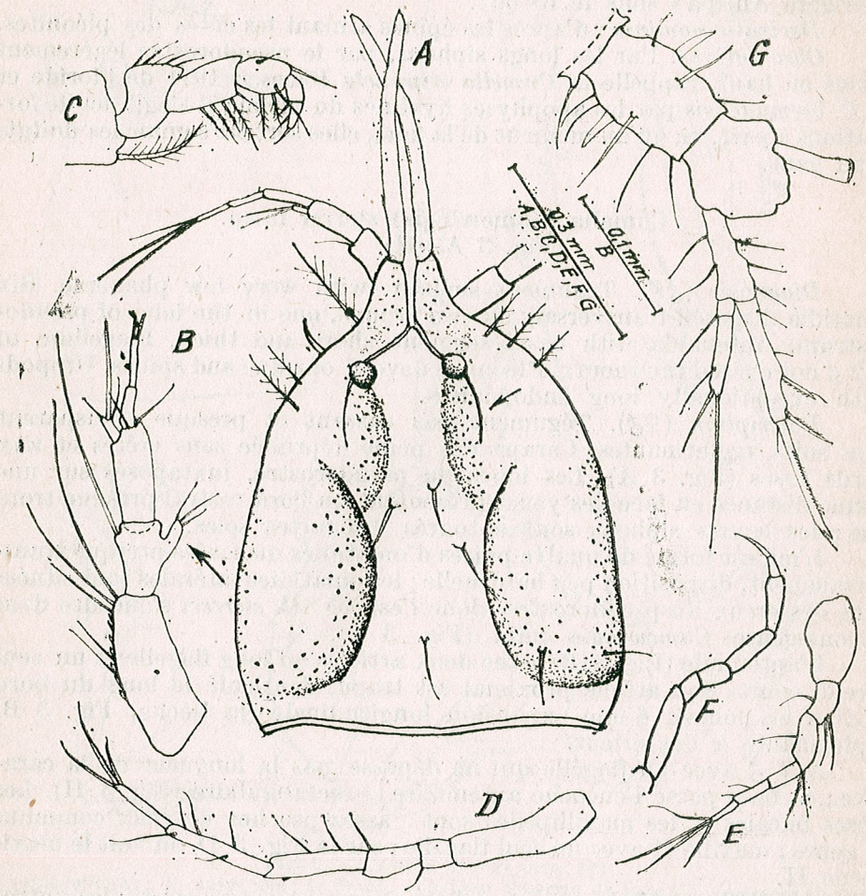

Fig. 2. — *Cumella spinosa* n.sp. (♀)
A, cephalothorax ; B. antennule ; la flèche ; l'apophyse dactyloïde ; C. maxillipède II ; D. péréiopode III ; E, prp. II ; F. prp. IV ; G. les derniers pléonites et l'uropode.

Pléonites plus longs que larges, pourvus de soies simples et avec une apophyse épineuse (Fig. 1 G) latérale qui manque chez les juv. Les endopodites des pattes, légèrement plus longs que les basis, avec deux sous-apicales *serrates setae*, plus celle apicale plus forte, toutes flagellées (Fig. 1 D). Antennule avec une apophyse dactyloïde sur la face interne du 2e article (Fig. 1B, la flèche). Maxillipède III avec une expansion extérieure de l'ischium (Fig. 3 C).

Pour les péréiopodes I et II, vois Fig. 1 D et E ; les autres (Fig. 3 F) avec des courts basipodites et sans exopodites.

Taille ♀ = 1,8 mm.

Matériel : 1 ♀, Walsingham Cave 29.X.1978. Holotype ♀ Muséum « Grigore Antipa » sous le n° 607.

Derivatio nominis : d'après les épines armant les côtes des pléonites.

Observations. Par les longs siphons, par le pseudorostre légèrement dirigé en haut, rappelle la *Cumella tripuncta* Băcescu 1971 de Floride et la *C. bermudensis* par les apophyses hyalines du pléon ; il s'agit ici de formations à part, vu qu'au moment de la mue, elles sortent comme les doitgts d'un gant.

Cumella (Cumewingia) sterrer in.sp.
(Fig. 3 A—H)

Diagnosis (♂♀). Tegument smooth with wery few phanerae. Six omatidia disposed transversaly en two groups, one in the lobe of pseudorostrum. Antennula with basal segment short and thick. Flagellum of A 2 ♂ not exceed the thorax. Pleonites devoid of setae and spines. Uropods with exceptionally long endopodites.

Description (♀♂). Tégument pas cassant et presque transparent sans soies agglutinantes. Carapace à peine déprimée sans crêtes et aux bords lisses (Fig. 3 A). Les lobes du pseudorostre, juxtaposés sur une bonne distance en face des yeux, présentent un bord rostral presque tronqué ; les courts siphons sont entourés des fortes soies.

L'œil est formé de quatre paires d'omatidies disposées presque transversalement, disposition peu habituelle ; les omatidies latérales sont situées dans des creux du pseudorostre, donc l'espèce *C. sterreri* s'encadre dans le sous-genre *Cumewingia* mihi (Fig. 3 A).

L'antennule (Fig. 3 B) avec deux articles au long flagelle et un seul à celui court. Son article proximal est trapu, denticulé le long du bord médian et pourvu d'une expansion longitudinale (la flèche, Fig. 3 B) agglutinante à l'extérieur.

A 2 ♂ avec un flagelle qui ne dépasse pas la longueur de la carapace ; sa base passe l'encoche antennaire ±rectangulaire (Fig. 3 H). Les pièces bucales et les maxillipèdes sont assez proches de ceux communs au genre ; maxille II avec un seul flagelle ; sur la Fig. 3 D on voit le maxilipède II.

Le péréiopode I (Fig. 3 F) grelle, avec un basis très court ; les autres pattes (Fig. 3 E, G) ont des coxes énormes. La base de l'uropode est légèrement plus longue que le dernier pléonite, mais plus courte que l'endopodite (Rp. 27 : 38) surtout ou ♀. La rame externe, fine, présente une longue soie simple apicale qui arrive au niveau de la soie apicale flagellée de l'endopodite. Taille : 1,4—1,7 mm.

Matériel. 2 ♀ et 1 ♂ ad., Cripplegate cave, 22.X.81.

Derivatio nominis: espèce dédiée au prof. W. Sterrer, directeur de la Station de Bermude. Hollotype ♂ déposé, Musée « Gr. Antipa » — Bucarest, n° 415. Paratype ♀, *ibid.* 415 b.

Observations. Les trois espèces nouvelles décrites maintenant confirment l'hypothèse (1) que le secteur caribbean est le centre génétique du genre *Cumella* et surtout de son sous-genre *Cumewingia* Băcescu 1971.

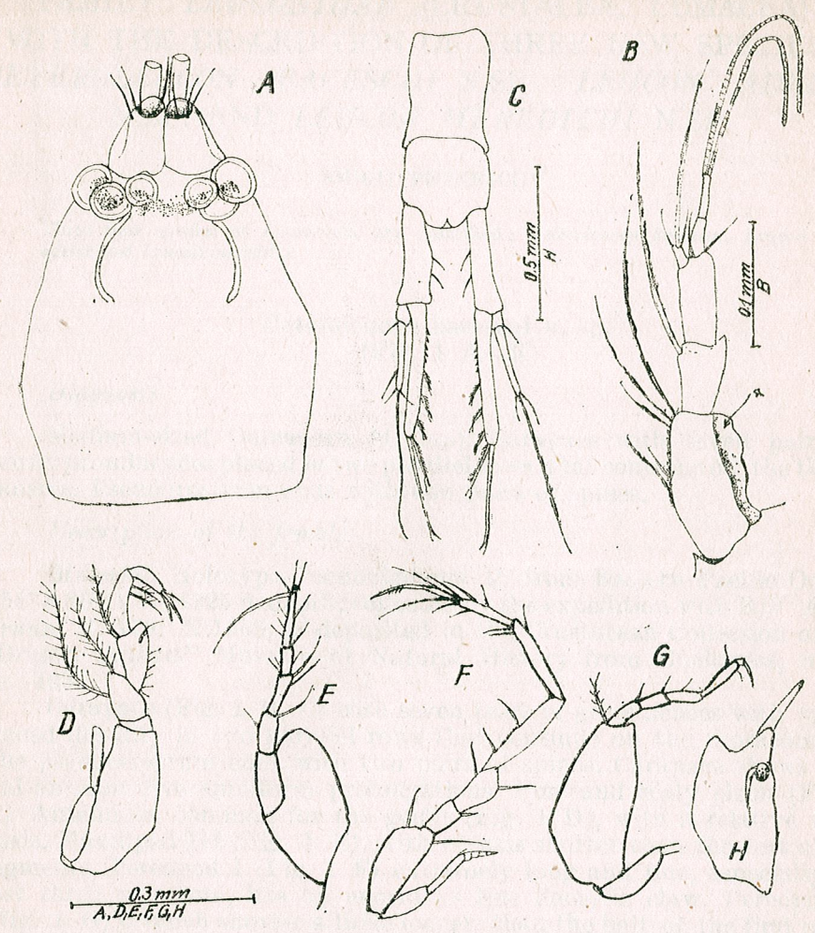

Fig. 3. — *Cumella sterreri* n.sp. (♂)
A, cephalothorax ; B. antennule ; la flèche, apophyse couverte de vase ; C. complexe caudal ; D. maxillipède II ; D. péréiopode II ; F. prp. I ; G, prp. IV ; H. carapace de profil.

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CONTRIBUTIONS TO THE KNOWLEDGE OF THE FAMILY *LEUCONIDAE* (CRUSTACEA, CUMACEA) WITH THE DESCRIPTION OF THREE NEW SPECIES: *HETEROLEUCON BACESCUI* N.SP., *LEUCON ADELAE* N.SP. AND *LEUCON MEREDITHI* N.SP.

IORGU PETRESCU

Three new species of *Leuconidae* are described: *Heteroleucon bacescui*, *Leucon adelae* and *Leucon meredithi*.

Heteroleucon bacescui n. sp.
(Fig. 1 A—K)

Diagnosis

Medium-sized Cumacean (4 mm). Carapace with seven pairs of thorny prominences placed in two parallel rows that continue on the thoracomeres. Pseudorostrum ends with two pairs of spines.

Description of the female

Material. Holotype- nonovigerous ♀, from Eastern Pacific Ocean, 8°54′ S 80°41'W, 4925 m depth, collected by the expedition with R/V *Anton Bruun*, st. 179, X.1965, is deposited in the Crustacean Collection of the "Grigore Antipa" Museum of Natural History from Bucharest, under no. 465.

Carapace (Fig. 1 B, C) with seven pairs of prominences with spines placed dorsally in two parallel rows that continue on the thoracomeres. The pseudorostrum ends with two pairs of spines. Carapace shows ventral-anterior five spiniform prolongations. Fine and scaly pleon (Fig. 1 A). Antennule, common for the genus (Fig. 1 D), with a relative short basis. Maxiliped III (Fig. 1 E), with a basis shorter than the rest of the segments. Pereopod I (Fig. 1 F) extremely long and fine, especially the last three segments, has an enormous fine dactylic claw. Pereopod II (Fig. 1 G) is much shorter a little longer than the half of the first pereopod. The other pereopods, like in Fig. 1 H—J. Uropods relative long and fine (Fig. 1 K). The peduncle is a little longer than the pleotelson. The uropodal rami equal, with few hairs. The terminal spines are broken.

Derivatio nominis. The species is dedicated to the honour of Academician Mihai Băcescu, my master.

REMARKS

Heteroleucon bacescui differs from the other species of the genus especially by the aspect of the carapace (1,2). It resembles with *H. heardi* Băcescu, 1979 (1), only by the generally shape of the pereopods and by the one-segmented endopodite, but differs by the antennule, maxiliped III and by the shorter and less hairy uropods.

REV. ROUM. BIOL.—BIOL. ANIM., TOME 36, Nos 1—2, P. 15—20 BUCAREST, 1991

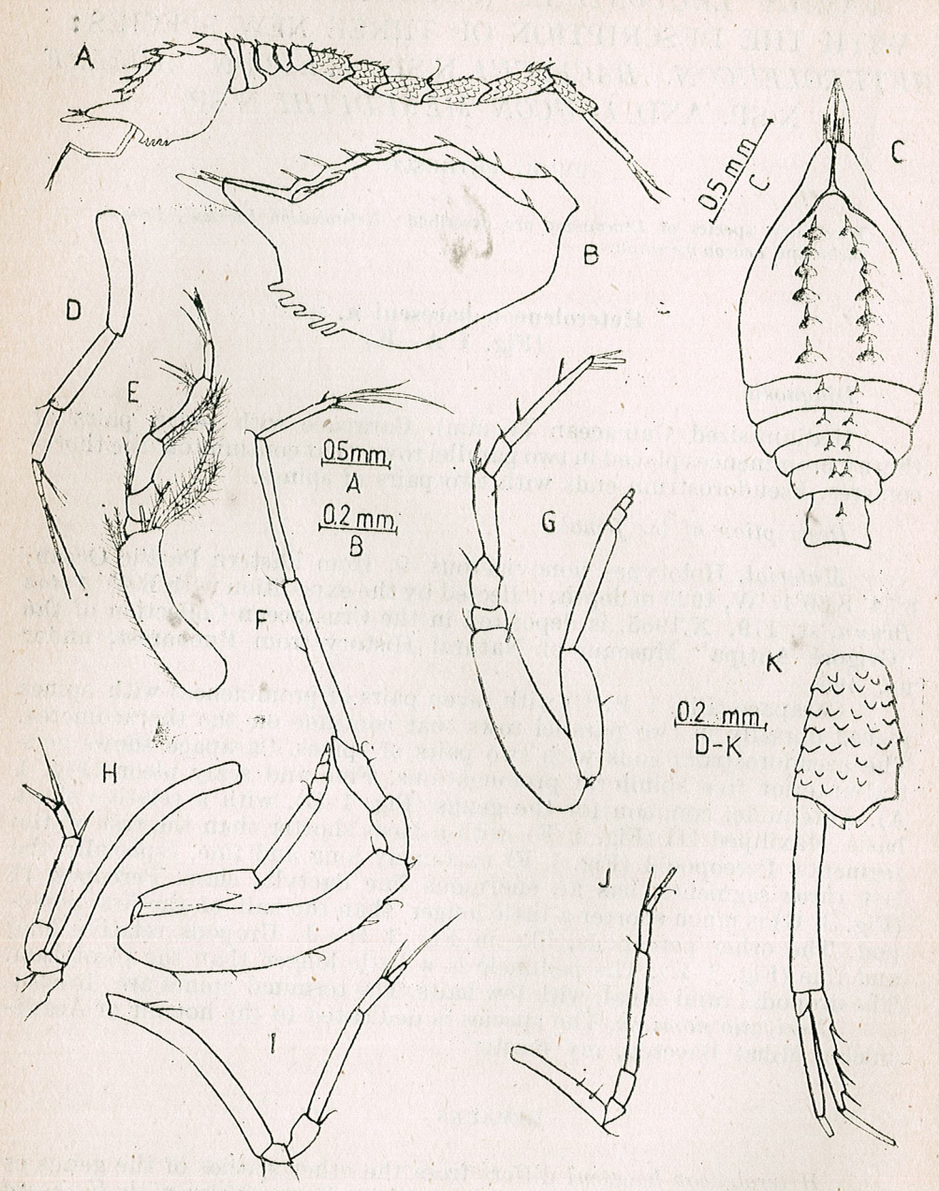

Fig. 1— *Heteroleucon bacescui* n. sp. ♀: A. body, lateral view; B, carapace, lateral view; C, carapace, dorsal view; D, antennule; E, maxilliped III; F, pereopod I; G, prp. II; H, prp. III; I, prp. IV; J, prp. V; K, left uropod.

Leucon adelae n. sp.
(Fig. 2 A—L)

Diagnosis

Carapace with a ridge ended with a denticle in the anterior third. The pleon is vigorously and scaly like the pereopods, too. Scaly and short uropods ended with long setae.

Description of the female

Material. Holotype-nonovigerous ♀, 4.58 mm, from the Southern Atlantic Ocean, South Georgia Islands, 54°18′S 39°23′W, 237 m depth, collected by R/V *Vema*, st. 21, at 5.III.1958, is deposited in the Crustacean Collection of the "Grigore Antipa" Museum of Natural History from Bucharest, under no. 466 : paratype-dissected ♀, from the same station and in the same collection under no. 467.

Carapace has a ridge ended with a denticle in the anterior third, like in juvenile *L. nasicoides* (4). The pseudorostrum and the anterior ventral margin of the carapace with few denticles (Fig. 2 B, C).

Robust and scaly pleon (Fig. 2 A). Antennule, common for the genus (Fig. 2 D). Scaly, vigorous maxiliped II with a flattened propodus (Fig. 2 E). Scaly maxiliped III (Fig. 2 F), common for the genus. Pereopod I (Fig. 2 G), shorter than the second, scaled, its basis is almost equal with the rest of the leg. Scaly pereopod II (Fig. 2 H) has the dactylus ended with two long pennate setae, like in *L. affinis* F age, 1951 (3). Pereopods III—V with scales (Fig. 2 I—K). The uropodal peduncle (Fig. 2 L) is short and equal in length with pleotelson that has two terminal setae like in *L. americanus* Zimmer, 1943 (8). The uropodal rami longer than the peduncle. Exopodite is a little longer and larger than endopodite. It shows three long pennate setae on the internal margin and three terminal ones, longer than those of the endopodite. Endopodite with spines on the internal side.

Derivatio nominis. The species is dedicated to my mother, Adela Petrescu.

REMARKS

It distinguishes from the other species of the genus by the scaly tegument of the pleon and of the pereopods. Uropods like in *L. sagitta* Zimmer, 1907 (7) and *L. americanus* with short peduncle, but differ by the ratio between the uropodal rami and its chaetotaxy.

Leucon meredithi n. sp.
(Fig. 3A—J)

Diagnosis

Carapace with a ridge formed by 9 denticles in its anterior part, ending on the tip of the optical lobe. Uropodal peduncle, short, unequal uropodal rami.

Description of the female

Material. Holotype-nonovigerous ♀, 3.15 mm, from the SE Atlantic Ocean, Argentinean coast, 54°23′S 65°35′W, 75 m depth, collected by R/V *Vema*, st. 14, at 19II.1958, is deposited in the Crustacean Collection of the "Grigore Antipa" Museum of Natural History, under no. 473.

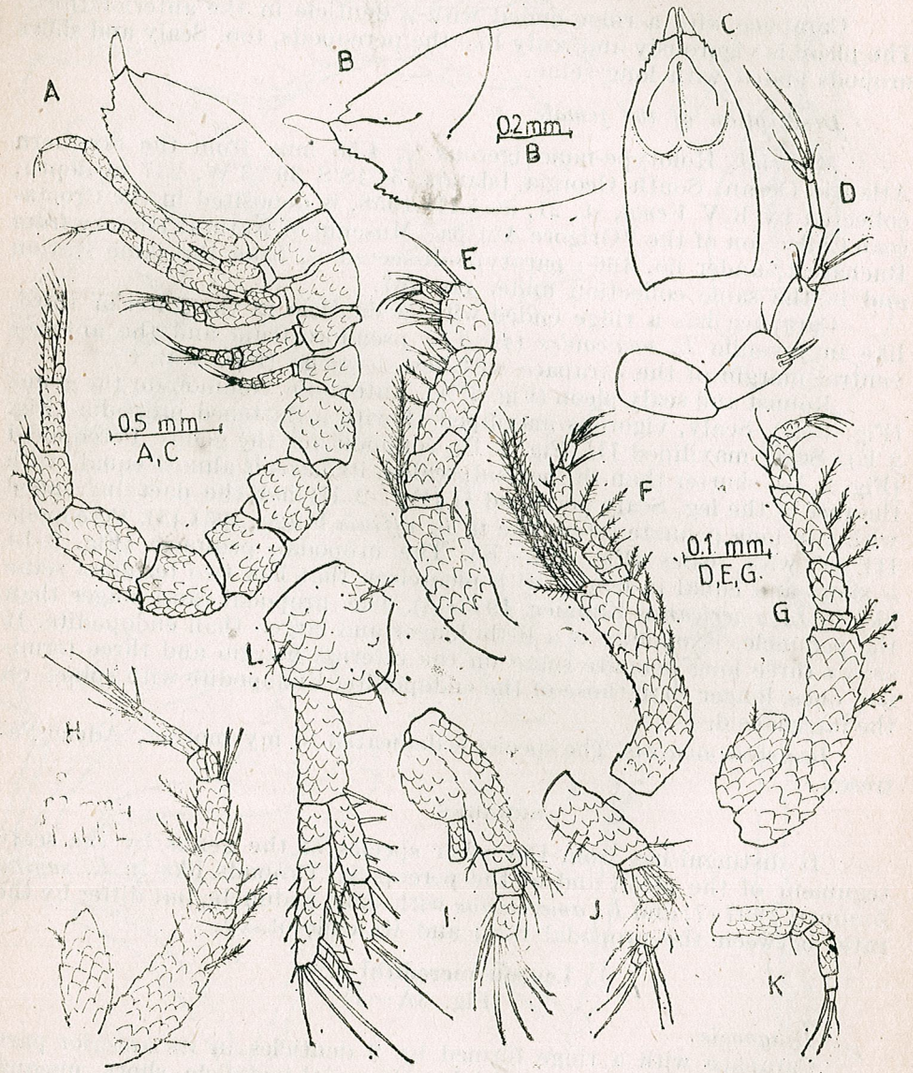

Fig. 2. —*Leucon adelae* n. sp. ♀: A, body, lateral view; B, carapace, lateral view; C, carapace dorsal view; D, antennule; E, maxilliped II; F, maxilliped III; G, pereopod I; H, prp. II; I, prp. III; J, prp. IV;K, prp. V; L, left uropod.

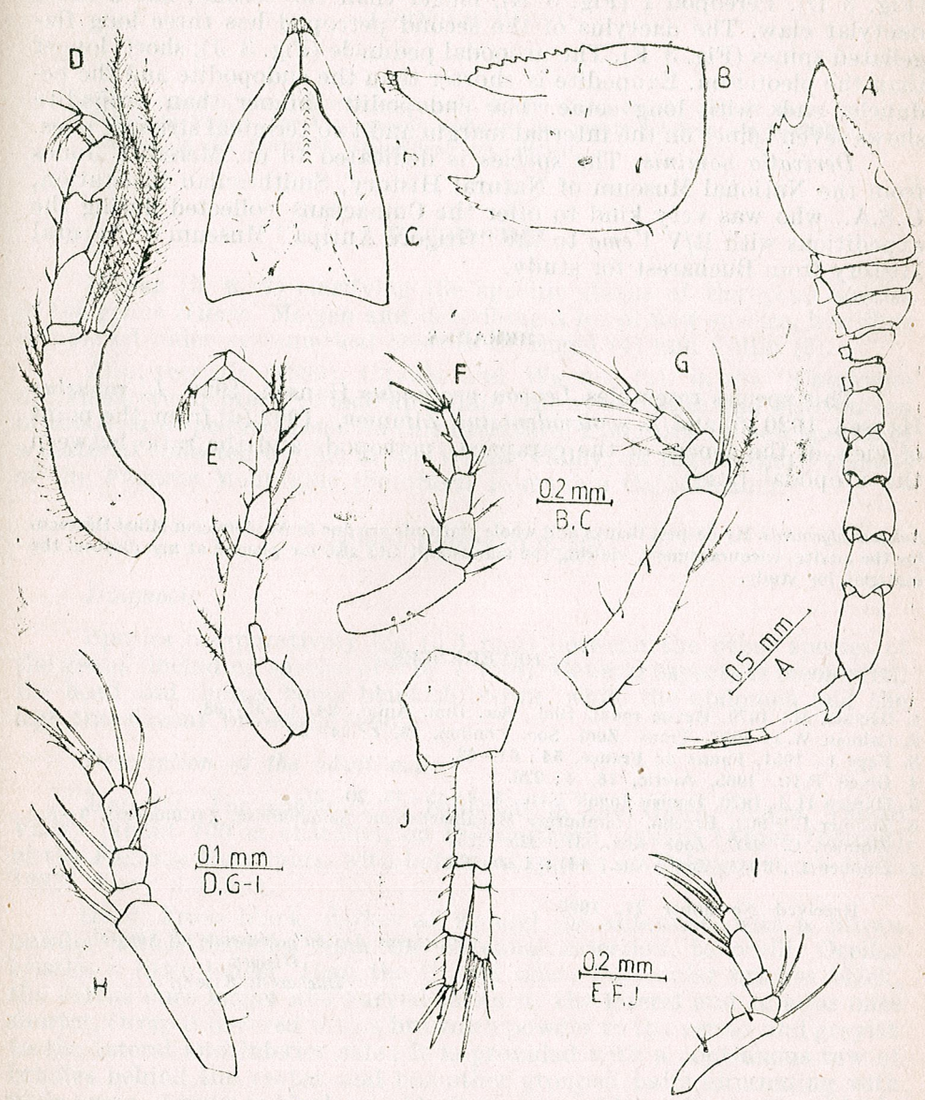

Fig. 3.—*Leucon meredithi* n. sp. ♀: A, body, lateral view; B, carapace, lateral view; C, carapace, dorsal view; D, maxilliped III; E, pereopod I; F, prp. II; G, prp. III; H, prp. IV; I, prp. V; J. right uropod.

Carapace has a ridge with 9 denticles in its anterior part (Fig. 3B, C). Its ventral margin has 6 denticles. Maxiliped III, common for the genus (Fig. 3 D). Pereopod I (Fig. 3 E), longer than the second, has a short dactylar claw. The dactylus of the second pereopod has three long flagellated spines (Fig. 3 F). The uropodal peduncle (Fig. 3 J), short, longer than the pleotelson. Exopodite is shorter than the endopodite and the peduncle, ends with long setae. The endopodite thinner than exopodite shows seven spines on the internal margin and two terminal stronger ones.

Derivatio nominis. The species is dedicated to dr. Meredith Jones from the National Museum of Natural History, Smithsonian Institution, U.S.A., who was very kind to offer the Cumaceans collected during the expeditions with R/V *Vema* to the "Grigore Antipa" Museum of Natural History from Bucharest for study.

REMARKS

This species resembles *Leucon profundus* Hansen, 1920, *L. robustus* Hansen, 1920 (5) and *L. septemdentatus* Zimmer, 1902 (6) from the point of view of the aspect of the carapace, pereopods and the ratio between the uropodal rami.

Acknowledgements. My deepest thanks and whole gratitude are due to Academician Mihai Băcescu, for the advice, encouragement, viewing the manuscript and also for placing at my disposal the material for study.

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"Grigore Antipa" Museum of Natural History
Bucharest, Kiseleff 1

HILARA REGNEALAI (DIPTERA : EMPIDIDAE), A NEW SPECIES FROM THE SOUTH-EAST EUROPE (ROMANIAN CARPATHIAN MOUNTAINS)

CORNELIU PÂRVU

Hilara regnelai, une nouvelle espèce de diptère (Empididés), trouvée dans la Vallée du Vâlsan, sur les versants sudiques des monts Făgăraș (de la chaîne méridionale des Carpates Roumaines) est décrite.

INTRODUCTION

Straka (5, 6, 7) clarifying the specific status of European species of the genus *Hilara* Meigen and describing a lot of new species, has thus completed older systematical treaties of Engel (4) and Collin (3).

Also, recently (1989), Chvála and Wagner (2), in the "Catalogue of Palaearctic Diptera", made up todate the systematics and distribution of the *Hilara* species. Based on this literature, I identified a new species in a material collected by me, in the Vâlsan Valley, on the southern versant of the Făgăraș Mountains (Southern Romanian Carpathians).

Hilara regnealai n. sp.

Diagnosis

Species comparatively big (5.5 mm) between the other species of the genus (including species of 2 to 7 mm). General aspects is bicoloured, the head and thorax being blackish-brown, while the abdomen and the legs translucent brownish-yellow.

Description of the adult male

Material. The specimen is collected in the Galeș village, Vâlsan Valley (about 700 m altitude), on the southern versant (Argeș District) of the Făgăraș Mountains, with botanical species of *Polygonum*, at 19.V. 1985. *Size*: 5.5 mm.

Head. *Frons* black, darker all around the antennae, with a brown powder which becomes from the antennal insertion, to ocelli. Ocellar bristles a little longer, than the frontal ones. Postocular bristles black, the vertex ones longer and curved forward, the lateral and inferior ones shorter. Occiput covered with a brownish powder to the vertex and greyish to the lateral and inferior side; it is provided with a continuous row of bristles behind the vertex and has other grouped hairs alternating with nude areas. *Antennae* black : a_1 black, a_2 brownish to the apex, a_3 black. *Palpi* : terminal article yellowish grey in the basal 2/3, with darker apex; terminal article has a very long black hair and others shorter brownish. *Proboscis* shorter than the length of the head.

Thorax, olive, with yellowish-brown powder, like pleura. Pronotum with 2 black bristles on lateral sides and with a row of shorter, translucent hairs between them. Humeral callus is provided with short pale hairs on all its area and in his posterior angle a black bristle.

The thoracal chaetotaxy : 1 intra-humeral bristle with some slight hairs in front of it, 1 post-humeral preceded by 3 short hairs, 6 little notopleurals, 3 pre-alars from among the middle one is the strongest, 1 supra-alar preceded by some short hairs, 1 post-alar ; the acrostichals are middle sized, biserial, dorsocentrals uniserial ending posteriorly in some long bristles, the last longer even than the 4 scutellar bristles. Halteres, squamae and squamal cilia, yellowish brown. *Wings* clear, with a fine yellowish brown tempt ; veins brown with an indistinct brownish stigma. Costal bristle not very strong, costal ciliation rather long, black-brownish. Posterior margin of the wing with long brown fringes, shorter and paler to the wing apex. **Legs** generally yellowish, except for the anterior side of coxa, slightly brown, trochanters with a little black spot, the distal tibial third and the tarsi are slightly brownish (the brown colour is more intense at leg I). *Leg. I.* Coxae yellowish with a brown tempt on the anterior side, where there is a short, fine and black pilosity and 4—5 black, longer and thicker preapical bristles.

Trochanters yellow with a black point in the apico-posterior zone, femora yellowish, tibia more yellowish at base, become darker-brown on the distal third. According to my drawing (Fig. 1, A), anterior side of tibia have 5 bristles and one preapical. Tarsi : basal article is approximately equal to the sum of the four following ones, dilated, inner side right, ornate with two types of hairs : some of them thick and short, others rarely, thinly and double longer ; the anterior side of this article is curved and from among short and dense hairs a series of 8 long curved bristles appear ; the second article is shorter than the third, the fourth is the shortest of all, and the fifth is approximately equal to the second. *Leg II.* Coxa brownish-yellow, with some black hairs on the anterior side (stronger than that of the fore coxae) ; trochanters with black apex, femora completely yellow, with 1—2 antero — dorsals on the basal part and 1 pre-apical bristle ; tibia, yellowish, becomes brownish to the apical third, tarsal articles brownish. *Leg III.* Coxa brownish-yellow, with a tuft of black hairs on the posterior side, trochanters yellowish with a black spot on the antero-apical zone, femora and tibia yellowish, becoming slightly brownish to the apex, provided with : 1 postero-dorsal bristles in the basal part, 4 antero-dorsal ones, 3 postero-dorsal ones, tarsi darker (to brown) than tibial apex. **Abdomen** yellowish brown, a little darker than legs ; anterior and posterior margins of the tergits and sternits are brown on a narrow stripe. *Hypopygium.* Left periandrial lamella with a bifid apical prolongation like two curved hooks a little overlapped at the tip — a unique aspect so far to the species of *Hilara* genus. Sternite VIII (Fig. 1, B) has also an unusual shape. I figured also the genital capsule in lateral view (Fig. 2, B), a detail of hipandrial apex (Fig. 2, A) of left paramera (Fig. 2, C), cerci (Fig. 2, E) and aedeagus with its apodema (Fig. 2, D).

Differential diagnosis. The new species resembles by the aspect of the leg I to a series of other species of this genus, e.g. : *Hilara matrona* Haliday, *H. matronella* Straka, *H. curtisi* Collin, *H. algeciracensis* Strobl. The last species is smaller (3.7—4 mm) than the new one and has 3 darker brownish stripes on mesonotum and blackish legs, aspects which netely

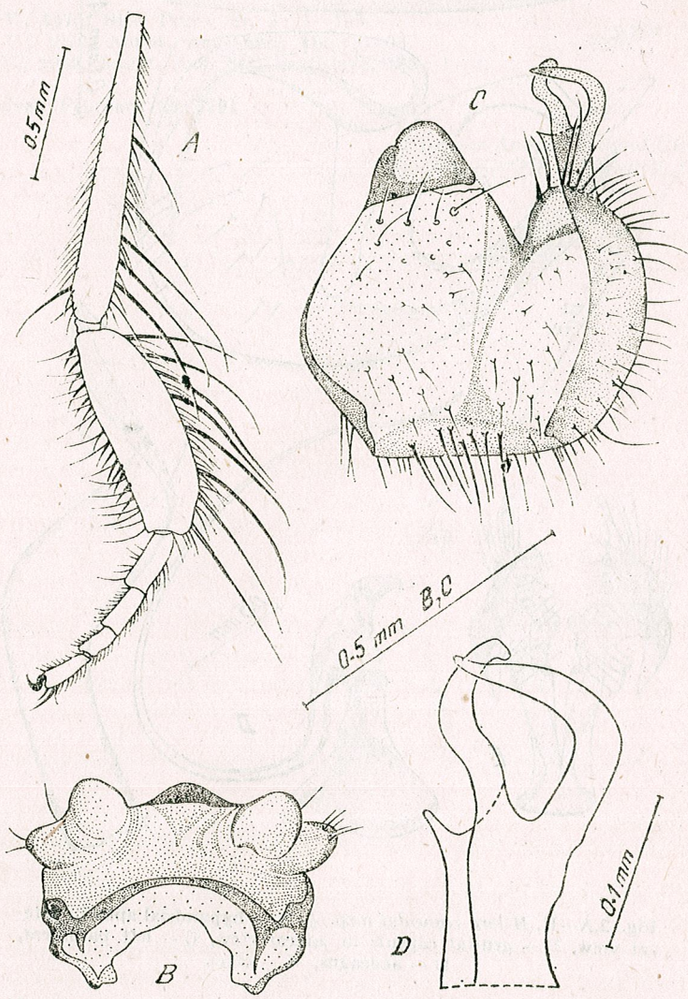

Fig. 1, A—D, *Hilara regnealai* n.sp., ♂, A — leg I, B — sternite VIII, C — left periandrial lamella, D — detail of the hooks of the periandrial lamella.

separate this species of n.sp. *H. regnealai* differ from all mentioned species and from all the species of the genus *Hilara* for which exist detailed drawings of the males genitalia, in the form of the sternite VIII and in the perian-

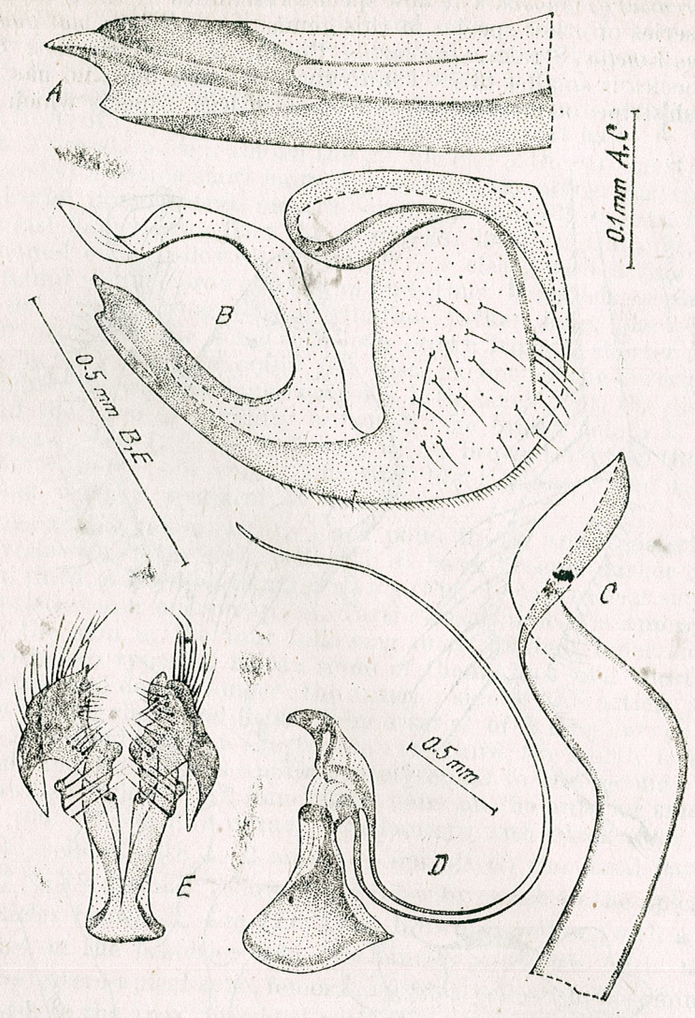

Fig. 2,A—E, *Hilara regnealai* n.sp. ♂, A — hypandrial apex in lateral view, B — genital capsule in lateral view, C — left paramera, D — aedeagus, E — cerci.

drial lamella (Fig. 1, C). The new species is nearest to the *H. matrona* Haliday.

Derivatio nominis. The species is dedicated to Dr. Mircea Regneală, the director of the Central University Library (Bucharest).

Acknowledgements. The author's thanks are addressed to Mrs. Corina Pavlov, librarian of the "Grigore Antipa" Museum and also to Mrs. Marinela Năzăreanu who redrew the figures in China ink.

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PRISTICEPHALUS CARNUNTANUS (PHYLLOPODA, ANOSTRACA), ESPÈCE NOUVELLE POUR LA FAUNE DE ROUMANIE

AURELIU STOICESCU

The fairy-shrimp *Pristicephalus carnuntanus* (Brauer, 1877), until now known from Hungary, Austria, southern Slovakia and north-western Yugoslavia, is recorded for the first time in Romania (two localities from the lowlands of western Banat). A detailed description and illustration of Romanian specimens supplements the description of this species in the classical monograph of Daday (3).

L'espèce *Pristicephalus carnuntanus* a été décrite en 1877 de Parndorf (Autriche), par Brauer, sous le nom de *Branchipus* (*Chirocephalus*) *carnuntanus* : Daday (3) la signale ensuite à Kecskemet en Hongrie sous le nom de *Pristicephalus carnuntanus* ; on l'a retrouvée depuis dans plusieurs localités en Tchécoslovaquie, Autriche, Hongrie et Yougoslavie.

Brtek (2) considère le genre *Pristicephalus* un synonyme de *Chirocephalus*. Etant donné qu'on ne connaît pas encore tous les stades larvaires de ces Anostracés, nous considérons ces deux genres distincts.

Botnariuc et Orghidan (1) mentionnent la possibilité de la présence du genre *Pristicephalus* en Roumanie, ce que nous confirmons dans le présent travail.

Matériaux : 2 mâles et 5 femelles prélevés du marais Überland, qui est situé au Nord-Est de la ville de Timişoara, le 10 avril 1985 ; 22 mâles et 33 femelles dans la localité Sînnicolaul-Mare, dans un marais situé près du remblai du chemin de fer vers Tomnatic, le 18 avril 1987. Les matériaux ont été récoltés par Dr P. Bănărescu[1].

Le mâle. La longueur entière du corps du front jusqu'à l'extrémité des cercopodes varie entre 13,4—17,5 mm.

La tête a le front arrondi (fig. 1). L'antenne I, avec la longueur entre 1,5—2,5 mm, n'est pas plus longue que l'article basal de l'antenne II. L'antenne II est biarticulée. L'article basal de l'antenne II a le bord externe arqué et le bord interne concave ; sa longueur peut être de 3,5mm pour les exemplaires les plus grands. Sur la partie dorsale de l'article basal de l'antenne II, près de la base, il y a un appendice antennal dorsal (serriforme) et, sur sa partie ventrale près du milieu, se trouve un prolongement digitiforme qui s'oriente en haut ayant le bout arrondi et rugueux (fig. 2).

L'appendice antennal dorsal (serriforme) tordu en spirale est musculeux avec de petits prolongements coniques sur le bord (fig. 3). L'appendice antennal dorsal (serriforme) est plus court que l'article basal de l'antenne II. L'article apical de l'antenne II est beaucoup plus long que l'article basal. L'article apical est courbé vers l'intérieur ayant à la base une apophyse telle une massue aux épines et orientéé vers l'intérieur (fig. 4). La moitié distale de l'article apical est plus amincie, alors que le bord intérieur est prévu d'un monticule telle une carène (fig. 5).

[1] Nous remercions le Dr P. Bănarescup our avoir mis à notre disposition ces materiaux

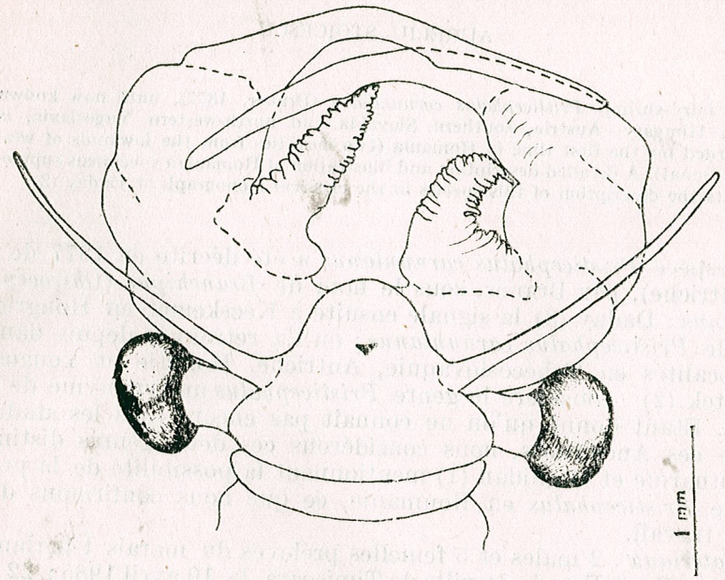

Fig. 1.—La tête (mâle).

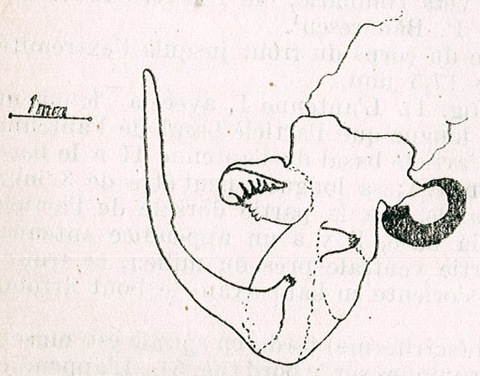

Fig. 2. — L'antenne II avec l'appendice antennal dorsal (serriforme) et le prolongement digitiforme (mâle).

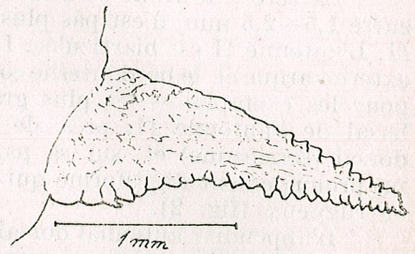

Fig. 3. — L'appendice antenal dorsal (serriforme) (mâle).

Le thorax avec les segments lisses a lameeuxl longueur entre 5,6—8mm. Toutes les pattes ont une structure approximativement semblable (fig.6a). Les paires de pattes I—X ont chacune deux lames branchiales (préépipodite) aux bords crenelés et dantelés; le sac branchial (épipodite) étroit aux bords lisses (fig. 6b). L'exopodite, dont le bout distal est arrondi, ne dépasse pas la moitié de la longueur des autres parties de la patte. L'endopodite de la première paire de pattes est conique (fig. 7). Sur le

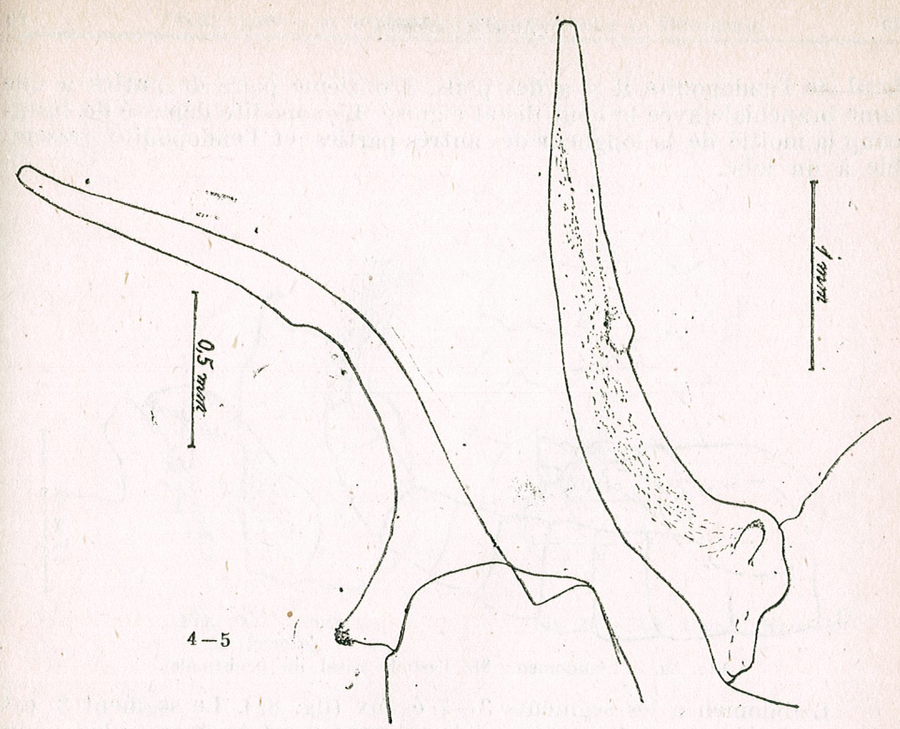

Fig. 4. — L'article apical de l'antenne II vu latéralement (mâle).
Fig. 5. — L'article apical de l'antenne II vu sur le bord intérieur (mâle).

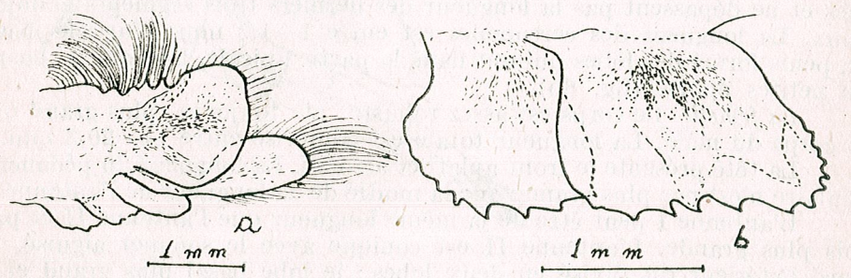

Fig. 6a. — La septième paire de pattes; 6b. les lames branchiales (préépipodite) (mâle).

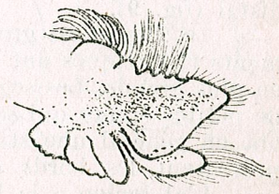

Fig. 7. — La première paire de pattes (mâle).

bord de l'endopodite il y a des poils. L'onzième paire de pattes a une lame branchiale avec le bout distal aiguisé. L'exopodite dépassé de beaucoup la moitié de la longueur des autres parties et l'endopodite ressemble à un lobe.

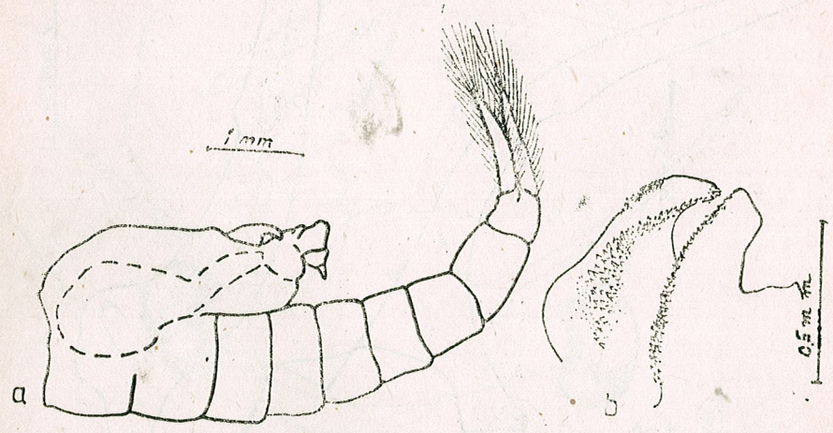

Fig. 8a. — L'abdomen ; 8b. l'article basal du penis(mâle).

L'abdomen a les segments 3—7 égaux (fig. 8a). Le segment 8 est un peu plus long que les autres et le segment 9 est toujours plus court Le bord postérieur et latéral des segments de l'abdomen est sans épines. La longueur de l'abdomen est entre 5—7 mm. Les cercopodes sont ensiformes et ne dépassent pas la longueur des derniers trois segments abdominaux. La longueur des cercopodes est entre 1—1,5 mm. L'article basal du penis formé des lobes qui ont dans la partie apicale, latérale et basale de petites épines (fig. 8b).

La femelle. Le corps est assez robuste et toujours plus grand que le corps du mâle. La longueur totale est comprise entre 14—20,3 mm.

La têtê présente le front aplati et arrondi. La longueur du pédoncule oculaire n'est pas plus grande que la moitié de la longueur de l'antenne I.

L'antenne I peut être de la même longueur que l'antenne II et parfois plus grande. L'antenne II est conique avec le sommet aiguisé. Le bord extérieur est divisé en deux lobes : le lobe basal plus grand et le lobe apical plus petit. Le bord intérieur dans le tiers distal présente une incision qui délimite deux lobes : le lobe proximal est plus grand que celui distal (fig. 9).

Le thorax est gros et il a la longueur entre 6,3—9,2 mm. Les segments thoraciques ont sur les parties des prolongements épineux de plus en plus grands. Les segments thoraciques 5—11 ont sur la partie dorsale de petites épines qui sont ordonnées sur deux lignes (fig. 10). Les pattes ont en général une structure pareille, avec deux lames branchiales qui présentent les bords crénelés.

L'abdomen a la longueur entre 5,2—8,4 mm. Le premier segment génital a sur les parties latérales une épine et sur la partie dorsale deux petites épines (fig. 11a). Le deuxième segment génital a sur les parties

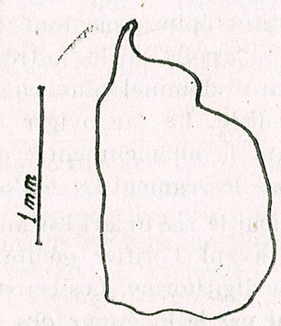

Fig. 9. — L'antenne H (femelle).

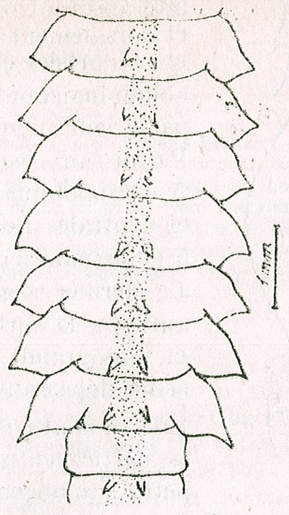

Fig. 10. — Le thorax (femelle).

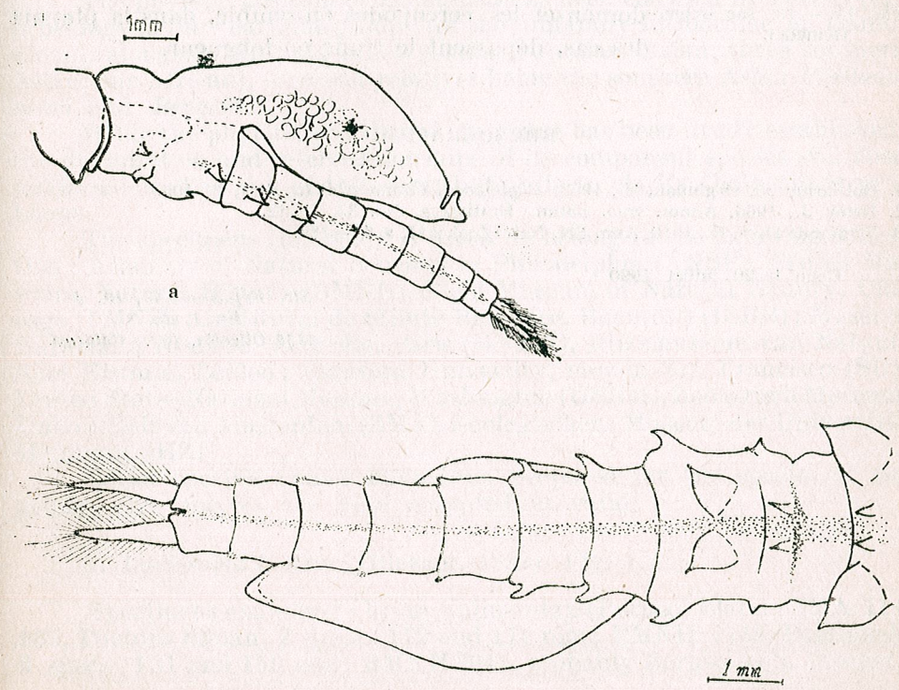

Fig. 11a. — L'abdomen vue latérale ; 11b. L'abdomen vu d'en haut (femelle).

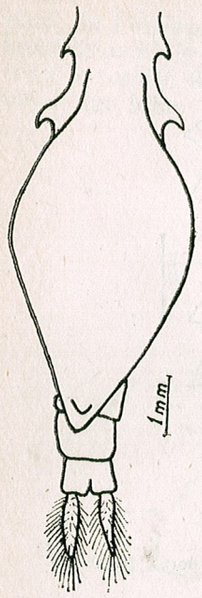

Fig. 12. — Le sac oviger (femelle).

latérales tout près du bord postérieur une forte épine et dorsalement deux grandes épines orienteés en haut et recourbées en arrière (fig. 11b). Les segments 3—4 abdominaux ont sur les parties latérales prés du bord pastérieur une forte épine recourbée en arriere. Parfois aux exemplaires les plus grands les segments 5—8 abdominaux présentent sur les parties latérales et ventrales de petites épines sur tout le segment 5 et des monticules aux poils sur les autres segments. Le dernier segment abdominal plus court que les autres a la surface lisse. Le sac oviger est allongé et plus grand, ayant la longueur entre 4,2—6,4mm et ne dépassant pas le segment 8 abdominal (fig. 12). Au bout postérieur le sac oviger est aminci et sur la partie ventrale, avant l'orifice génital, il y a un petit prolongement digitiforme. Les cercopodes ensiformes ne dépassent pas la longueur des deux derniers segments abdominaux. Sur le bord des cercopodes il y a des poils disposés uniformément. L'abdomen et les cercopodes en-semble, dans la plupart des cas, dépassent le tronc en longueur.

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A REVIEW OF THE SPECIES OF *LUCIOSOMA* (*PISCES, CYPRINIDAE*)

PETRU M. BĂNĂRESCU

The five species of Luciosoma are reviewed. The differences between *L. bleekeri* and *L. setigerum* are pointed; it is suggested that the presumed *L. setigerum* recorded in the basins of Chao Phraya and Mae Klong rivers in the Thailand actually are *L. bleekeri*. The population of *L. setigerum* from the Baram River basin, Kalimantan island, may represent a distinct subspecies. A lectotype is designed for *L. pellegrinii*. A cladogram of the species is suggested and the significance of their distribution for the general zoogeography of the south-south-eastern Asian aquatic fauna is mentioned.

Luciosoma Bleeker, 1855 (type species : *Barbus setigerus* Valenciennes) is a southern Asian genus of cyprinid fishes present in the eastern half of the Indo-Chinese subcontinent (Mekong and Menam Chao Phraya basins and some smaller rivers), the Malay Peninsula and the three large western Indonesian island; it is absent from East Asia proper (that also includes the north of Vietnam), from the Philippines and from the western half of Indo-China (Salwin, Irrawaddy and Sittang rivers). This distribution is similar with that of numerous other genera of primary freshwater fishes and invertebrates.

The phyletic position of *Luciosoma* has been clarified by Howes (5) : it belongs to the "bariliine group" (of the subfamily Rasborinae or Danioninae), alongside with six other genera (two East Asian, three southern Asian, one African), its closer relatives being the southern Asian *Parluciosoma* and *Bengala*.

While the phyletic position of the genus has been firmly established, the delimitation and interrelationships of its component species still need clarification. The aim of this contribution is to clarify these minor problems.

The specimens this study is based on belong to the following collection : Academy of Natural Sciences in Philadelphia (ANSP), British Museum, Natural History (BMNH), Field Museum of Natural History, Chicago (FMNH) ; Institutul de Științe Biologice, București (ISBB) ; Muséum National d'Histoire Naturelle, Paris (MNHN), Rijksmuseum van Natuurlijke Historie, Leiden ; Stanford University, now in San Francisco (SU), United States National Museum, Washington (USNM), Zoölogisch Museum, Universiteit van Amsterdam (ZMA), Zoologischens Museum der Universität Hamburg (HZI).

Seven specific names have been proposed for the species of the genus ; five species are here accepted as valid.

1. *Luciosoma trinema* (Bleeker, 1852). Fig. 1.

Specimens examined : From Kalimantan (Borneo) island : ZMA 114. 955, Poetoes Sibian, 2 specs., 172 and 174 mm ; RMNH 7758, Pontianak, 2 specs., 131 and 159 mm ; RMNH 7041, probably Borneo (coll. Bleeker),

one, 172 mm; MNHN 91—405/408, Borneo, 4 specs., 62—123 mm.; SU 31170, Sarawak, two specs.,163.5 mm. From Sumatera: ZMA 1151, Palembang, two specs., 163—164 mm; ZMA 115191, Djambi, two specs., 84 and 113 mm. From the Malay Peninsula: BMNH 1931,7.20: 29, Tasek Bera, Pahang, two specs., 100 and 172 mm.

Both pairs of barbels rudimentary. Pelvics and anal fins prolonged into a filament. A row of roundish spots extends on body sides from behind the opercle to above the anal fin; a broad blackish stripe on the caudal peduncle, continuing along the upper caudal lobe; another stripe on the lower caudal lobe.

D 3/7; A 2/6; L. lat 43—45; Circum-peduncular scales 16 or 18 ($3^1/2$ or $4^1/2$ above and $2^1/2$ below the lateral line scale on each side); Sp. br. (9) 10—12; 20—21 predorsal scales; D. phar. 5.4.2—2.4.5.

Range: the Malay Peninsula, island Sumatera (eastern slope) and Kalimantan or Borneo (western, southern and south-eastern slopes; absent from the north-east of the island); not recoreded and probably absent from Djawa island; absent from the Indo-Chinese subcontinent (1; 12; 2; 3; 4; 11; 6; 7; 8).

2. *Luciosoma bleekeri* Steindachner, 1879

Synonyms: *Luciosoma harmandi* Sauvage, 1880; *L. setigerum* (not of Valenciennes): Smith, 1945.

Specimens examined: from Mekong River basin: holotype of *L. harmandi*, MNHN A 2398, Mekong River in Laos, 73 mm; from Chao Phraya (or "Menam") River basin: USNM 103293, one spec., 153 mm, determined *L. setigerum*; USNM 103267, one 77 mm; USNM 102891, Bangkok, one, 173 mm, USNM 107882, northern Thailand, two, 111—122 mm; ANSP 87246 three, 109—144 mm; BMNH 1897.10.8: 134—135, Bangkok, two, 104—106 mm; BMNH 1934.12.18: 8, Notaburi, one, determined, *L. setigerum*, SU 28797, Lante, one, 99 mm; FMNH 50785 and ISBB 4120, Kam Peng Pett Province, Thailand, 13 specs. in all, 103—127 mm, from Meklong River basin, a smaller river, sw. from the Chao Phraya USNM 103268, three specs., 102—108 mm.

D 3/7; A 2/6; L. lat. 45—47 (48); circum-peduncular scales 16 ($4^1/2$ above and $2^1/2$ below the lateral line scale on each side); Sp. br. 10—12; predorsal scales 20—21.

Barbels well developed. Neither the anal, nor the pelvics are prolonged into a filament. Either a continuous longitudinal dark stripe on body sides, often prolonged on the central part of the caudal fin (Fig. 2), or a row of closely set roundish spots, often followed by an elongate spot on the caudal fin (Fig. 3).

Range: restricted to the basins of Mekong River in southern Vietnam, Laos, Kampuchea and Thailand, of Chao Phraya ("Menam") and Mae Khlong rivers in Thailand; absent from the Malay Peninsula (inclusively the part that belongs politically to Thailand) and Indonesia. It is the only member of the genus present in the Mekong basin (10, 11, 7, 8) and, almost surely in the two other basins, too. Smith (11) mentions

the presumed occurrence of *L. spilopleura* in the Chao Phraya based on an old (1865) paper of Bleeker; this is surely a misidentification. The species is not listed from this basin in the recent review of the Indochinese inland water fish fauna (8).

The presumed *L. setigerum* from the Chao Phraya listed by Smith (11) are, in my opinion, *L. bleekeri*. Smith seems not to have been aware of the main differences between both species (pelvics prolonged in the former, not prolonged in the latter), mentioning only the number of circumpeduncular scales and differences in colour pattern. All available specimens from the Chao Phraya basin (including USNM 103293 and BMNH 1934.12.18: 8, determined *L. setigerum*, the former probably by Smith himself) actually are *bleekeri*, having 16 circumpeduncular scales and pelvics not prolonged.

3. *Luciosoma setigerum* (Valenciennes, 1842)

Synonyms: *Barbus setigerus* Valenciennes, 1842; *Luciosoma setigerum* auct.; *Luciosoma weberi* Popta, 1905.

Specimens examined: from Kalimantan or Borneo island: syntype of *L. weberi*, RMNH 7624, Boelit, Kapuas River basin, one spec., 176 mm; RMNH 7755, Raoeven, one, 173 mm; RMNH 7040, probably Borneo, Bleeker's collection, one, 151 mm; BMNH 1898.11.14: 4, upper Baram River, one, 152 mm (determined *L. spilopleura*); BMNH 1895.7.2: 73—75, Baram district, two, 107 and 131 mm;

from Sumatera island: ZMA 115193, Sidjoeng, two, 180 and 209 mm; ZMA 114954, Djambi, eight specs., 55—115 mm;

from Djawa island: ZMA 115192, Kediri, one 113, mm;

from the Malay Peninsula: USNM 101203, Perak, one, 104 mm; USNM 101196, eastern Perak, one, 186 mm; SU 31167, Muar River, one, 120 mm.

D 3/7; A 2/(5) 6; L. lat. 43—46; circum-peduncular scales 14 ($3^1/2$ above and $2^1/2$ below the lateral line scale); Sp. br. 10—13; predorsal scales 22—25.

Both pairs of barbels well developed. Pelvic fins prolonged into a filament; anal not prolonged. Colour pattern similar to that of *L. bleekeri*, but the lateral stripe is always continuous (at least in the available specimens) and extends also on the head, to the nostrils. On the contrary, this stripe does not extend on the middle rays of the caudal (as in *L. bleekeri*), while each lobe of the caudal fin has an inframarginal dark band.

This species is closest to *L. bleekeri*, differing from it in having the pelvics prolonged, 14 circum peduncular scales (not 12 as mentioned in the bibliography: 12 and 11) as against 16 in *bleekeri* and a different colour pattern.

The five available specimens from Baram River basin, north-western Kalimantan island, differ from the others in having much longer rostral barbels, reaching to, or almost to the margin of the opercle, while in other populations they never reach beyond the middle of the pre-opercle. The Baram basin population may represent a distinct subspecies.

The range of the species encompasses the three large western Indonesian islands and the Malay Peninsula (both slopes); the so-called *L. setigerum* from the Chao Phraya basin are probably *L. bleekeri*.

4. *Luciosoma spilopleura*

Specimens examined: ZMA 114953, Air Panata, Sumatera, four specs., 176—246 mm; SU 33615, Sarawak, north-western slope of Kalimantan island, one spec., 126 mm.; RMNH 7756, Indonesia (no locality), four 30—56 mm; MNHN 03195, one, 44 mm;

D 3/7; A 2/6; L. lat. 41—43; Circum-peduncular scales 14 (3 1/2 above and 2 1/2 below the lateral line scale) or 12 (3 1/2 above and 1 1/2 below); Sp. br. 9—12; predorsal scales 19—23. D. phar. 5.4.2—2.4.5.

Barbels well developed. Pelvic fins prolonged into a filament in adult specimens, not in juvenile ones; anal not prolonged. A longitudinal row of brownish spots on sides, above the lateral line, which can no more be recognized in preserved specimens (Fig. 7); below them, on the lateral line, 4—8 minute black spots. A brown blackish bar on the dorsal and anal fins; a dark large spot at the base of the caudal fin.

Recorded only from the western and north-western slope of Kalimantan island and on the eastern one of Sumatera (1; 9; 12); absent from the north-eastern slope of Kalimantan (6), Djawa and the Malay Peninsula; erroneously reported from the Chao Phraya basin in Thailand (11), probably on the base of mislabelled or misidentified specimens.

5. *Luciosoma pellegrinii* Popta, 1905

Specimens examined: syntypes, RMNH 7625, Bo River (tributary of Mahakam River, eastern slope of Kalimantan or Borneo island), three specimens, 179—196 mm; the second large one, 183 mm, is here declared lectotype (Fig. 8).

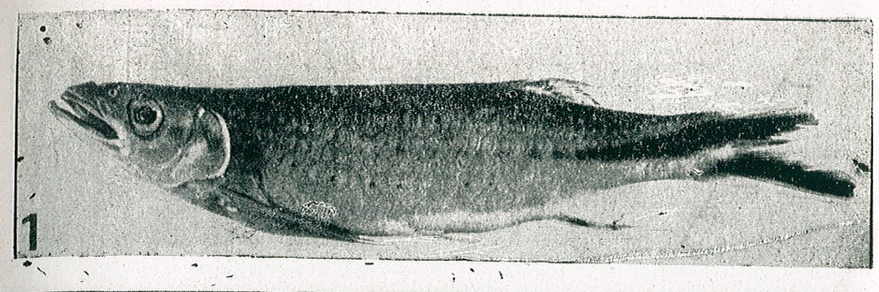
FMNH 68209 and ISBB 2940, Kinabatangan River, north-eastern slope of Kalimantan island (North Borneo; politically in Malaysia), 12 specimens.

D3 /7; A2 /6—8; L. lat. 36—46; Circum-peduncular scales 14(3 1/2 above and 2 1/2 below the lateral line scale on each side); predorsal scales 20—22; D. phar. 5.4.2—2.4.5.

Barbels well developed. Pelvic fins prolonged into a filament also in juveniles; anal not prolonged. Colour pattern similar to that of *L. spilopleura*, but there are no marginal dark bands on the caudal fin lobes.

Weber and de Beaufort (12) consider this species a synonym of *L. spilopleura*; actually it differs from this in the number of branched anal rays (*L. pellegrini* is the only species of the genus that has not constantly six branched rays), the pelvics prolonged in a filament also in juveniles, colour pattern and a few morphometric characters.

The species is endemic to the north-central part of Kalimantan (Borneo) island, having been reported only from two rivers: Bo (a tributary of Mahakam River) on the eastern slope (9) and the Kinabatangan River in North Borneo, Malaysia (6); both rivers belong to the eastern slope of the island.



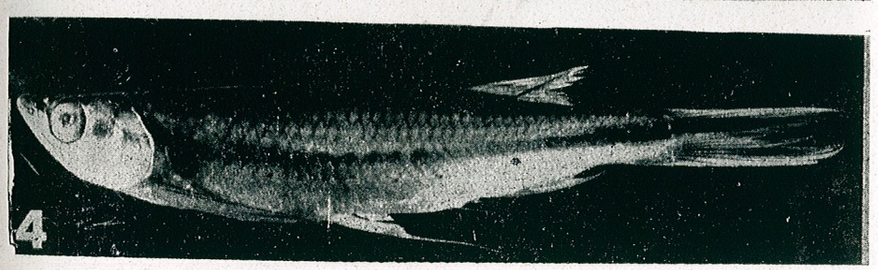


Fig. 1. — *Luciosoma trinema* (Bleeker), ZMA, 114955.
Fig. 2. — *Luciosoma bleekeri* Steindachner, BMNH 1879.10,8 : 134 : Chao Praya River, Bangkok.
Fig. 3. — *Luciosoma bleekeri* Steindachner, USNM 103267; same locality.
Fig. 4. — *Luciosoma setigerum* (Valenciennes) ZMA 114954; Djambi, Sumatera.

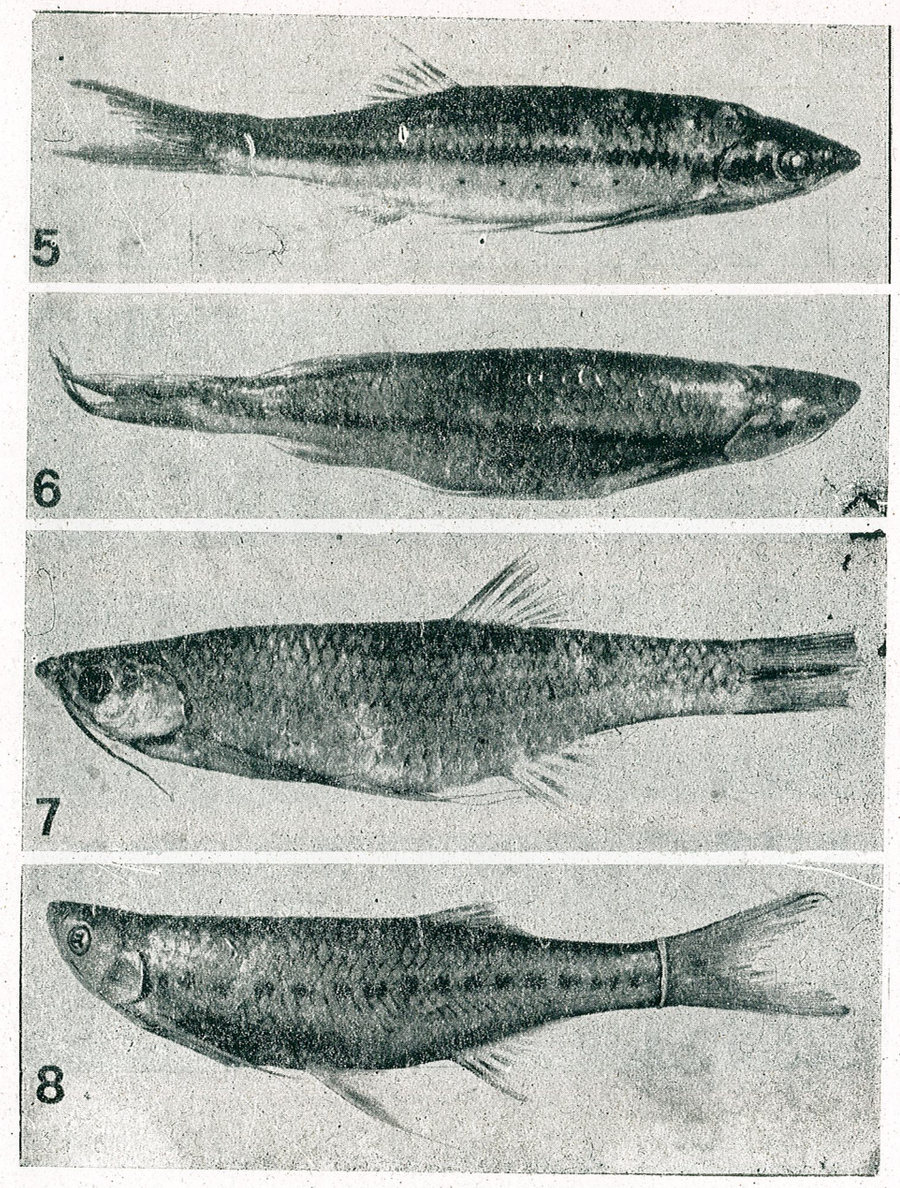


Fig. 5. — *Luciosoma setigerum*. BMNH 1998.11.14 : 4 ; Baram River, northern slope of Borneo (population with long barbels).
Fig. 6. — *Luciosoma setigerum* (Valenciennes), holotype of *L. weberi* Popta RMNH 7624. Boelit ; Kapuas R. basin, Kalimantan.
Fig. 7. — *Luciosoma spilopleura* Bleeker, ZMA 114953 ; Air Panata, Sumatera.
Fig. 8. — *Luciosoma pellegrinii* Popta, lectotype, ZMA 7625 ; Bo River, Kalimantan island,

Interrelationships of the spici es and zoogeography

L. trinema is the most distinct species of the genus, being the sister on the trunk including the five other species. It is the only species with rudimentary barbels (either a primitive or a derived character) and with the anal fin prolonged (a derived character). It can be considered apomorphic comparatively to the other species.

The four other species comprise two pairs of sisters : *bleekeri setigerum* and *spilopleura/pellegrinii*. Both members of each pair have vicariant ranges : *bleekeri* lives in the Indochinese subcontinent, *setigerum* in the Malay Peninsula and Indonesia ; *spilopleura* on the eastern slope of Sumatera and on the western one of Kalimantan, *pellegrinii* on the eastern slope of the latter island.

The relationships of the five species can be expressed as follows :

bleekeri
setigerum
spilopleura
pellegrinii
trinema

It is worth mentioning that Bleeker (1) had already realized the special position of *trinema*, assigning it to a distinct subgenus *Trinematichthys*.

A single species lives in the subcontinent : (Mekong, Chao Phraya and Meklong rivers basins) : *L. bleekeri*, endemic. Two species are present in the Malay Peninsula and the Indonesian islands : *L. trinema* and *L. setigerum* ; the two others are exclusively Indonesian (the sisters, *L. spilopleura* and *L. pellegrinii*. The fish fauna of continental south-eastern Asia is, generally speaking, richer than that of Indonesia ; the genus *Luciosoma* is an exception, including more species in Indonesia. The occurrence of two species in Indonesia and the Malay Peninsula confirms the fact that the aquatic fauna of the latter is closer to that of the Archipelago than with the Indochinese one, while the distribution of the two members of the pair *spilopleura/pellegrinii* demonstrates that the water divide between the western and eastern slopes of Kalimantan island is an older barrier than the sea arm between this island and Sumatera.

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A PRELIMINARY REPORT ON THE GEOGRAPHICAL DISTRIBUTION OF AMPHIBIANS IN ROMANIA

DAN COGĂLNICEANU

Provisional up-to-date dot maps of 15 species of Amphibians of Romania are presented. The maps used have a U.T.M. grid system with 10×10 km squares. A short comment follows, concerning the distribution, habitat and life histories of the species.

The publication of Amphibia by Fuhn in 1960 (5), in the Romanian Fauna Series, was a turning point for herpetology. Since then no attempt to summarize further herpetological recordings has been done. This preliminary report is at present the most complete account on the distribution of Amphibians in Romania. It is based on:
a) The distribution records from "Amphibia" (5), (570 localities);
b) Published faunistical records in scientific papers between 1960 and up to now (551 localities);
c) Personal information from reliable informers (433 localities);
d) Author's own faunistical records (221 localities).

When more reports were available for a given locality, only the most recent report was considered and counted in consequence.

This paper does not include information on the *Rana esculenta* complex, due to the unprecise available data and also because of the publication of a recent general survey report on Green Frogs in Romania (23).

The information on the distribution of Amphibians in Romania is incomplete, large areas having received no attention from herpetologists. A purpose of this paper is to point out the areas where intensive herpetological studies are necessary.

The maps used have a U.T.M. grid system with 10×10 km squares (11). Five species of Urodela and 10 of Anura (not including the *Rana esculenta* complex) are known to occur in Romania. Of these, 3 species and 2 subspecies have limited ranges. The list of species together with a brief comment follows. Only the most relevant references were included in the text.

Salamandra salamandra salamandra

It occurs mainly in the mountainous and hilly country, in forested areas. It was reported at altitudes ranging between 100—1500 m. The eastern limit of its range in Europe is reached in Moldavia (2, 4, 6, 9, 17).

Triturus vulgaris

Two subspecies are known to occur in Romania. The nominal subspecies is widely distributed, from the Danube Delta up to 1500 m altitude in the Carpathians. The *ampelensis* subspecies is restricted to Tran-

sylvania, generally at higher altitudes. The exact range of the latter subspecies and the integradation area between subspecies are not precisely known (1, 7, 9, 10, 13, 16, 17, 19).

Triturus cristatus

Recently *T. cristatus* was stated to be a superspecies, the 4 taxa encompassed until now as subspecies being elevated to full species (24). Two subspecies were known to occur in Romania, *cristatus* and *dobrogicus*. Since most of the faunistical records published until now do not distinguish clearly between the two subspecies and due to the still precarious systematic position, the early subspecies rank was maintained. The exact ranges of the subspecies are not known. Some localities recorded from literature as *cristatus* may in fact refer to *dobrogicus*. While *dobrogicus* is a lowland species, restricted to the Danube meadow and Delta, *cristatus* is widely distributed up to 1000 m altitude (1, 4, 7, 9, 13, 17, 19).

Triturus alpestris alpestris

Distributed in hilly and mountainous areas, from about 400 m altitude up to 2000 m. The eastern limit of this species range are the Oriental Carpathians (2, 9, 13, 17, 19).

Triturus montandoni

It is a montane species restricted to the Oriental Carpathians. It is known to hybridize with *T. vulgaris* all over their overlapping ranges. The extant and intensity of the hybridization area are unknown (2, 9, 13, 17, 19).

Bombina bombina

A typical lowland species, although some records (mostly from Transylvania) are from hilly areas at 4—500 m altitude. Widely distributed along the Danube and in the Danube Delta. Many localities where *B. variegata* also occurs have intermediate hybrid populations. The hybridization zone of these two related species was not studied in Romania (1, 3, 4, 6, 7, 16, 17).

Bombina variegata variegata

Widely distributed in hilly and mountainous areas from 200 m up to 1600 m. Very resistant to pollution and habitat destruction. Hybridizes with *B. bombina* throughout their overlapping ranges (1, 2, 6, 9, 10, 13, 16, 17, 18).

Pelobates fuscus fuscus

A strictly nocturnal, secretive animal, with burrowing habits, generally confined to areas with sandy soils. Distribution everywhere were conditions allow, at altitudes between 0—500 m (6, 7, 16, 17).

Pelobates syriacus balcanicus

Very similar in habits with *P. fuscus*. Found only in the S.E. of Romania where it reaches the north limit of its range. Although very restricted in distribution it forms large populations. The areas where it occurs should be partially protected by law, mostly in what concerns the availability of water bodies for breeding, since agriculture represents a serious threat (6).

Bufo bufo bufo

A widespread species, mainly nocturnal, with terrestrial habits. Found at altitudes between 50—1500 m (1, 2, 4, 6, 9, 13, 17).

Bufo viridis viridis

Widely distributed all over the country from sea-shore up to 1700 m in the mountains. Frequently seen around inhabited areas. Maybe the most common and adaptable species (1, 2, 4, 6, 7, 8, 16, 17).

Hyla arborea arborea

Distributed throughout the country between 0—1000 m altitude. Shows a marked preference for areas with trees and bushes. Threatened by human activities, should be protected locally (1, 3, 4, 6, 7, 9, 13, 16, 17).

Rana dalmatina

Widely distributed from less than 50 m altitude up to 800 m. Found only in damp habitats, mostly woods and swampy areas (1, 4, 6, 13, 15, 16, 17, 20, 21).

Rana temporaria

Widespread in hilly and mountainous areas from 200 m up to 2000 m Largely terrestrial, can be encountered in almost any moist place. Often found at high altitudes, where no other amphibian species ventures (1, 2, 3, 4, 8, 9, 13, 16, 17, 20, 21, 22).

Rana arvalis

Restricted in distribution to the northern half of Romania, shows preferences for damp fields, meadows and sphagnum bogs, mostly in sandy and clay areas. A lowland species, does not go beyond 500 m altitude. Both *arvalis* and *wolterstorffi* subspecies occur but since the validity of *wolterstorffi* is disputed only the species range was considered. It reaches the S.E. range limit in Romania (12, 13, 14, 16).

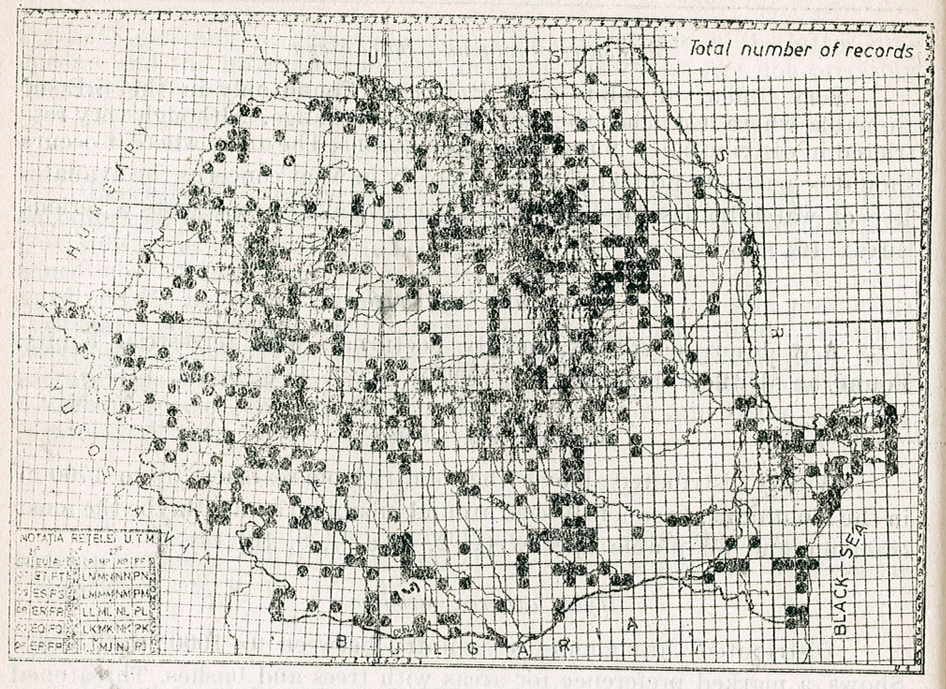


Fig. 1

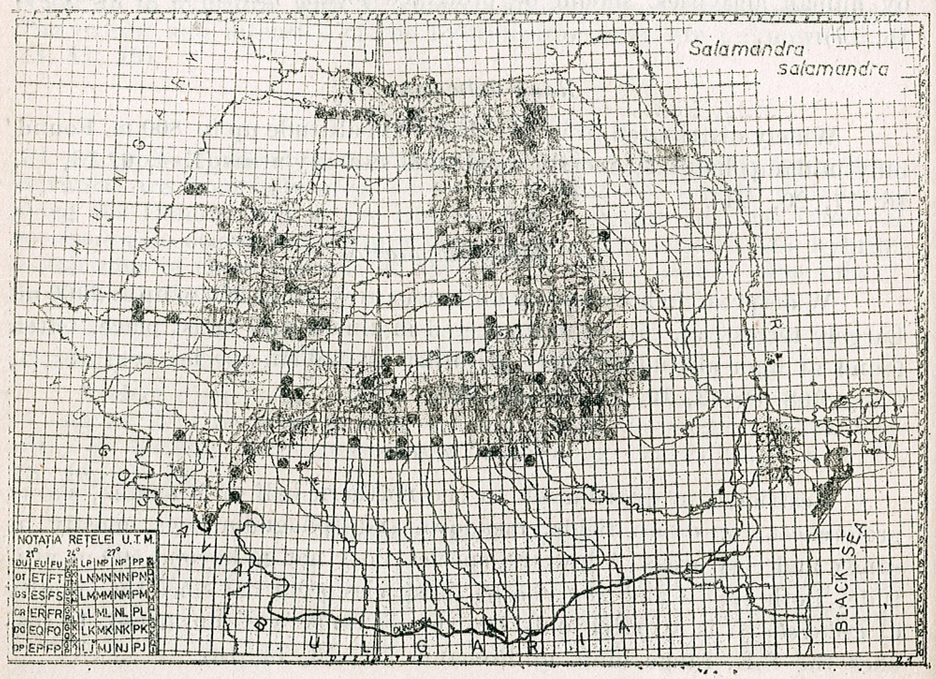


Fig. 2

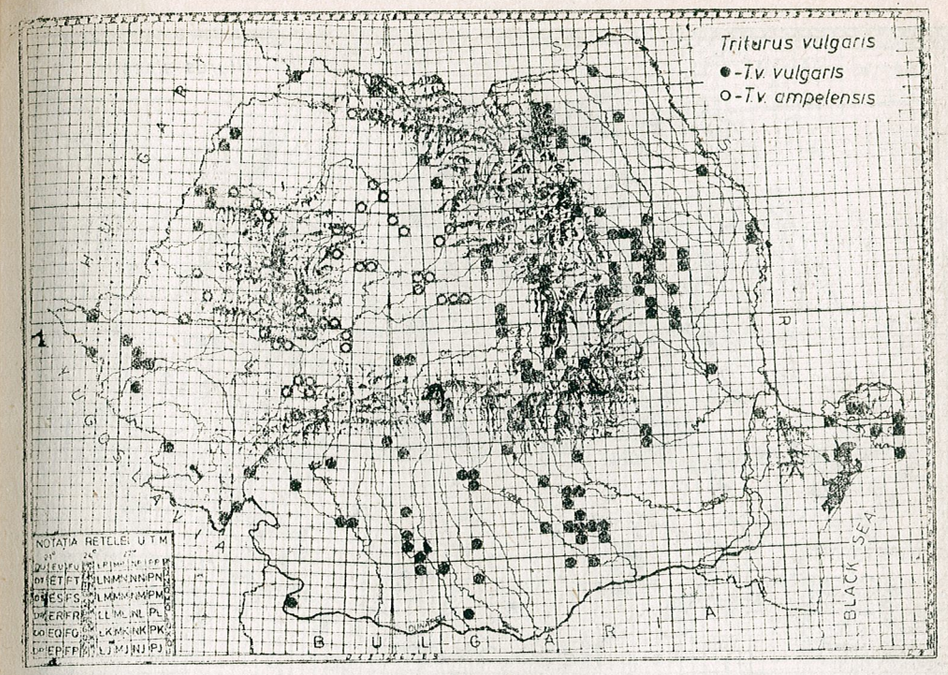


Fig. 3

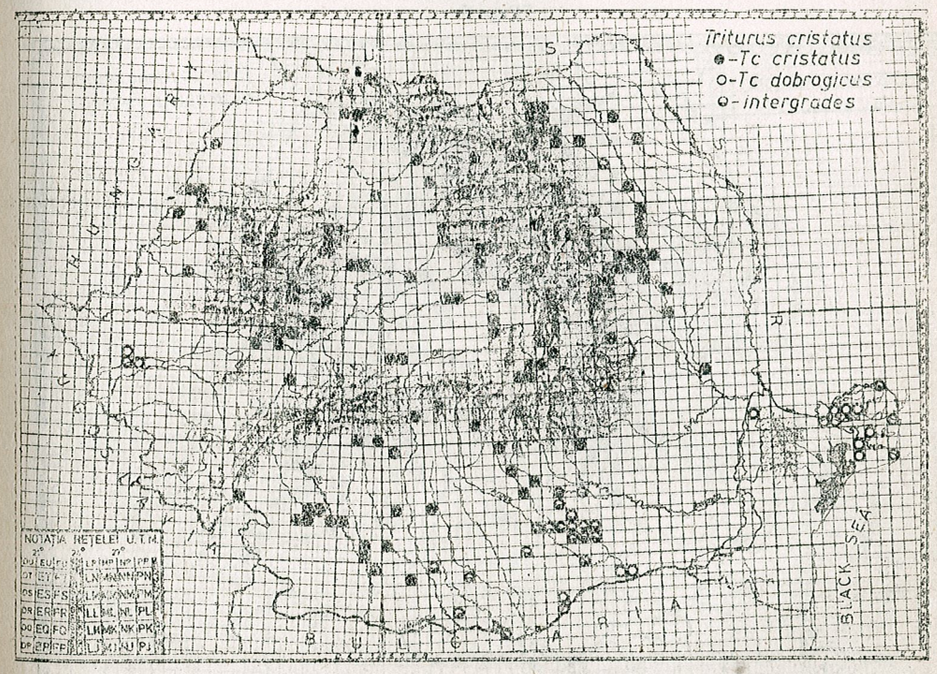


Fig. 4

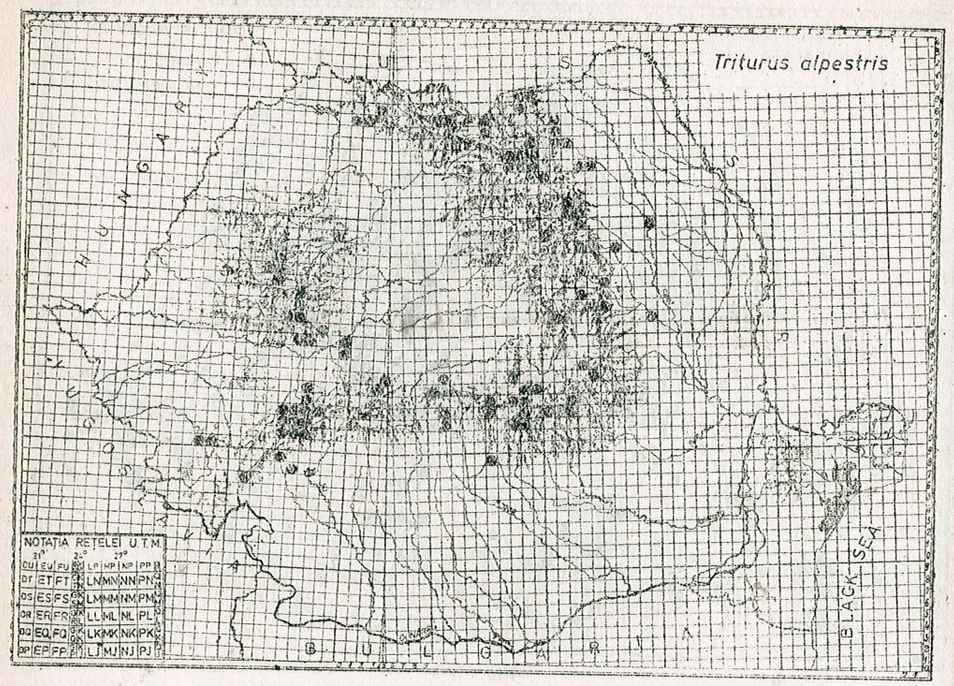
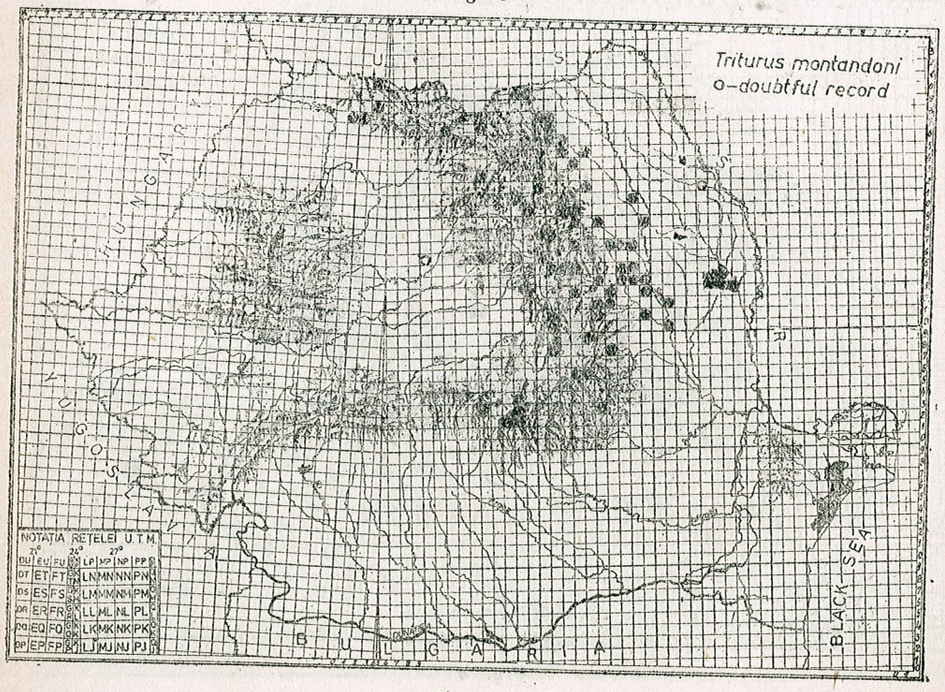


Fig. 5



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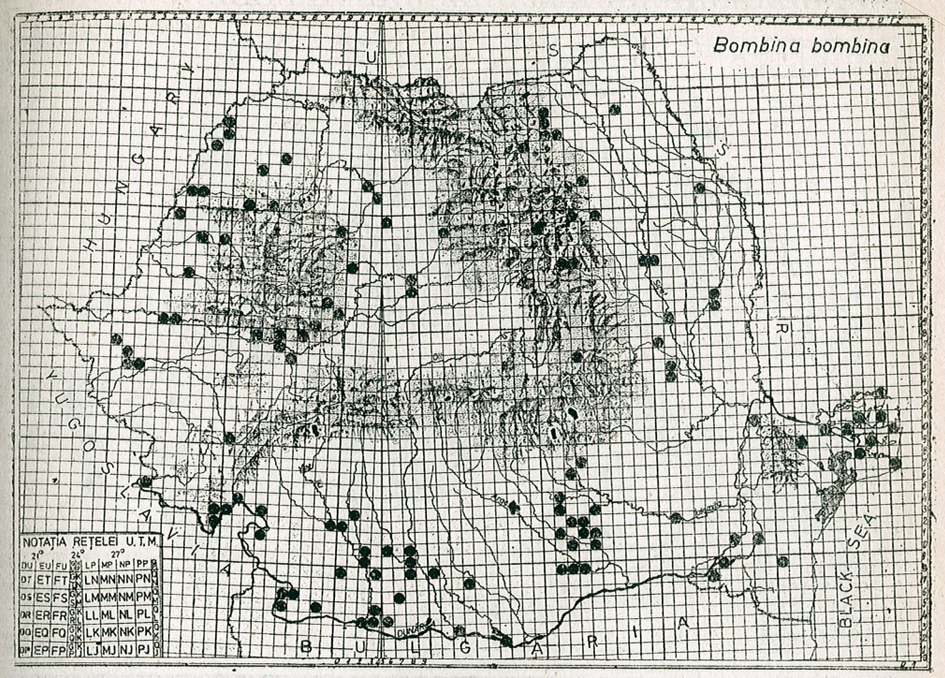


Fig. 7

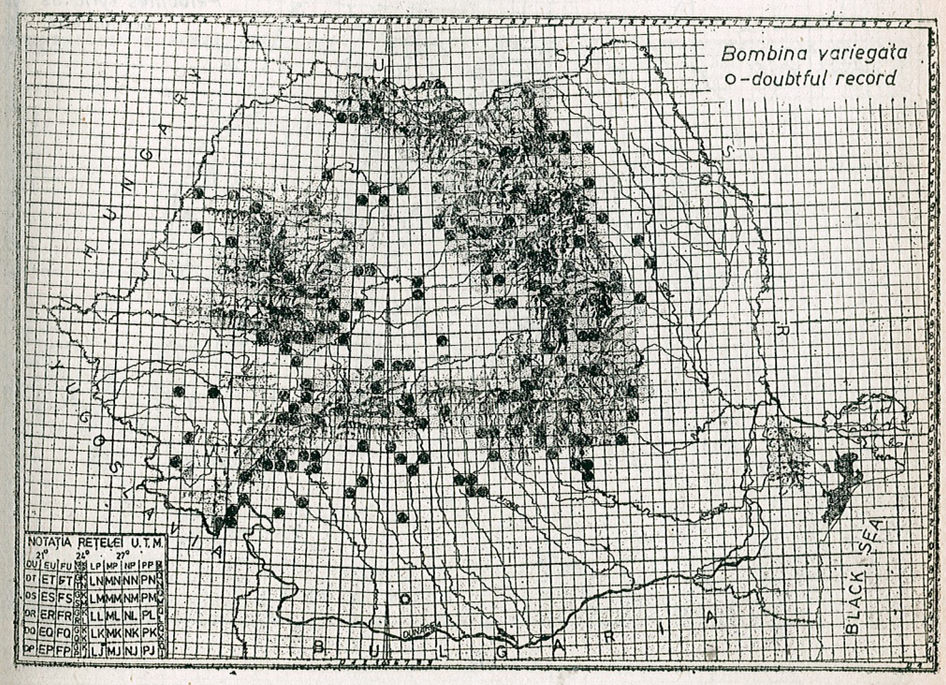
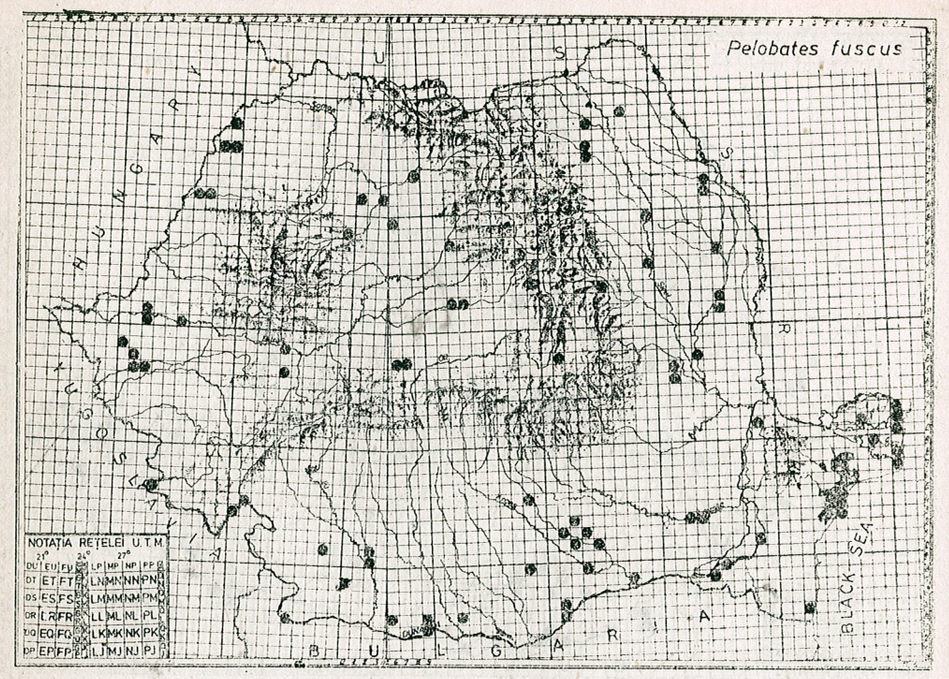


Fig. 8



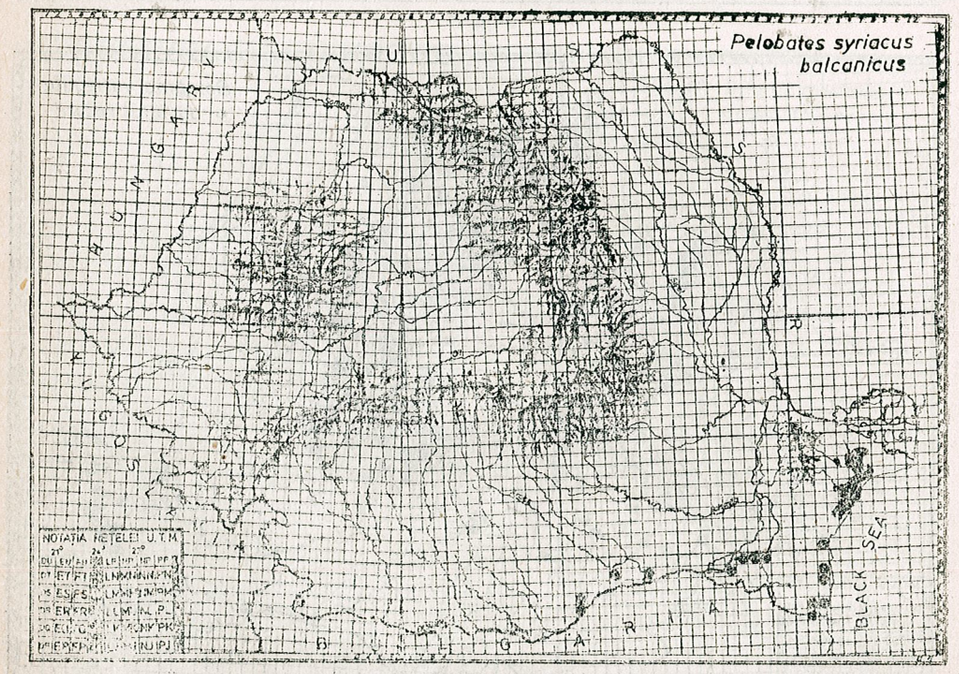
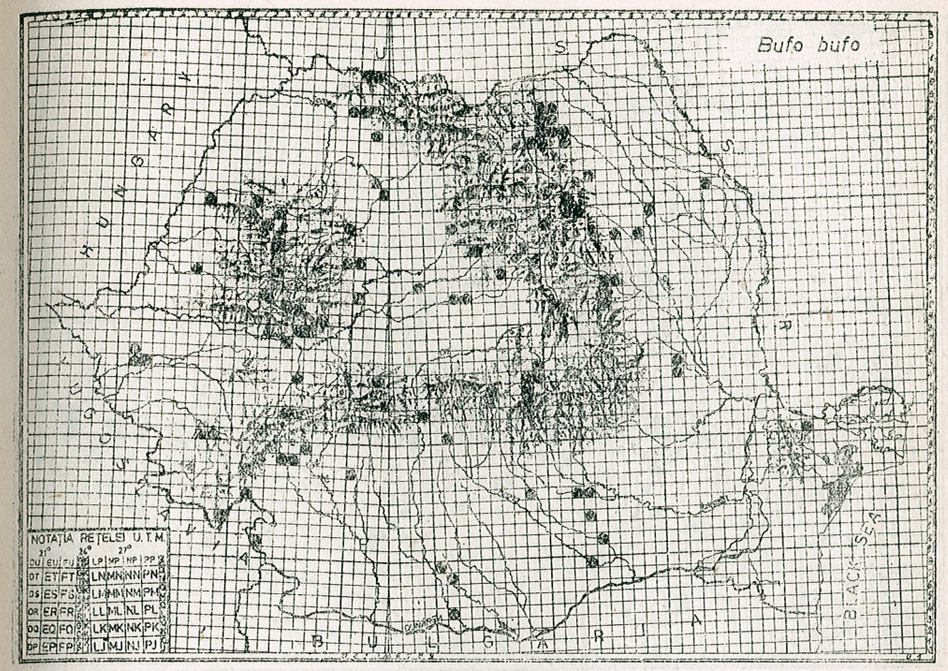


Fig. 10



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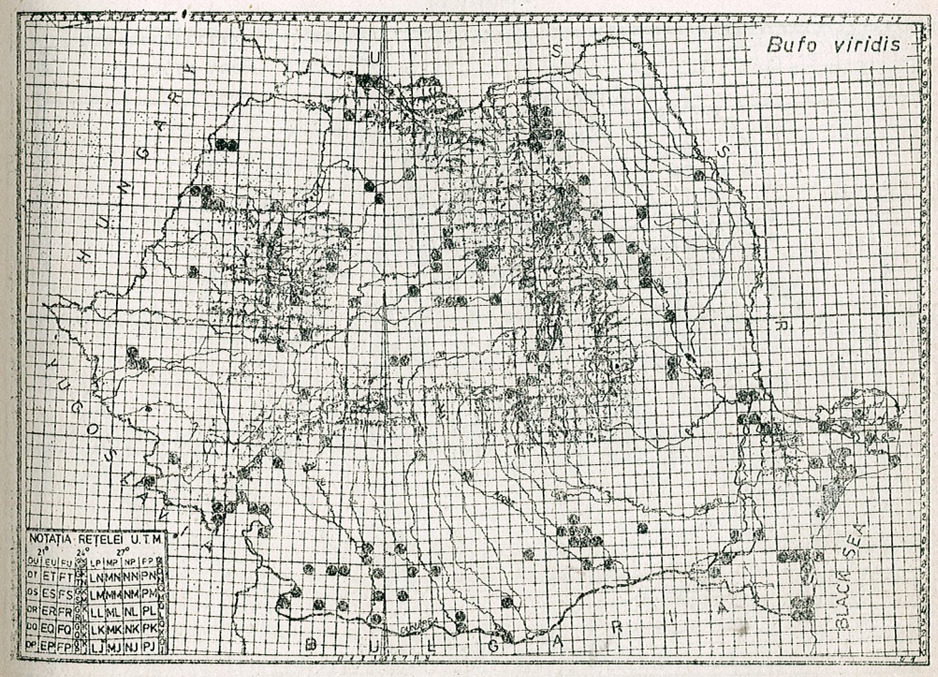

Fig. 12

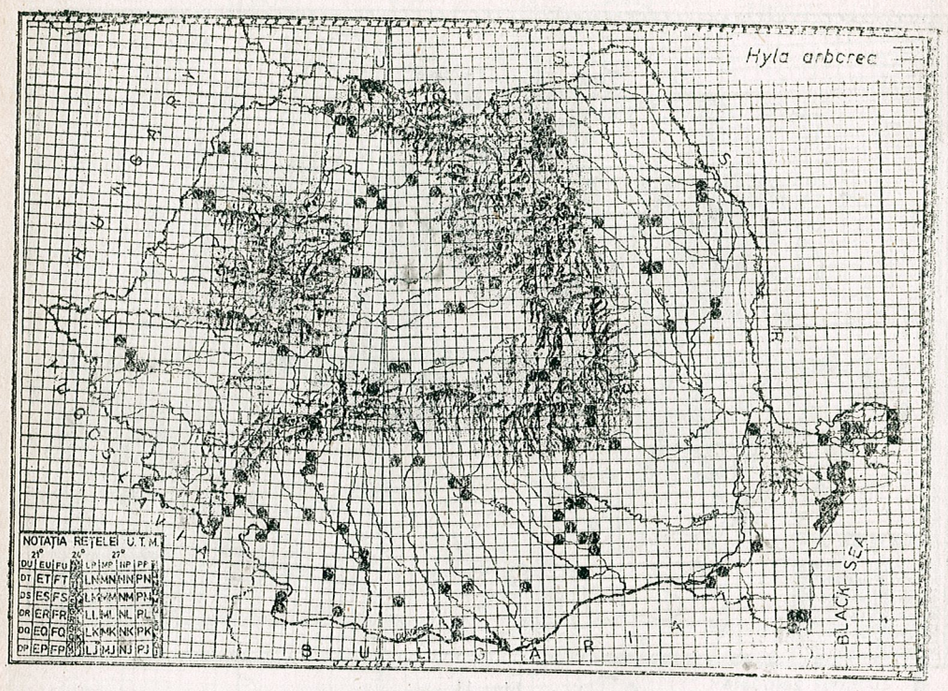

Fig. 13

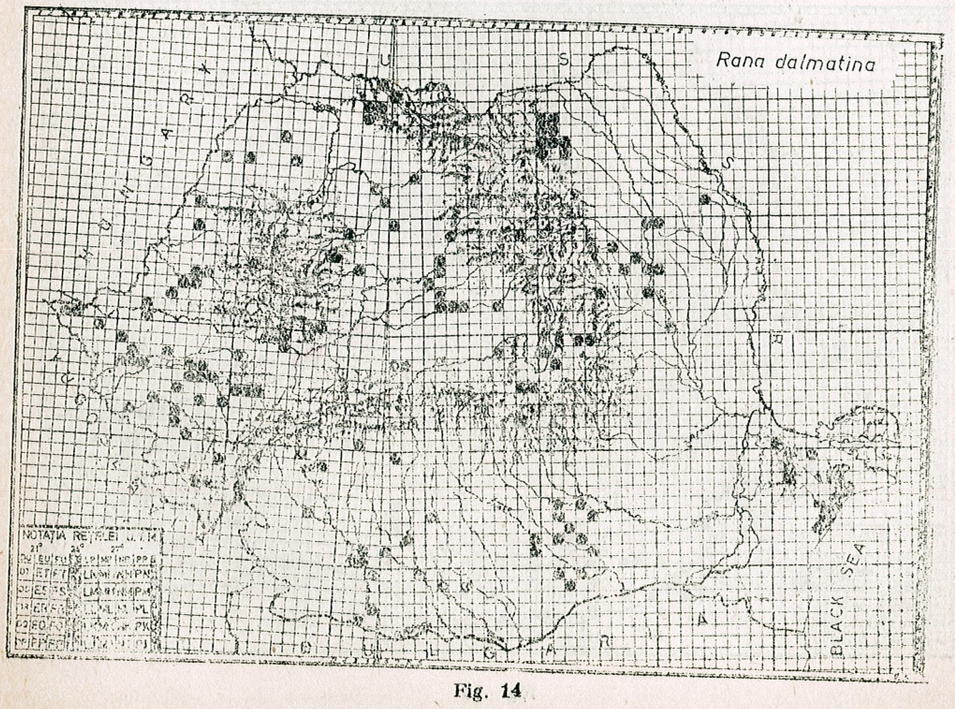

Fig. 14

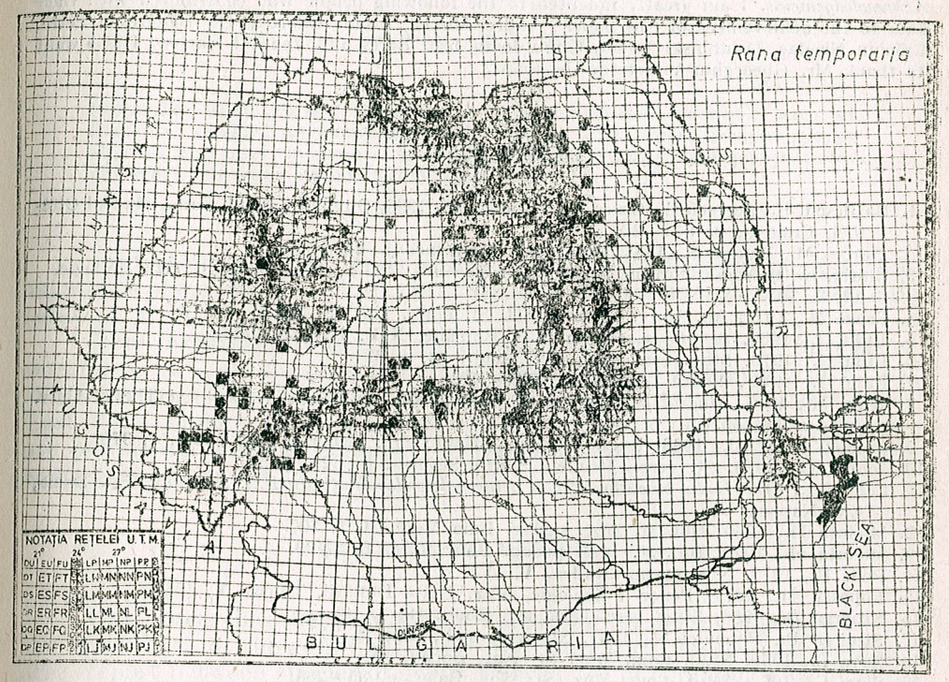

Fig. 15

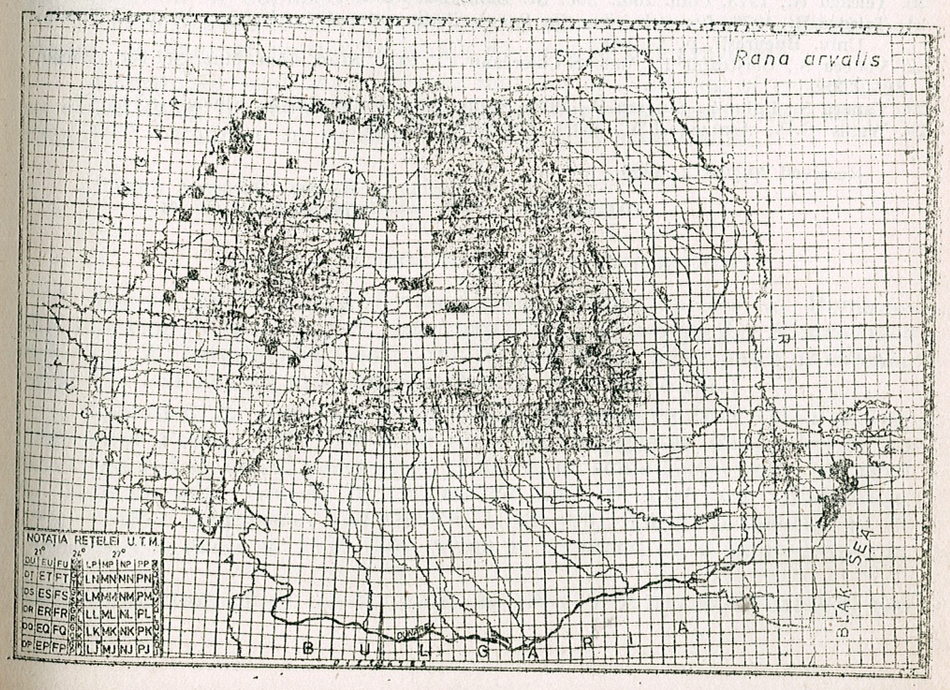

Fig. 16

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RECENT RESEARCHES REGARDING CEREAL BUGS (*EURYGASTER* SPP.) IN ROMANIA

C. POPOV and I. ROȘCA

Researches have followed spreading of bugs populations on whole area of Romania, establishing numerical levels, damage area, report between species of genus Eurygaster in common with level of parasitisation of oophagous parasites and crops protection against heavy infestations.

Protection of cereal crops against the attack of cereal bugs is considered as one of the most important stage of the technologies of these cultures (4, 6, 9, 11, 18).

This because, nowadays, cereal bugs and particularly *Eurygaster integriceps* Put., are the most important cereal pests in Romania (1,5, 6, 10). Wheat crops are particularly damaged on a wide area in the South and East of the country. In order to restrict their outbreak, chemical treatments are performed annually against the overwintering adults and moreover against the nymphs (17, 18).

MATERIAL AND METHOD

Our researches have followed spreading of bugs populations on the whole area of the country establishing numerical levels, and damage area, report between species of genus Eurygaster in common with level of parasitisation of oophagous parasites.

These researches have been done in close co-operation with district centers of plant protection to which we present our gratitude.

Researches regarding biological features of evolution of cereal bugs, as establishing of economic threshold or damages, have been done at I.C.C.P.T. Fundulea in natural and laboratory conditions.

RESULTS AND DISCUSSION

SPECIFIC COMPOSITION AND DISTRIBUTION

Among the 14 *Eurygaster* world-wide known species, in Romania the following are present: *E. integriceps* Put., *E. maura* L., *E. austriaca* Sch. and *E. testudinaria* Geoff., the former three being pests of cereals, while the last has an unsignificant faunal occurrence (6).

Recorded in Romania even since the past century (Montandon, 1885 after Popov, 1977) (6) member s of Eurygaster genus began to be considered as cereal pests after 1920, when some losses were recorded in Transylvania, caused by *E. maura* and *E. austriaca* (Rădulescu, 1938 — after Rădulescu, and Gruiță, 1942) (20). The first mention of *E. integriceps* is made in 1938 (20), when it occurred on a limited area in

the South-East of the country. Economically, cereal bugs began to act as pests of outmost significance after 1963 in the South-East of the country and particularly in Dobrudja, through the species *E. integriceps*, *E. maura* and *E. austriaca* (1, 4).

As it results from Table 1, cereal bugs occur throughout the country, however their damaging area cover only zones where *Eurygaster integriceps* became prevalent, accounting for 60—75% of all species. Historically, evolution of its damaging area was as follows: 1963—2 districts (Tulcea and Constanţa); 1968—7 districts (Călăraşi, Ialomiţa, Brăila, Galaţi, Giurgiu); 1970—12 districts (Teleorman, Olt, Agricultural Sector Ilfov, Dolj, Mehedinţi); 1977—14 districts (Argeş, Dîmboviţa); 1982—17 districts (Bacău, Vaslui, Vrancea); 1986—22 districts (Buzău, Iaşi, Prahova, Vîlcea and Botoşani). At present, their damaging area is permanent and occupies, with some exceptions, all wheat-cropped zones in the above mentioned districts (17). One can state that the cereal bugs problem in Romania is that of *E. integriceps*.

Table 1

Evolution of the ratio between Eurygaster species in wheat cropping zones of Romania during 1938—1990

Zone	Period	Species (%)			
		E. integriceps	*E. maura*	*E. austriaca*	*E. testudinaria*
Dobrudja	1938—39	73	17	10	0
	1970—76	97	2	1	0
	1980—90	98	1	1	0
Bărăgan	1938—39	30	31	38	1
	1970—76	94	2	3	0
	1980—90	97	1	1	1
Central Wallachia	1938—39	21	39	40	0
	1970—76	82	10	7	1
	1980—90	98	1	1	0
Oltenia	1938—39	0	86	14	0
	1970—76	64	16	19	0
	1980—90	98	1	1	0
Central Moldavia	1938—39	0	80	20	0
	1970—76	61	26	7	0
	1980—90	93	5	2	0
Northern Moldavia	1938—39	0	75	25	0
	1970—76	38	44	18	0
	1980—90	90	6	4	0
West Plain	1938—39	0	82	18	0
	1970—76	2	54	43	1
	1980—90	2	60	38	0
Plain and Plateau of Transylvania	1938—39	0	85	15	0
	1970—76	0	83	17	0
	1980—90	0	85	15	0

LIFE-CYCLE AND INFESTATION LEVEL

The species *E. integriceps* is monovoltine and its diapause takes place in oak forests (*Quercus* spp.) (1). Otherwise, presence of oak forests in alternance with wheat crops, at distances rarely exceeding 15—25 km,

constituted, along with climate conditions, one of the factors favourizing the rapid spread and numerical increase of cereal bugs (6). Diapause mortality is usually 15—30%, rarely higher values being recorded (17, 18). Spring migration to wheat crops begins regularly between 5 and 10 April, and ends in the first decade of May (18). Mass egg-laying takes place during May, and nymphal development in June.

During the last decade of June, mass appearance of the new adults occurs, these completing the process of fat accumulation before the harvest start. Researches demonstrated (tables 2—4) the major significance on the fat-body level both on diapause survival and on fecundity, even in a differentiated manner by species (7, 8, 17).

Table 2

Influence of fat-body (%) established at the start of diapause on mortality during diapause in *E. integriceps* (multiannual average)

Females		Males	
fat-body (%)	mortality (%)	Fat-body (%)	Mortality (%)
27.8	81.0	25.9	95.0
28.4	60.5	28.0	77.5
33.2	57.5	31.4	59.0
35.4	40.0	32.7	40.0
37.7	36.0	33.7	40.5

Table 3

Influence of fat-body (%) on fecundity in *E. integriceps* under controlled conditions

Fat-body (%) at the end of diapause	Fecundity (eggs/female)	
	mean	maximum
22.5	33.3	138
26.3	45.3	150
27.7	62.6	212
29.3	80.5	216

Table 4

Influence of fat-body on reproductive potential in *Eurygaster* species (multiannual average)

Species	Fat-body (%) at the a end of diapause	Sterile females (%)	Fecundity (eggs/female)	
			mean	maximum
E. integriceps	28.6	10.0	67.65	272
E. maura	23.9	13.5	48.12	186
E. austriaca	21.4	27.5	24.07	95

Within the damaging area, the adult infestation level in spring is usually 2—8 specimens/m², though, in some instances more than 8 specimens/m² being found. The economic damage threshold for treatment applications against the overwintering adults is differentiated in terms of vegetation state of crops and evolution of climate conditions : for well grown, tillered cultures — 7 individuals/m²; for poorer crops, emerged in winter and without tillers, and in droughty springs 3—5 specimens/m². The time for application of this treatment is usually during the first decade of May (1, 6, 17, 18).

Nymph infestation is much higher than that of overwintering adults, commonly between 15 and 40 specimens/m², locally even with higher densities, especially in the southern part of the damaging area. The economic damage threshold is 3 specimens/m² for wheat crops intended for consumption and 1 specimen/m² for those for seed production. The application period covers the former half of June, with slight variations from a year to another as depending on climate conditions (17, 18).

NATURAL PARASITIZATION

Under the ecological conditions prevailing in Romania, the main parasitary factor encountered in cereal bugs are the oophagous parasites (3, 6, 14, 15, 17). As it could be seen in Table 5, during 1988—1989

Table 5

Recording of medium level of parasitisation of bugs eggs by oophagous parasites in damage area in the last years

District	Medium level of parasitisation (%)	
	1988	1989
Argeş	11.8	29.0
Bacău	38.7	70.5
Botoşani	16.4	60.2
Brăila	7.7	48.0
Buzău	4.5	20.9
Călăraşi	33.9	58.0
Constanţa	22.8	35.4
Dîmboviţa	15.0	40.2
Dolj	41.8	65.8
Galaţi	20.4	35.0
Giurgiu	26.8	39.7
Ialomiţa	19.7	34.0
Iaşi	35.0	85.0
Mehedinţi	48.5	80.0
Olt	39.6	53.0
Prahova	25.0	38.2
Teleorman	16.4	30.8
Tulcea	52.8	72.0
Vaslui	8.2	38.2
Vilcea	18.2	37.4
Vrancea	15.4	42.5
Agricol Sector Ilfov	14.8	32.5
Average	24.2	47.5

an important part of the bug populations were destroyed by oophagous parasites. The high parasitization levels recorded in nearly all districts are to be retained, this confirming the highest ability of parasites adaptation. One can state that egg parasitization in cereal bugs by oophagous species is important even if this alone cannot maintain pest populations below the economic damaging threshold, it can contribute to a large extent to reduce pest populations.

Composition of oophagous parasite range recorded in our country outlines the high share of *Telenomus chloropus* (80%) among the eight species recorded. This species, together with *Trissolcus grandis* and *T. simoni*, detain the main role in parasitizing cereal bugs in their damaging area. The other species (*Trissolcus rufiventris*, *T. pseudoturensis*, *Ooencyrthus telenomicida* and *Anastatus bifasciatus*) occur sporadically and are devoid of economic significance.

CHEMICAL CONTROL

Crops protection against heavy infestations is achieved by chemical treatments, these allowing to lower the pest density below the economic damaging threshold. The most commonly used insecticides are dimethoate and trichlorphon (2, 4, 19); recent tests (16, 17) also revealed other insecticides and particularly synthetic pyrethroids (Table 6).

Table 6

Results obtained in field conditions (verifying plots) with tested insecticides in the order of registration for control of sun pest

Product	Dose g/ha		Mortality (%)	Difference confronted by standard	Year of approbation
	s.a.	p.c.			
Sumicombi 30	225	750	99.2	+0.9	1983
Marshal 25	250	1000	98.4	+0.1	1983
Cybolt 10	50	500	94.8	+0.3	1984
Ekalux S 32	400	1250	96.9	+0.9	1984
Gastac 10	15	150	98.5	+1.3	1985
Decis 2,5	10	400	97.6	+2.8	1986
Karate 2,5	10	400	98.4	+3.6	1987

CONCLUSIONS

1. Species *Eurygaster integriceps* Put., is the most important pest of wheat cultures from Romania.
2. Damage area of this pest includes 22 districts situated in Moldavia, Dobrudja, Wallachia and Oltenia.
3. Level of infestation records as a rule, density of 2—8 exemplars/m² for hibernated adults and 15—40 exemplars/m² for nymphs.
4. Parasitisation determined by oophagous is important but it cannot ensure maintaining of bugs under economic threshold of damages.
5. Economic threshold of damages is depending on the vegetation state of culture to the 7 exemplars/m² in the case of hibernated adults

and 3 nymphs in consumption wheat cultures and one nymph in seeds producing areas.

6. Chemical control is the main way of protection of cultures, using produces based on dimethoate, trichlorphon and synthetic pyrethroids.

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BIOMASS AND PRODUCTIVITY OF COLLEMBOLA FROM THE SOILS OF SOME MOUNTAIN FORESTRY ECOSYSTEMS

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Six sites in the Bucegi, Gîrbova and Retezat Mountains have been studied for a two year period. Biomass of species was established by weighing a definite number of individuals and multiplying their weight by the density number of each species. Biological productivity was established during April-November under the form of growing biomass for each month. It was emphasized that the biomass species hierarchy was different, compared with the species hierarchy established on the basis of numerical density. At the same time biological productivity of Collembola was different in the biotopes from the Gîrbova, Bucegi and Retezat mountains. The highest value of biomass was obtained in the humus of the biotope with the vegetal association *Piceetum carpaticum* and the smallest one in the biotope with the vegetal association *Abietum dacicum*.

The biomass of Collembola shows, besides numerical dominance, the role of each species in the activity of nutrient cyclings and energy flow in an ecosystem. Since numerical dominance shows the position of each species in these processes, depending on the individual number, biomass shows ecological dominance of species depending on the substance quantity possessed at a given time. Since the differences between these biotopes consist, among others, in plant associations, we have tried to find out certain characteristics of biomass and productivity of Collembola, depending on plant associations and type of soils.

MATERIAL AND METHODS

Six sampling sites were selected as follows: Gîrbova Mountain (I-ecosystem with vegetal association *Abietum dacicum*, soil pH=6.8, 800 meters altitude); Bucegi Mountains, two sites (II-ecosystem with vegetal association Abieto-Fagetum, soil pH=6.1, 910 meters altitude; III-ecosystem with vegetal association *Fagetum dacicum*, soil pH=5.4, 1290 meters altitude); Retezat Mountains, three sites (IV-ecosystem with vegetal association Festuco (drymeae)-Fagetum, soil pH=4.5, 850 meters altitude; V-ecosystem with vegetal association *Piceetum carpaticum*, soil pH=3.5—4.4, 1250 meters altitude; VI-ecosystem with vegetal association *Pinetum mugi carpaticum*, soil pH=3.8, 1800 meters altitude.

A study area of 1250 square meters (50/25 meters) was sampled in each site from April to November. Eight samples were taken at random from each site, from litter and humus. The surface area of a sample unit was 33 square centimeters. The method for extraction individuals was a Tullgren funnel modified and adapted to a set of 60 V lights installed at the upper surface of the sample units. The period of extraction was a week.

Biomass species was established by weighing and multiplying the average of a sample with numerical density. Biological productivity was established for the April-November period under the form of growing biomass for each species and for each month.

RESULTS

Species biomass shows that *Folsomia quadrioculata* is the most representative species from all studied biotopes. It is a species with a high ecological plasticity, ubiquistic one, almost each month having the highest biomass values (Table 1). It is followed by *Isotoma violacea*, another species well represented in all samples. These two species are followed by other 4 species — *Isotomiella minor*, *Folsomia inoculata*, *Onychiurus armatus* and *Isotomina bipunctata*, after which all the remaininig species show small values of biomass. Comparing the biomass density of all six sampling biotopes was established that the highest biomass value was presented by the biotope with the vegetal association *Piceetum carpaticum*, from the Retezat Mountains and the smallest one, by the biotope with vegetal association *Abietum dacicum*, from the Gîrbova Mountains. It is to notice that the biomass of Collembola from the Retezat biotopes is higher than those from the Gîrbova and Bucegi. One explanation of that might consist in the existence of some better conditions in the Retezat Mountains since the samplings were taken out in the Natural Reservation, without man's influence for many years. This situation, which confirms data established on the base of numerical density (1), has created conditions for a better stability of the structure of Collembola populations from the Retezat biotopes, as compared with Gîrbova and Bucegi, opened to tourism, wood cuttings and other man activities.

Biomass presents a clear vertical stratification, the highest quantity being identified to the humus level. Seasonal dynamics of Collembola biomass shows nearly the same characteristics with those of numerical dynamics with a biomass peak during the summer and a considerable decrease during spring and autumn.

BIOLOGICAL PRODUCTIVITY

On the basis of biomass as dry weight per square meter, it was possible to estimate the biological productivity for each biotope (Figures 1—4). As a result of an important increase of individual numbers at the beginning of summer, the biomass has increased accordingly for each species. By adding month by month increased biomass during the April-November period the biological productivity for each biotope and for each year was obtained. Significant growings of productivity were registered between June and July in all six biotopes. At this time imature individuals, reaching 80% from all population numbers during the spring time, became adults with a corresponding increasing in biomass. Comparing with the beginning of spring with a high mortality rate of survival adults during the winter, in June and July mortality was accidental for juveniles. In August-September biomass increasing stopped because of a new mortality

Table 1

Biomass density of Collembola species the from Gîrbova, Bucegi and Retezat Mountains (mg dry matter/sq.m.)

Species	Biotope	Year of study	Level of collecting samples	Months of collecting samples								Total
				IV	V	VI	VII	VIII	IX	X	XI	
0	1	2	3	4	5	6	7	8	9	10	11	12
Folsomia quadrioculata	*Abietum dacicum* I	1	L	—	46.86	91.93	100.53	114.4	88.45	207.97	24.25	674.39
			H	—	51.98	211.45	90.14	334.59	86.67	41.64	124.78	941.25
		2	L	15.66	26.08	41.59	214.93	13.86	12.22	19.12	1.8	345.26
			H	78.06	93.57	62.41	140.48	114.4	12.22	133.51	10.30	645.04
	Abieto-Fagetum II	1	L	—	211.45	79.71	306.83	119.66	98.88	54.13	55.46	926.12
			H	—	100.53	45.07	285.9	74.63	43.38	97.05	150.87	797.43
		2	L	27.72	51.98	72.8	249.57	79.71	48.5	58.94	22.6	611.82
			H	206.33	48.5	91.93	185.51	88.45	178.6	202.85	104.33	186.50
	Fagetum dacicum III	1	L	—	13.86	81.54	275.66	150.64	98.88	116.18	34.68	771.44
			H	—	202.85	114.4	209.8	137.00	194.11	273.82	315.47	1447.45
		2	L	62.41	12.22	34.64	152.51	128.26	58.93	50.33	3.46	502.76
			H	218.36	273.87	206.19	310.29	116.18	178.59	204.5	121.3	1629.28
	Festuco (drymeae) Fagetum IV	1	L	—	112.84	35.64	90.1	34.64	332.76	69.32	152.52	827.82
			H	—	—	—	—	—	—	—	—	—
		2	L	147.39	135.17	265.22	554.65	377.84	155.99	26.08	11.48	1673.82
			H	313.06	348.46	169.86	393.48	279.09	261.79	286.04	214.88	2466.66
	Piceetum carpaticum VI	1	L	—	95.13	173.33	348.46	415.95	643.48	261.1	218.36	2184.81
			H	—	—	—	—	—	—	—	—	—
		2	L	606.09	464.5	503.03	1222.06	596.25	201.02	93.57	163.59	3385.61
			H	—	—	192.46	955.1	670.84	344.98	588.19	329.33	3545.40
	Pinetum mugi carpaticum VI	1	L	—	—	—	249.57	81.08	644.74	—	—	975.40
			H	—	—	—	—	—	—	—	—	—
		2	L	—	—	—	249.57	587.64	750.59	540.79	—	2128.59
			H	—	—	—	611.9	422.91	341.5	279.09	—	1655.4

0	1	2	3	4	5	6	7	8	9	10	11	12
TOTAL	*Abietum dacicum* I	1	L	—	106.06	246.36	349.09	361.81	301.51	403.93	51.21	1819.97
			H	—	130.9	416.96	223.33	496.66	188.78	141.21	301.51	1899.35
		2	L	45.45	113.03	174.84	763.63	116.66	130.9	159.09	1173.33	2676.93
			H	293.63	296.96	298.78	749.09	560.6	319.39	299.99	90.9	2909.34
	Abieto-Fagetum II	1	L	—	477.27	202.72	651.81	303.93	201.81	169.39	156.66	2263.59
			H	—	214.24	134.84	850.6	209.39	170.9	262.12	394.54	2236.63
		2	L	143.63	147.57	229.39	1164.84	292.69	174.54	229.69	129.39	2511.47
			H	538.78	191.21	435.15	673.33	466.06	723.63	411.21	432.42	3872.33
	Fagetum dacicum III	1	L	—	24.24	201.51	783.93	429.69	256.66	259.39	114.24	2069.66
			H	—	548.48	360.6	578.78	354.24	487.57	700.6	1089.09	4119.36
		2	L	187.27	79.69	90.91	650.3	343.93	278.48	189.69	8.78	1829.05
			H	711.81	906.66	553.72	1379.99	623.63	611.81	811.81	430.9	6029.33
	Festuco (drymeae)-Fagetum IV	1	L	—	230.63	128.48	178.48	613.33	648.48	350.3	343.63	2493.33
			H	—	—	—	—	—	—	—	—	—
		2	L	358.18	347.87	599.39	861.81	475.15	203.93	47.51	44.84	2944.74
			H	1215.45	551.51	508.48	881.51	641.51	546.66	599.39	512.42	4663.93
	Piceetum carpaticum V	1	L	—	201.81	298.78	814.54	859.69	1757.57	859.09	607.21	5428.75
			H	—	—	—	—	—	—	—	—	—
		2	L	—	622.72	866.05	1798.78	763.33	428.18	259.39	374.54	5112.99
			H	73.03	1321.21	591.51	2267.57	1319.99	761.81	1243.93	933.63	8512.68
	Pinetum mugi carpaticum	1	L	—	—	—	—	—	1509.39	—	—	2280.26
			H	—	—	—	—	—	—	—	—	—
		2	L	—	—	—	985.15	1189.39	1121.21	1305.45	—	4601.2
			H	—	—	—	1241.21	1064.54	636.05	435.75	—	3377.55

1 = first year of study
2 = second year of study
L = litter; H = humus

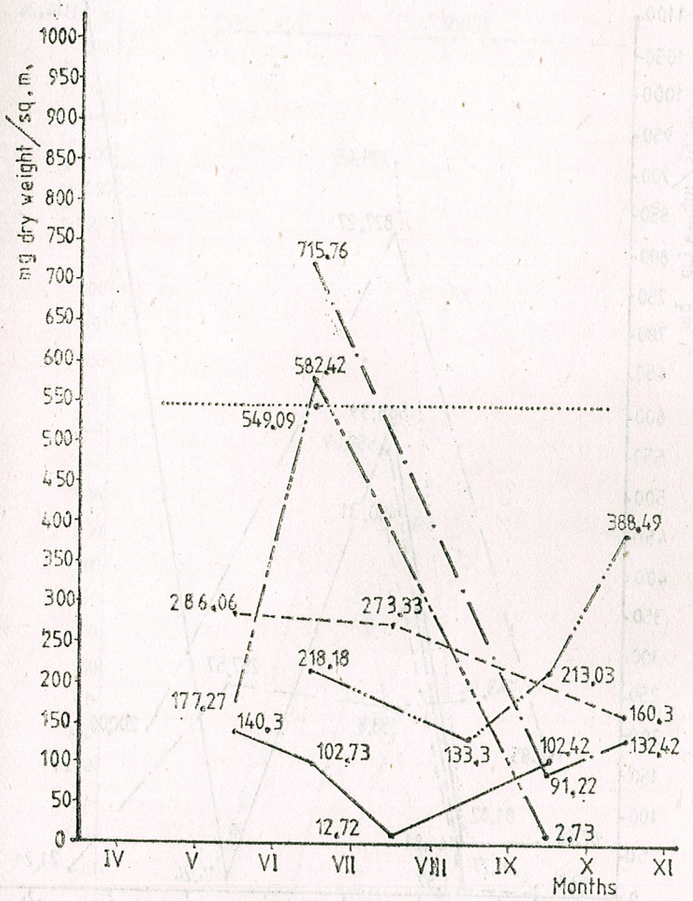

Fig. 1. — Biological productivity of Collembola from the Gîrbova and Bucegi Mountains in the first year of study I.

Figs. *1* and 2:

——— litter of the biotope with vegetal association
I *Abietum dacicum* = 358.17 mg dry weight/sq.m.
II *Abietum dacicum* = 1846.56 „
– – – – humus of the biotope with vegetal association
I *Abietum dacicum* = 719.69 „
II *Abietum dacicum* = 755.17 „
. . . . . litter of the biotope with vegetal association
I Abieto-Fagetum = 549.09 „
II Abieto-Fagetum = 1080.6 „

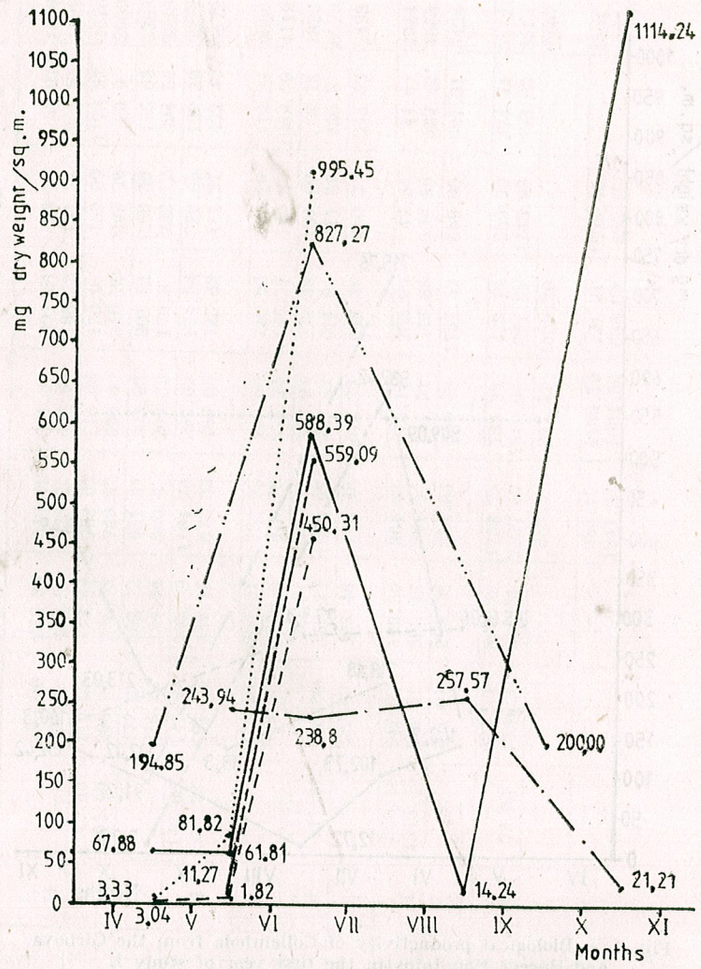

Fig. 2.— Biological productivity of Collembola from the Gîrbova and Bucegi Mountains in the second year of study II.

Figs. 1 and 2

— . — . — . — humus of the biotope with vegetal association
I Abieto-Fagetum = 939.4 „
II Abieto-Fagetum = 761.52 „
——— litter of the biotope with vegetal association
I *Fagetum dacicum* = 762.42 „
II *Fagetum dacicum* = 570.36 „
— . . . — . . . — humus of the biotope with vegetal association
I *Fagetum dacicum* = 953 „
II *Fagetum dacicum* = 1222.12 „

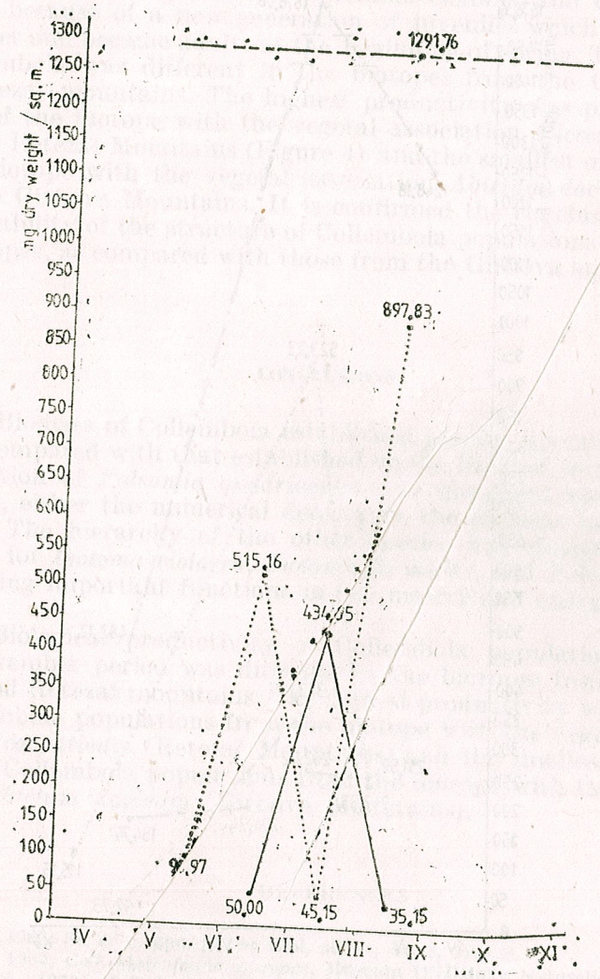

Fig. 3.—Biological productivity of Collembola from the Retezat Mountains in the first year of study I.

Figs. 3 and 4:

——— litter of the biotope with vegetal association
I Festuco (drymeae) Fagetum = 520 „
II Festuco (drymeae) Fagetum = 513.94 „
— — — — humus of the biotope with vegetal association
II Festuco (drymeae) Fagetum = 425.76 „

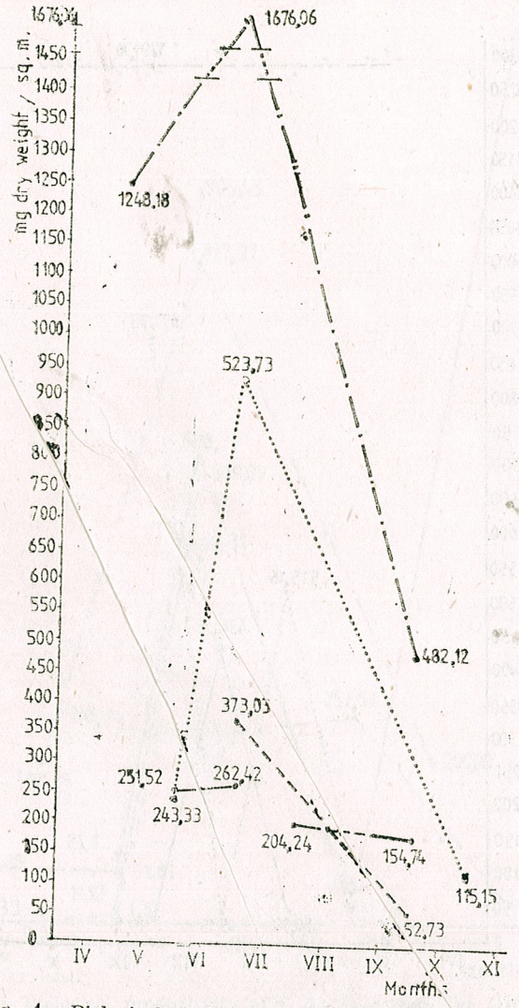

Fig. 4.—Biological productivity of Collembola from the Retezat Mountains in the second year of study II.

Figs 3 and 4:

. . . . . litter of the biotope with vegetal association
I *Piceetum carpaticum* = 1555.11 „
II *Piceetum carpaticum* = 882.21 „
— · — · — · — humus of the biotope with vegetal association
II *Piceetum carpaticum* = 3406.36 mg dry weight/sq.m
— — — — — litter of the biotope with vegetal association
I *Pinetum mugi carpaticum* = 1291.76 „
II *Pinetum mugi carpaticum* = 358.98 „

of adults caused by low soil humidity and high temperatures. The small increasing productivity during September-October and October-November was because of a new generation of juveniles which resisted during the winter and became adults at the beginning of spring. The productivity of Collembola was different in the biotopes from the Gîrbova, Bucegi and Retezat mountains. The highest productivity was presented by the humus of the biotope with the vegetal association *Piceetum carpaticum*, from the Retezat Mountains (Figure 4) and the smallest one by the litter of the biotope with the vegetal association *Abietum dacicum* (Figure 1) from the Gîrbova Mountains. It is confirmed the conclusion regarding a better stability of the structure of Collembola populations from the Retezat biotopes, as compared with those from the Gîrbova and Bucegi mountains.

CONCLUSIONS

1. Biomass of Collembola established another hierarchy of the species, as compared with that established on the basis of numerical density. The position of *Folsomia quadrioculata*, as dominant species, remained the same, either the numerical density or the biomass being taken into account. The hierarchy of the other species was changed with higher positions for *Isotoma violacea*, *Isotomiella minor*, and *Folsomia inoculata*, they having important functions in the matter and energy transfer processes.

2. Biological productivity of Collembola populations during the April-November period was different in the biotopes from the Gîrbova, Bucegi and Retezat mountains. The highest productivity was produced by the Collembola populations from the biotope with the vegetal association *Piceetum carpaticum* (Retezat Mountains) and the smallest one was produced by Collembola populations from the biotope with the vegetal association *Abietum dacicum* (Gîrbova Mountains).

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CYTOSTATIC AND ANTITUMOR ACTIVITY OF THE POLYPHENOLIC PREPARATION PA2

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The *in vitro* and *in vivo* action of the PA 2 polyphenolic preparation on HeLa cell cultures and on the evolution of solid or ascitic Guérin T-8 lymphotropic epithelioma and of Walker 256 carcinosarcoma was investigated. The results obtained allow to consider this preparation as a potential cytostatic and antitumoral agent.

Among other things, the neoplasic cell is characterized by a profound alternation of the energetic metabolism as a consequence of the irreversible diminution of the oxidative reactions and of redox processes, of the maximum compensatory intensification of anaerobic glycolysis caused by some intracellular biochemical lesions, (4), (16), (17), (25). Thus, the normalization of the tumoral cell by remaking of the aerobic system, for the assurance of the necessary energy to reactivation, represents one of the ways of action on the malignant process (2), (3), (5), (8), (9), (11), (14), (25). For example, the restoration of the intracellular levels of NAD and cyclic AMP induced a cytostatic action (3), (5), (14). The stimulation of the redox processes by another donors or acceptors of electrons — therefore with redox properties — is also used in antitumoral therapy (6).

The phenolic type structures can be implied in modulation of the cell redox systems activity as nonenzymatic protectors and electron donors (23). On this hypothesis a cytostatic and antitumoral action of these structures by stimulation of redox processes and therefore by cellular energetic recovery was supposed by us.

In the present paper the results obtained by testing of the "in vitro" cytostatic action on HeLa cells of the polyphenolic preparation PA 2 and its "in vivo" antitumoral activity on rats bearing of different experimental tumoral systems are exposed.

MATERIAL AND METHODS

The PA 2 preparation — a polyphenolic fraction separated and purified from a crude alkaline vegetal extract — was isolated at the Macromolecular Chemistry Institute "P. Poni" Iassy.

The cytostatic action was assessed "in vitro" by comparative follow-up of the total protein dynamics during the evolution of the HeLa cell cultures incubated with PA 2 preparation and of control.

The test tubes were inoculated with 1×10^5 cells and after 24 hours the culture medium was replaced with a medium containing 1.5 mg/ml of PA 2 polyphenolic preparation. At 24, 48 and 72 hours of cultures development the medium was discarded from the test tubes and the cell layer was washed with TFS and subjected to total protein determination (13), (15).

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Five culture tubes were used for each type of culture and time interval and the statistical analysis was performed using Student's "t" test.

White Wistar rats of 125—150 g bearing either Guérin T-8 lymphotropic epithelioma of solid and ascitic form, or Walker 256 carcinosarcoma were used for "in vivo" testing of the PA 2 antitumoral activity.

24 hours after the tumoral transplant, the treatment started and lasted for 16 days in the case of solid Guérin T-8 tumor and 19 days in the case of Walker 256 tumor or until the death of the last control animal for the ascitic tumor. The treatment was applied by daily intraperitoneal administration of PA 2 drug in different doses.

The estimation of the antitumor activity was based on the comparative follow-up of the mean tumor weight (MTW) at the sacrifice or of the mean survival time (MST) of ascitic rats in the treated and control groups.

The evaluation of antitumor activity was made by the percentage determination of mean tumor regression (%MTR) or of mean survival time prolongation (%MSTP) and by the calculation of the statistic significance and the T/C value (where T=MTW or MST for the treated groups and C=MTW or MST for the controls).

The appreciation of cytostatic and antitumoral effects was performed by comparative analysis of our evaluation index values with those imposed, by the selection criteria of antitumoral substances established by the screening programs of the Institute of Microbiology and Experimental Therapy from G.D.R. (10) and of Cancer Chemotherapy National Institute from U.S.A. (12), for these preliminary steps.

RESULTS

The experimental data obtained in the "in vitro" investigation of the action of PA 2 preparation on HeLa cell cultures development as compared to controls are shown in Fig. 1.

The protein dynamics of the cultures treated with PA 2 reveals significant decreases ($p<0.001$) of the protein values during the evolution of HeLa cell cultures. Thus, the protein concentrations, recorded at 48 and 72 hours, illustrate an inhibition of cultures development of 28.3% and 50.0%, respectively.

The results of the "in vivo" testing of the PA 2 antitumoral activity on rats bearing solid Guérin T-8 tumor are presented in Table 1.

In comparison with the control group it is observed that the daily treatment with the PA 2 polyphenolic preparation in a dose of 40 mg/kg.b.w. induced a significant decrease ($p<0.001$) of MTW which allows an estimate of a MTR of 40.3% and a T/C value of 0.59.

The "in vivo" investigation of the PA 2 treatment effect on Walker 256 tumor development gave the data included in Table 2.

Once again, comparatively with the control group, it can be seen that the PA 2 administration in a dose of 57 mg/kg.b.w./daily was correlated with a significant antitumoral action ($p<0.001$), illustrated by a MTR of 47.3% and a T/C value of 0.52.

The testing of the antitumor activity of the polyphenolic preparation PA 2 was extended on a tumoral line of ascitic type (Table 3). On the contrary, as compared with the control group, in the case of PA 2 treatment a significant decrease ($p<0.01$) of MTS was observed. Therefore, this preparation had not an antitumoral effect on the ascitic system.

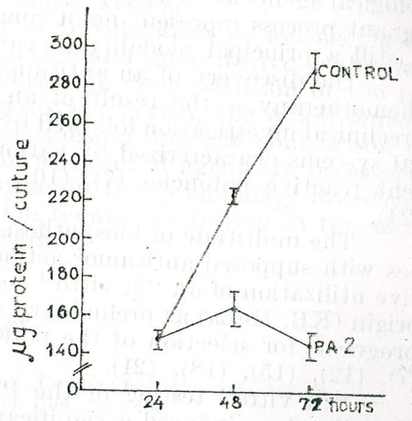

Fig. 1. — Protein content of HeLs cell cultures incubated with the PA2 polyphenolic preparation (1.5 mg/ml).

Table 1

Testing of the antitumoral activity of the PA 2 treatment (40 mg/kg.b.w./daily/ip) on rats bearing solid Guérin T-8 tumor. Figures in brackets indicate the number of animals

Group/treatment	M.T.W. (g)	% M.T.R.	T/C value	Statistical significance
Control	15.4±0.8(14)	—	—	—
PA 2 preparation	9.2±1.1(10)	40.3	0.59	$p<0.001$

Table 2

Cancerostatic action of the PA 2 preparation, administered in a dose of 57 mg/kg.b.w/daily/ip, on Walker 256 tumor

Group/Treatment	M.T.W. (g)	%M.T.R.	T/C value	Statistical significance
Control	14.6±1.2(15)	—	—	—
PA 2 preparation	7.7±1.1(10)	47.3	0.52	$p<0.001$

Table 3

Mean survival time of the rats bearing of ascitic tumor treated with PA 2 polyphenolic preparation (40 mg/kg.b.w./daily/ip)

Group/Treatment	M.T.S.(days)	%M.S.T.P	T/C value	Statistical significance
Control	25.8±1.0(14)	—	—	—
PA 2 preparation	22.2±0.4(10)	−14.0	0.86	$p<0.01$

DISCUSSION

Although there is a continuous progress in cancer diagnosis and treatment, the antineoplastic chemotherapy is characterized by a low efficiency. Therefore, the identification of new cancerostatic pharmacological agents as well as the discovery of new ways of action on the malignant process represent main concerns at present, when chemotherapy is still a principal modality in cancer treatment.

The discovery of an antitumoral drug and its use in antineoplastic chemotherapy is the result of an "in vitro" and "in vivo" complex preclinical investigation followed by a clinical trial on some testing biological systems characterized by various levels of organization and by different reactive potencies (7), (10), (12), (15), (18), (19), (20), (21), (22), (24).

The multitude of biosynthesis, semisynthesis and synthesis substances with supposed antitumor action imposed the introduction and intensive utilization of an "in vitro" test on neoplastic cell cultures of human origin (KB, HeLa) as preliminary step of the chemotherapeutic screening programs for selection of the potential cytostatic and/or cytotoxic drug (7), (12), (15), (18), (21).

"In vitro" testing of the PA 2 polyphenolic preparation showed that the drug induced a significant inhibition of HeLa cell cultures development (50%). The profound alteration of the total protein dynamics allowed us to consider it as a cytostatic agent.

Although "in vitro" testing system has some advantages — offers the possibility of a direct pursuit of the cytostatic action in a short time; requires small amounts of substances; "in vitro" cytostatic activity was positively correlated sometimes with " in vivo" antitumor action (1), (7), (19), (21), (24) — it represents only a preliminary step followed by "in vivo" tests on experimental tumoral systems. For the characterization of a drug as a cancerostatic agent a multistage screening on tumor bearing animals is required because of the internal selfdefence and control mechanisms functioning in an animal organism which can prevent the malignant transformation and evolution of some cells.

Therefore, we performed an "in vivo" test of the antitumoral effect of the PA 2 polyphenolic preparation on rats bearing either of Guérin T-8 lymphotropic epithelioma of solid and ascitic type or of Walker 256 carcinosarcoma.

"In vivo" testing of this biologically active product revealed a significant tumor regression for the solid type tumors and a significant decrease of the survival time in the case of ascitic tumor.

The appreciation of the PA 2 antitumoral activity was performed by comparative analysis of our evaluation index values with those imposed by the selection criteria of cancerostatically active agents established, for the first step, by the multistage screening programs of the Institute of Microbiology and Experimental Therapy in G.D.R. (10) and of the Cancer Chemotherapy National Institute in the U.S.A. (12).

According to these screening programs in a first stage the experimental antitumoral treatment must induce, on at least one tumoral system out of the three systems tested, a MTR of at least 35% for solid

tumors and an increase of the MST of 30—50% for ascitic tumors in the treated groups compared to the controls, or give a T/C value of 0.54—0.64 for solid tumors and 1.25 for ascitic tumors.

In the light of the above reference values, our data emphasize the antitumoral therapeutic effect of the PA 2 polyphenolic preparation on Guérin T-8 and Walker 256 solid tumoral systems.

The preclinical characterization of a substance as antineoplastic agent is the result of a complex qualitative and quantitative evaluation of this specific activity. To this purpose, the demonstration of the reproducibility and stability of the cancerostatic action, the establishment of the existence of a dose-response relationship and the comparison of the induced specific effect with that of a standard agent are necessary.

Therefore, the results obtained by us in this preliminary investigation with PA 2 polyphenolic preparation require its testing in the next steps of the preclinical screening program.

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FURTHER EVIDENCE ON CANCEROSTATIC ACTIVITY OF THE NEW BIOSYNTHESIS ANTIBIOTIC PREPARATION A 37.4

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The possibility of optimization of the antitumoral effect by therapeutic dose manipulations, as a consequence of the existence of a dose-response relationship, was established by "in vivo" testing on rats bearing either Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma of the different doses of A 37.4 antibiotic, which was used in a combined therapy together with the semisynthesis polyene NsMC 2.

The significant effectiveness of the A 37.4 antitumoral therapy on Walker 256 tumor with different degrees of development was also evidenced. The results complete the preclinical experimental evidence which characterize the new biosynthesis antibiotic A 37.4 as a potential active cancerostatic agent.

The preclinical characterization of a new drug as antitumoral agent is the result of a complex evaluation of the specific action. Chemotherapeutic screening programs aimed to identify new cancerostatic agents require a multistage investigation (1), (2), (3), (7), (9), (10), (11).

In previous papers we evidenced the cancerostatic therapeutical effect of the new antibiotic preparation A 37.4 and the reproducibility and stability of this action on adequate experimental models, these being steps of the qualitative evaluation (4), (5), (6).

In the present investigation the "in vivo" testing of the action of A 37.4 different doses, used in a combined therapy with chemically modified nystatin 2 (NsMC 2) on rats bearing various tumoral systems, is performed. At the same time an estimation of the A 37.4 therapeutic efficiency on tumors with different degrees of development is made.

MATERIAL AND METHODS

White Wistar female rats of 150 g, bearing either Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma were used as experimental animals.

24 hours and in a case 3, 5, 7 days after transplant the antitumoral treatment started and lasted for 16 days for the Guérin T-8 tumor and 19 days in the case of Walker 256 tumor.

The intraperitoneal (ip) treatment was applied by administration of different doses (mg/kg.body weight) of A 37.4 at 3-days intervals concomitantly with a daily injection of the association agent NsMC 2 (a semisynthesis polyene) in a dose of 50 mg/kg.b.w.

The estimation of the antitumoral activity was based on the follow up of the mean tumor weight (MTW) at sacrifice in the treated groups comparatively with the control.

The evaluation of the cancerostatic effect was made by the percentage determination of mean tumor regression (%MTR) and by the cal-

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culation of the statistic significance using Student's "t" test and theT/C value (where T = MTW for the treated group and C=MTW for the control).

Antitumoral therapeutic effectiveness of the A 37.4 antibiotic was appreciated by comparative analysis of our evaluation indices values with those imposded by the reference programs from G.D.R. (2) and U.S.A. (3) for these tages of the screening.

RESULTS

The antitumoral activity of the different doses of A 37.4, used in the combined treatment with NsMC 2, on rats with Guérin T-8 tumor, is given in Table 1.

Table 1

Antitumor activity of different doses of A 37.4 antibiotic preparation (mg/kg.b.w./at 3 days/ip), administered in association with NsMC 2 (50 mg/kg.b.w./daily/ip), on Guérin T-8 tumor. Figures in brackets indicate the number of animals

Group/Treatment	M.T.W.(g)	% M.T.R.	T/C value	Statistical significance
Control	13.5±1.7(13)	—	—	—
A 37.4(0.025mg)+NsMC 2	10.5±1.5(9)	22.3	0.78	N.S.
A37.4(0.050mg)+NsMC 2	8.3±1.8(8)	38.6	0.61	$p < 0.02$
A37.4(0.075mg)+NsMC 2	6.7±1.1(9)	50.6	0.49	$p<0.01$
A37.4(0.1 mg)+NsMC 2	12.0±1.7(9)	11.2	0.89	N.S.

In comparison with the control group it was observed that this combined therapy induced :

— nonsignificant decrease of the MTW in the case of the groups treated with 0.025 and 0.1 mg, which allow an estimate of a MTR of 22.3% and 11.2%, respectively and a T/C value of 0.78 and 0.89 respectively ;

— significant antitumoral activity ($p<0.02$) illustrated by a 38.6% MTR and by a T/C value of 0.61 in the case of the group treated with 0.050 mg ;

— maximum and significant cancerostatic effect ($p<0.01$), revealed by a 50.6% MTR and by a T/C value of 0.49, in the case of the group treated with 0.075 mg.

The results obtained in antitumoral activity testing of different doses of A 37.4, administered in association with NsMC 2, on rats with Walker 256 tumor are presented in Table 2.

It can be seen that — in comparison with the control group — the progressively therapeutical dose increase is correlated with a limited augmentation of the antitumoral efficiency.

Thus, the minimum effect was observed when A 37.4 was administered in a dose either of 0.025 mg (32.1% MTR, 0.68 T/C value) or of 0.1 mg (17.0% MTR and 0.68 T/C value), the medium action was recorded when the dose was increased to 0.050 mg (42.0% MTR and 0.58

T/C value) and the maximum antitumoral activity was noticed when the antibiotic dose was of 0.075 mg (58.1% MTR and 0.42 T/C value).

Table 2

Antitumor activity of different doses of A 37.4 antibiotic preparation (mg/kg.b.w./at 3 days/ip), administered in association with NsMC 2 (50 mg/kg b.w./daily/ip), on Walker 256 tumor. Figures in brackets indicate the number of animals

Group/Treatment	M.T.W.(g)	% M.T.R.	T/C value	Statistical significance
Control	11.2±1.7(12)	—	—	—
A 37.4(0.025mg)+NsMC 2	7.6±2.0(8)	32.1	0.68	N.S.
A 37.4(0.050mg)+NsMC 2	6.5±1.5(8)	42.0	0.58	$p<0.05$
A 37.4(0.075mg)+NsMC 2	4.7±1.7(8)	58.1	0.42	$p<0.02$
A 37.4(0.1 mg)+NsMC 2	9.3±1.9(8)	17.0	0.83	N.S.

The antitumoral treatment efficiency of the A 37.4 preparation, administered in association with NsMC, on Walker 256 tumor with different degrees of development can be seen in Table 3.

Table 3

Cancerostatic effect of the associated therapy with A 37.4 (0.075 mg/kg.b.w./at 3 days/ip) and NsMC 2 (50 mg/kg.b.w./daily/ip) on Walker 256 tumor with different degreas of development. Figures in brackets indicate the number of animals

Group/Treatment	M.T.W.(g)	% M.T.R.	T/C value	Statistical significance
Control	12.5±1.7(15)	—	—	—
A 37.4+NsMC2/24 hours	7.0±1.8(9)	44.0	0.56	$p<0.05$
A 37.4+NsMC 2/3 days	6.2±1.5(9)	50.4	0.49	$p<0.05$
A 37.4+NsMC 2/5 days	6.9±1.1(9)	44.8	0.55	$p<0.05$
A 37.4+NsMC 2/7 days	5.7±1.2(9)	54.4	0.46	$p<0.02$

Comparatively with the control group it was observed that this treatment induced significant antitumoral effects indifferently of the therapy beginning.

Thus, the treatment started 24 hours after transplant is correlated with a MTR of 44.0% and a T/C value of 0.56. When the treatment started 3 days after transplant there were registered a MTR of 50.4% and a T/C value of 0.49. In the case of the treatment started 5 days after transplant the MTR and T/C values were of 44.8% and 0.55, respectively. Finally, when the therapy started 7 days after transplant the antitumoral indices has 54.4% MTR and 0.46 T/C values.

DISCUSSION

The characterization of a new substance as an active cancerostatic agent — the final purpose of the preclinical chemotherapeutic screening programs — is based not only on the qualitative evaluation of the speci-

fic action but also by the evidence of its pharmacotherapeutical effect and by demonstration of the reproducibility and stability of its antitumoral effect (2), (3), (10), (12).

Thus, the programs elaborated by the Institute of Microbiology and Experimental Therapy from Germany (2) and the Cancer Chemotherapy National Institute from the U.S.A. (3) and the national methodology of pharmacological preclinical research (12) require additional investigation stages in order to assure the quantitative evaluation of cancerostatic activity. Among other things, these steps must appreciate the therapeutic effectiveness by the establishment of the existence of a dose-response relationship and by the recording of a significant antitumoral effect on tumors with different degrees of development.

These experimental aspects represented the objective of the present study, carried out in laboratory conditions using the A 37.4 antibiotic preparation and other two tumoral systems besides the ascitic line, which was used in a previous investigation (6).

By modifying the dose of A 37.4, in the combined therapy of the Guérin T-8 lymphotropic epithelioma and Walker 256 carcinosarcoma, the dependence of the antitumoral effect upon the drug dose has been followed, in the condition of a daily injection of the same dose of the association agent, NsMC 2 (50 mg/kg.b.w.).

The augmentation of the A 37.4 dose from 0.025 to 0.050 mg/kg.b.w. is correlated with an intensification of the antitumoral action of 16.3%, in the case of Guérin T-8 tumor and of 9.9%, in the case of Walker 256 tumor.

The increase of the drug dose from 0.050 to 0.075 mg/kg.b.w. has shown a potentiation of the cancerostatic effect of 12.0% on Guérin T-8 tumor and of 16.1% on Walker 256 tumor.

The analysis of the experimental results registered in these conditions revealed the existence of a relationship between the therapeutic dose and the intensity of the induced cancerostatic effect. This appreciation is based on the criterion established for this step of investigation by the reference screening programs. According to the German and American programs (2), (3) the dose-effect relationship is confirmed if it is obtained a T/C value of 0.42—0.54 at least one of the used doses and if a progressive potentiation of the activity in relation to the dose increase is registered.

The dose-response relationship has, however, a limited character since the administration of 0.1 mg/kg.b.w. did not induce a significant antitumoral activity. The explanation of this phenomenon should have an answer in the intensification of the secondary effects, related to the toxicity of the A 37.4 product, as the drug dose increases. In the conditions in which the drug toxicity was not sufficiently balanced by the protective effect induced by NsMC 2 association agent (4), (5), (6), the possibility of manifestation of the antitumoral activity specific to the new antibiotic preparation decreased.

The therapeutic effectiveness of the A 37.4 antibiotic preparation administered at different time intervals after the tumoral transplant, together with the association agent, was also investigated in the present paper. Comparatively with the standard values of the evaluation indices imposed by the reference programs — a MTR at least of 35% and a

T/C value of 0.54—0.64 (2), (3) — our results evidenced the significant efficiency of the new antibiotic preparation on Walker 256 carcinosarcoma with different degrees of development.

The possibility of optimization of the antitumoral action by therapeutic dose manipulation and the significant therapeutic effectiveness on tumor with different degrees of development allow us to appreciate the antibiotic preparation A 37.4 as a potential cancerostatic agent. Also, they impose the comparison of the A 37.4 specific activity with those of some standard agents in order to assure a complete quantitative evaluation of its pharmacological antitumoral effect.

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EFFECT OF DICHLOROPINACOLONE ON GLYCEMIA AND GLUCOSE UPTAKE BY ISOLATED DIAPHRAGMS AND RENAL SLICES IN FEMALE WISTAR RATS

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Female young Wistar rats, weighing 160—200 g, were fed for 90 days with dichloropinacolone-containing (1% and 5% from LD 50/kg b.w./day) laboratory diet. After a fasting period of 18 hrs and 34 hrs following the cessation of the treatments, the glycemia as well as the "in vitro" glucose uptake from 1 ml glucose-containing (16.7 mM, p.a. "Merck") Krebs-Henseleit bicarbonate solution (pH = 7.4) by the isolated hemidiaphragms and renal slices were investigated under aerobic conditions (gas phase = 95% O_2 + 5% CO_2), at 37.6°C, for 2 hrs. It was established that dichloropinacolone elicited a moderate hyperglycemia, as well as an enhancement of diaphragmatic glucose uptake independently of the applied doses, while the renal glucose consumption increased in a direct manner with the daily doses of this fungicide.

Earlier experiments from our laboratory revealed that some dichloroderivate pesticides, depending on their doses and on the duration of the administration with the food, in white rats affect both the glycemia homeostasis and the glucose uptake by skeletal muscles (5), (6), (13), (17) and kidney (3). Starting from these establishments and from the large agricultural application of fungicides, in the present study we followed the dynamics of fasting glycemia levels as well as the rate of "in vitro" glucose uptake by isolated hemidiaphragms and renal slices from young Wistar female rats, after chronic daily administration with the food of two different doses of fungicide, dichloropinacolone.

MATERIAL AND METHODS

For the experiments young female Wistar rats (weighing 160—200 g) were used. The animals were divided into 3 groups, as follows : I. Normal control group ; II. Group to which was administered with the food 1% daily doses from LD 50/kg dichloropinacolone for 90 days (low dose) ; III. Group treated for 90 days with daily doses of 5% from LD 50 dichloropinacolone/kg by feeding (high dose).

The animals were fed with standard diet and kept under standardized bioclimatic laboratory conditions (temperature = 22.5°C ; humidity = 60%), drinking tap water being provided *ad libitum*.

Dichloropinacolone was freely homogenized with the laboratory food before feeding of the individuals.

The animals were sacrificed by cervical dislocation, decapitation and exsanguination after the above treatments.

For the determination of glycemia, 0.1 ml blood samples were used and deproteinized. The glucose content of the supernatant fluid was determined enzymatically, using GOD-Perid Kit "(Boehringer", GmbH., Mannheim, Germany) and the method of Werner *et al.* (21).

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After sacrification of animals, the diaphragms and kidneys were quickly isolated and immersed for 20 minutes in ice-cold Krebs-Henseleit bicarbonate buffer (4°C; pH=7.4), without glucose. From each diaphragm a hemiorgan of approximately 75—100 mg and from the left kidney of each individual a transversally sectioned slice of 0.5—0.8 mm (60—80 mg) were used for incubation. As incubation medium for the tissue pieces, 1 ml glucose-(p.a. "Merck"; 16.7 mM) and gelatine-containing (p.a. "Merck"; 2 mg/ml) was used. The incubation for 2 hrs, at 37.6°C, was carried out in an original device (10), applying our procedures, described elsewhere (3), (11), (12), (18). The gaseous phase of the incubation system was carbogen (95% O_2+5% CO_2) and the shaking velocity of 90 oscillations/minute and 5 cm amplitude.

The initial and postincubation content of glucose in the incubation medium was tested enzymatically, according to Werner and coworkers (21). The rate of glucose uptake by the tissue pieces was calculated and expressed as micromole/100 mg fresh tissue for 2 hrs.

Data are expressed as means ±S.E. for the number of observations. Probabilities (P) of chance difference between groups were calculated according to Student's two-tailed *t*-test, the differences at $P<0.05$being considered statistically significant.

RESULTS

Table 1 shows the overall picture of fasting blood glucose levels as well as the "in vitro" glucose uptake of hemidiaphragms and renal slices

Table 1

Blood glucose levels and „in vitro" glucose uptake by the isolated hemidiaphragms and renal slices in female young Wistar rats under normal conditions (control) as well as after the administration for 90 days of low doses (1% from LD5 0/kg/day) or high doses (5% from LD 50/kg/day) of dichloropinacolone (DCP)

Group	Blood glucose (mg/100 ml)	Micromole glucose uptake by 100 mg tissue for 2 hrs	
		Hemidiaphragms/Renal slices	
Control	75±1.59 (9)	3.439±0.299 (8)	1.725±0.314 (8)
DCP 1%	85±3.64 (8) $P<0.02$*	5.820±0.322 (8) $P<0.001$*	2.822±0.121 (8) P < 0.01*
DPC 5%	84±2.66 (10) $P<0.01$* $P<0.50$**	6.273±0.239 (8) $P<0.001$* $P>0.10$**	5.022±0.298 (8) $P<0.001$* $P<0.001$**

Results are expressed as mean ± S.E. Number of experiments is given in parentheses. *P* — values are calculated vs. the control* as well as vs. the group treated with low dose of fungicide **. LD 50 is the lethal dose of DPC (1973 mg/kg), at which the mortality of rats was 50%.

isolated from normal and from chronically treated rats with different dichloropinacolone doses. Figure 1 depicts the percent modifications of the above parameters vs. the corresponding control values.

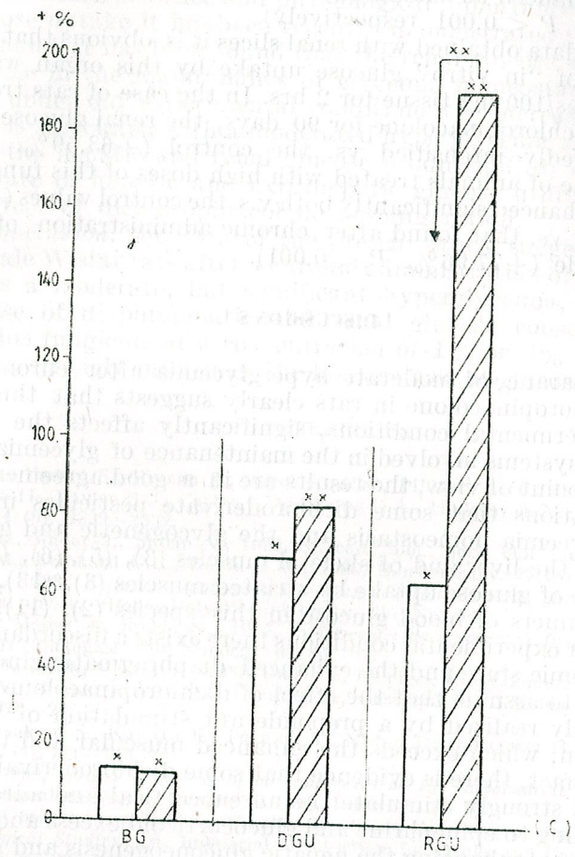

Fig. 1. — Percent modifications vs. the control values (C) of glycemia (BG) and of glucose uptake by isolated hemidiaphragms (DGU) and renal slices (RGU) in wistar rats after administration for 90 days of low doses (white columns) or high doses (shaded columns) of dichloropinacolone.
* $P<0.02$ or <0.01; ** $P<0.001$. The arrows indicate the significance of differences between the effect of low and high doses (in the case of renal slices).

Under normal conditions the mean value of fasting glycemia was 75±1.59 mg%. The chronically administered low or high doses of dichloropinacolone elicited a moderate, but statistically significant increase in glycemia vs. the control values (+13.3%, $P<0.02$ and +12.0%, $P\leqslant 0.01$, respectively).

The rate of glucose penetration from the incubation medium into the hemidiaphragms isolated from the normal group was equal to

3.439±0.299 micromoles/100 mg fresh tissue for 2 hrs. Under the influence of administered low or high dichloropinacolone doses for 90 days, the glucose uptake by isolated diaphragmatic muscle was similar, but significantly enhanced as compared to the control (+69.23%, $P < 0.001$ and +82.41%, $P < 0.001$ respectively).

From the data obtained with renal slices it is obvious that in normal state the rate of "in vitro" glucose uptake by this organ was 1.725±0.314 micromoles/100 mg tissue for 2 hrs. In the case of rats treated with a low dose of dichloropinacolone for 90 days, the renal glucose consumption was markedly intensified vs. the control (+63.59%, $P < 0.01$), while in the case of animals treated with high doses of this fungicide, the phenomenon enhanced significantly both v.s. the control values (+191.43% $P < 0.001$) and vs. that found after chronic administration of low doses of this fungicide (+77.96%, $P < 0.001$).

DISCUSSIONS

The appearance of moderate hyperglycemia after chronic administration of dichloropinacolone in rats clearly suggests that this fungicide, under our experimental conditions, significantly affects the activity of the regulatory systems involved in the maintenance of glycemia homeostasis. From this point of view, the results are in a good agreement with our earlier observations that some dichloroderivate pesticides in rats may affect both glycemia homeostasis and the glycogenetic and glycogenolytic capacity of the liver and of skeletal muscles (3), (5), (6), (13), (17) as well as the rate of glucose uptake by striated muscles (3), (13), considered as major consumers of blood glucose in this species (2), (11), (19), (20). Since under our experimental conditions there exists a discordance between the hyperglycemic state and the enhanced diaphragmatic glucose uptake, it is pertinent to assume that the effect of dichloropinacolone administration was mainly realized by a preponderant stimulation of hepatic glucose production, which exceeds the enhanced muscular and liver glucose utilization. In fact, there is evidence that some dichloroderivate pesticides in Wistar rats strongly stimulate the adrenocortical and adrenomedullar activities, leading to epinephrine and glucocorticoid excess and to a hyperglycemic state by enhancing the hepatic gluconeogenesis and glucose production (13). At the same time, it is well established that the glucocorticoid excess in white rats exerts moderating effects upon the muscular glucose utilization by its antiinsulinic activity (10). Therefore, the discrepancy between the dicloropinacolone-induced moderate hyperglycemia and enhanced diaphragmatic glucose uptake, under our experimental conditions, seems to be well correlable. In fact ,it has been convincingly demonstrated that at the level of working striated muscles catecholamine excess may potentiate the activity of circulating insulin upon the transmembranal glucose penetration from the blood into the striated muscle fibres, by stimulating the activity of glucose-transport systems at sarcolemmal level (2), (4), (19), (20).

The results obtained on isolated renal slices from dichloropinacolone-treated rats demonstrate that this fungicide, in a direct relationship with the applied doses, strongly stimulates the glucose utilization. In

this stimulatory effect seems to be involved either a direct renal effect of this fungicide, or an effect realized by stimulation of renal insulin-receptors, of which presence and physiological importance in the enhanced renal glucose uptake it has been recently demonstrated (1), (7), (16). On the other hand, it has been found that the renal glucose uptake plays an essential role in the maintanance of glycemia homeostasis in white rats (9). Since under our experimental conditions the increased renal glucose utilization is associated with a moderated hyperglycemic state, we assume that both the hepatic and renal glucose production (8) significantly exceed the rate of hepatic and extrahepatic glucose utilization under the chronic effect of dichloropinacolone administration.

In conclusion, the data of the present study suggest that in adult young female Wistar rats after a chronic administration of dichloropinacolone elicits a moderate, but significant hyperglycemia, associated with the increase of diaphragmatic and renal glucose consumption. Consequently, this fungicide at a concentration of 1% or 5% from LD 50/kg for 90 days, strongly influences the homeostasis of glucose metabolism.

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HYPOCHOLESTEROLEMIC EFFECT OF OAT-BRAN HYDROALCOHOLIC EXTRACT

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In male Wistar rats weighing 180—200 g, were induced hypercholesterolemia by triton 1339 WR (150 mg/kg b.w./day) for 21 days. The hypercholesterolemic rats were treated with *Avena sativa* hydroalcoholic extract (15 ml/kg b.w./day; the extract containing 0.533 mg water-soluble fibers and 0.64 mg saponins/ml), for three weeks. A significant decrease of cholesterolemia in hypercholesterolemic rats receiving *Avena sativa* hydroalcoholic extract was obtained.

It was found that plant water-soluble fibers (gums, mucilages as well as polysccharides) have a hypocholesterolemic effect in men (3, 16, 19) and animals (5, 6) too. The same hypocholesterolemic effect using a plant saponins rich feed was obtained (4, 8, 12, 15, 17, 18).

The present study was undertaken to assess the effectiveness of oat-bran hydroalcoholic extract on serum cholesterol in hypercholesterolemic rats.

MATERIALS AND METHODS

Oat-bran hydroalcoholic extract was obtained by a repercolation method, with ethanol 50%, and the ratio oat-bran/solvent was 1 : 1 (9). Our extract contained 0.533 mg water-soluble fibers (gravimetric evaluation) and 0.64 mg saponins/ml (20).

Our experiments were performed on male Wistar rats weighing 180—200 g reared in the stockfarm of our laboratory and kept under standardized feeding and bioclimatic laboratory conditions. The animals were divided into three groups : control group (C); hypercholesterolemic group (T) — the animals were daily, subcutaneously, injected with triton 1339 WR (a hypercholesterolemic agent; 150 mg/kg b.w.) for all period of experiment (21 days); hypercholesterolemic group receiving oat-bran extract (TA) : the animals were injected with triton (the dose was as in T group) and received, by intragastric sonde, daily 15 ml oat-bran extract/kg b.w.

Seric cholesterol was evaluated by Watson's method (13).

All the results were expressed as means ± S.E. Differences were checked up by Student's "t" — test; Differences of $p < 0.05$ were taken as significant.

RESULTS

From the data summarized in Table 1 we can show that triton administration caused an increase of seric cholesterol in T group. In the case of triton treated animals, as compared with those from the control group, cholesterolemia was +132.1% an the 4th day, up to +500% an the 9th day, and +278.7% an the 21th day of the experiment. In the group

receiving triton and oat-bran extract simultaneously, cholesterolemia was higher than control values, but smallerthan triton treated group values. It is remarkable that cholesterolemia was significantly lowered in TA group when it was compared with T group, an the 9th and 21th days of the experiment: -41.6% ($p < 0.05$) and -30.4% ($p < 0.001$), respectively (see Table 1).

Table 1

Values of cholesterolemia in the control (C), triton treated (T) and triton treated and oat-bran extract receiving (TA) rats

Animal group		Cholesterolemia (mg/100 ml serum)			
		0 days	4 days	9 days	21 days
C	$\bar{X}\pm SE$	105.3±7.3	110.2±6.2	108.4±5.1	112.5±6.8
	n	7	7	7	7
T	$\bar{X}\pm SE$	109.8±6.3	255.8±24.2	651.2±109.2	426.0±13.6
	n	7	7	7	8
	p	—	<0.001	<0.001	<0.001
	±C%	+4.3	+133.1	+500.7	+278.7
TA	$\bar{X}\pm SE$	106.7±5.2	238.2±13.2	380.3±39.5	296.7±20.2
	n	7	7	8	8
	p	—	<0.001	<0.001	±0.001
	±C%	+1.3	+116.2	+250.8	+163.7

Note: In Table 1 are given means±standard error; n seems number of animals; p = statistical significant modification vs. controls.

DISCUSSIONS

Using literature data that we have known, we are going to explain some of the mechanisms by which oat-bran extract lowers seric cholesterol.

1. Water soluble fibers inhibit 3-hydroxy-3-methyl-glutaryl CoA reductase activity. This enzyme is implicated in cholesterol synthesis (7).

2. The saponins are able to make some complexes with the cholesterol in the gut (10, 17). Inhibition of 3-hydroxy-3-metyl glutaryl CoA reductase by water soluble fibers, on the one hand, and the capacity of saponins to make complexes with the cholesterol in the gut, on the other hand, show us a possible direct action of our oat-bran extract upon the synthesis and absorption of cholesterol in rats.

3. The water soluble fibers and saponins from oat-bran hydroalcoholic extract could be able to influence indirectly the decrease of cholesterolemia. In the colon, the metabolic products of the bacterial action upon water soluble fibers are largely methane, CO_2, water and short chain or volatile fatty acids such as acetate, butyrate and propionate (6, 7). Most of these short chain or volatile fatty acids were absorbed into the portal

circulation, and at such concentration seen in the portal vein of oat-bran fed rats, could attenuate hepatic cholesterol synthesis (1). It was established that water soluble fibers (2, 11) and saponins (12, 15, 18) rich diets usually increase fecal bile acid excretion. The loose of bile acids may be a cause of the hypocholesterolemic effect of the oat-bran extract. It was shown that in small intestine water-soluble fibers and saponins could make gel systems (2) or mycelial systems respectively (14). These gel and micelian systems can include bile acids that will be loose by feces. Saponins can promote the neutre sterols loose by feces (14, 18). These neutre sterols can be precursors in cholesterol synthesis. We do not exclude the existence of the other mechanisms that could be involved in the hypocholesterolemic effect of our oat-bran extract.

This study suggests that oat-bran hydroalcoholic extract lowered cholesterolemia in experimental hypercholesterolemic rats.

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BIOCHEMICAL CHANGES INDUCED BY OJ-ASCITES CARCINOMA IN RAT THYMUS DURING INVOLUTION

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The intraperitoneal transplantation of OJ-ascitic carcinoma in female Wistar rats induced a rapid thymus involution with significant inbalance of nucleic acid and protein metabolism. Three days after tumor transplantation, an important decrease of total nucleic acids, deoxyribonucleic acid and especially of ribonucleic acid, a decrease of total proteins, and an increased activity of the glutamate-oxalacetate-transaminase (GOT) and glutamate-pyruvate-transaminase (GPT) were observed. Between the 3rd and the 9th days after tumor transplantation, relatively stable values of the ribonucleic acid were found, while the total protein content increased concomitantly with the massive decrease of transaminase activity. After this delay, a marked decrease of thymus nucleic acids and total proteins was coincident with the mortality of animals. These changes are assigned to the increased glucocorticoid secretion in tumor-bearing hosts and are related to the status of the antitumoral immune respunse.

Previous findings (20, 24) suggest that the constant and irreversible thymus involution in tumor-bearing hosts is brought about, at last partially, by an endocrine mechanism, consisting in the activation of the hypothalamo-pituitary-adrenal-axis, as a principal part of the stress (19) reaction induced by the tumoral growth. The special sensibility of thymus lymphocytes for cortisol is explained by the existence of glucocorticoid receptors (25). A participation of the thymus to an immune response of the host against the tumor is also discussed (7, 8, 13).

Taking into consideration appreciable quantities of thymic nucleic acids (17, 22), which exibit important changes during acute thymus involution induced experimentally by cortisol administration (4, 17, 18, 23), or during thymus regeneration, we studied the dynamics of nucleic acids, in relation with other parameters of the protein metabolism during the thymus involution induced by OJ-ascites carcinoma in rats.

MATERIAL AND METHODS

Female Wistar rats provided by the stockfarm of the Oncological Institute of Cluj, weighing 160—170 g, were transplanted intraperitoneally with 1 ml ascitic fluid of OJ-ascites carcinoma (16), containing 32.000 cells/mm^3. The animals were sacrified 1, 3, 9 and 17 days after tumor transplantation. Eight rats with the same biological characteristics were sacrified to serve as controls. All animals were necropsied and the thymuses were weighed.

The thymic concentration of total acids (TNA) was determined by the differential spectrophotometric method of Spirin (21) and that of the desoxyribonucleic acids (DNA) by Ogur and Rosen's technique, modified by Abraham and Pora (2). The differences between TNA and DNA enabled us to establish the ribonucleic acid content (RNA). Finally, the glutamate-oxalacetate-transaminase (GOT) and the glutamate-pyruvate-transaminase (GPT) activities of the thymus were determined by

Reithman-Frenkel's method (9), while the protein level in the thymus was estimated by the method of Robinson and Holgen, modified by Korpaczy (12).

RESULTS

The values of the metabolic parameters obtained by us in the control group were given in Table 1.

Table 1

The values of some metabolic parameters obtained in the control group : thymus weight (mg) ; TNA (mg/g) ; RNA (mg/g) ; DNA (mg/g) ; protein content in the tthymus (mg%) ;GOT and GPT (micrograms of pyruvate used/minute/g tisuse)

Metabolic Parameters							
	Thymus weight	TNA	RNA	DNA	Protein content	GOT	GPT
$\bar{X}$	208.37	33.07	5.93	26.77	27.77	35.20	66.33
SE	12.05	1.19	0.13	1.15	2.77	7.40	7.50
n	8	8	8	8	8	8	8

Note : In Table 1 are given means ($\bar{X}$) with their standard error (SE) and number of individual values (n).

1. *Thymus weight.* Seventheen days after tumor transplantation, the thymus of the tumor-bearing rats is significantly decreased both as volume and weight; the weight reduction representing 64% ($p < 0.01$) of the control values.

2. *Nucleic acids and proteins.* The dynamics of the nucleic acids in thymus (TNA and RNA) is characterized by a significant decrease of their quantity, already 24 hours after transplantation. After the three days, a relative stabilization of the phenomenon is observed; the 19% decrease of the RNA being highly significant ($p < 0.001$) As the tumor is growing extensively, the quantity of thymic nucleic acids is decreasing progressively. In the 17th days, TNA is reduced by 34%, DNA by 35%, and RNA by 27%, as compared to the values obtained in the thymus of the control group.

The total thymic proteins are reduced by 48% ($p < 0.001$) in the third days of the experiment, but after 9 days the diference v.s. the control values is no statistically significant (−17%). In the seventeen days the total proteins are again decreased by 40% ($p < 0.001$).

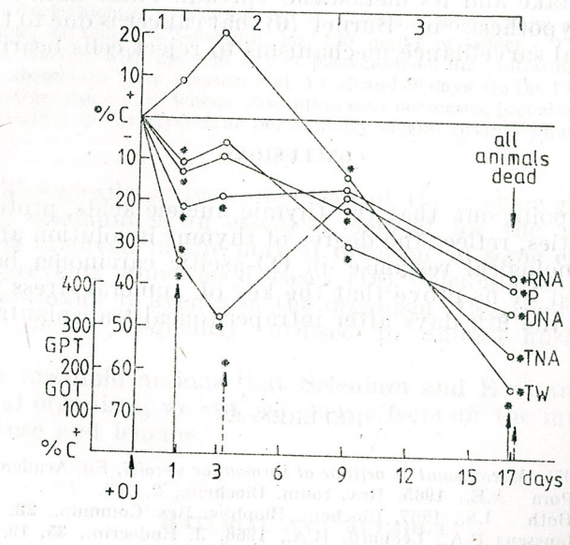
3. *GOT-transaminase activity* already exhibits statistically significant enhancements both on the first day (+398%) and on the seventeenth day (+120%), after tumor transplantation.

4. *GPT-transaminase activity* is also significantly increased on the third days (+196%; $p < 0.001$), and on the seventeenth day following tumor induction (+74%; $p < 0.05$).

DISCUSSIONS

Our results show that the progressive and ireversible thymus involution, induced by a transplanted tumor, is accompanied by characteristic changes in nucleoprotein and protein metabolism.

As a result, immediately after the tumor transplantation, on the background of nucleic acids metabolic inbalance, a decrease of the total protein quantity, associated with an increase of the GOT- and GPT-transaminase activities in the thymus become apparent. Later, between the third and the ninth days of the experiment, concomitantly with the



Fip. 1. — Pegercante differences of the thymus weight (TW), total nucleic acids (TNA), DNA, RNA, total protein content (P), as well as of GOT and PTG activities in the thymus of OJascites carcinoma bearing-rats, compared with the control (C). The asterisc marked statistically significant differences; 1 = alarm phase · 2 = ≡ stress adaptation phase ; 3 = exhaustion phase.

suppression of transaminase activities the decrease of thymic nucleic acids and proteins is observed.

In this way, a decrease of thymic nucleic acids, especially of RNA, is evident already 24 hours after tumor transplantation. This observation is consistent with the fact that the inhibition of the RNA synthesis (10, 14), the decrease of the RNA-polymerase activity (1, 11), and the increase of ribonuclease activities (3) are considered as the major consequence of the early effects of a glucocorticoid excess. This suggests that by tumoral growth induced stress, leads, in the thymus to an inhibition of the RNA "template activity", of the DNA transcription on RNA, as well as of the translation of this information on the polypeptide chains (10). At the same time there are proofs that in the thymus of chicken leukosis virus, which caused transplantable hepatoma, the basal thymidine-kinase activity was lower than those of healthy bird (15).

The nucleic acids and proteic changes in the thymus (23) correspond structurally with a lymphocyte depletion in the thymus, which begins on the first days after tumor transplantation, leading in several weeks to a complete thymus involution (5, 11, 20).

Nine days after tumor transplantation, when the rats begin to die, the quantity of nucleic acids in the thymus, especially that of RNA, decreases in a very similar manner to that observed after 3 days of cortisol administration (4, 23). Thus, thymus responses in tumoral-bearing hosts can illustrate two moments of immunological crisis corresponding to the tumor intake and its metastasic spread. These data are in agreement with the hypothesis of Burnet (6) that cancer is due to the inability of immunological surveillance mechanisms to reject cells bearing neoplastic mutations.

CONCLUSIONS

Our data point out that the thymic nucleic acids, protein or GOT and GPT activities, reflect the degree of thymus involution and respectively the immunological response in OJ-ascitic carcinoma bearing rats. The tests showed by us prove that the key of tumoral stress takes place on the 3rd and the 9th days after intraperitoneal transplantation of the neoplasm.

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THE EFFECTS OF SELENIUM AND E VITAMIN ADMINISTRATION ON THE INTESTINAL ABSORPTION OF GLUCOSE AND LEUCINE IN CHICKENS

RODICA GIURGEA and CORINA ROȘIORU

Cornish-Rock chicknes were administered Selenium or Selenium and E vitamin (0,3 mg/kg of fodder — Selenium, and 120 mg/kg of fodder — E vitamin) for 21 days, starting with the 5-th day of posteclosional life. Intestinal glucose and leucine absorption were measured at 13, 20 and 56 days. On the 13-thr and 20-th day intestinal glucose and leucine absorption were decreased, probably as a result of a modification of the membrane permeability or/and thyroid function.

As a biologically active component of the enzyme glutathione peroxidase (21), Selenium is an essential element for the animal organism (6, 22). It is involved in the normal function of some endocrine glands (7, 8) and prevents numerous diseases (16). Selenium and E vitamin are important antioxidant agents in the organism (19), their associated administration being frequently utilised in animal husbandry (19,23, 15, 20).

Of the mainfold actions that Selenium and E vitamin accomplish in the animal organism, we studied their effects on the intestinal absorption of glucose and leucine.

MATERIAL AND METHODS

Experiments were conducted on Cornish-Rock chickens, which entered the experimental period the 5-th day of their posteclosional life. Three experimental groups were formed, of 60 animals each, as follows: the control group (C); the Selenium-treated group (Se); the group which received both Selenium and E vitamin (SeE). Both compounds were administered in the food, in doses of 0.3 mg/kg of fodder (organic Selenium) and 120 mg/kg of fodder (E vitamin). The administered compounds were from Huhtamäki Oy Novamed. The treatments lasted for 21 days (from the 5-th to the 25-th posteclosional day) afterwhich the chickens received only normal fodder, proper to their age. Fodder and water were given ad libitum and the housing conditions approximated those in avian farms.

The chickens were sacrificed by decapitation between 9 and 11 hours in the morning, after a 16-hours fasting, in the 13—, 20— and 56-th day of posteclosional life.

A 4—5 cm of the jejunum was immediately sampled, everted, rinsed, and ligatured ("everted intestinal sac") (18, 24). Krebs-Henseleit saline, containing 5 mM glucose was introduced in the sac before making the second ligature. It was then incubated in a stopped vial containing 3 ml previously oxygenated saline (as above, but containing 10 mM glucose) to which either ^{14}C-D-glucose or ^{14}C-L-leucine was added; the labelled substances were from the Radioisotope Production Center, Institute for

Physics and Nuclear Engineering, Măgurele. Incubation was performed in a thermostated (40±0.2°C) shaking bath, for one hour. Then, the intestinal sacs were rinsed, blotted and opened, and the fluid of each sac was sampled, its volume measured, and used integrally for liquid scintillation determination of its radioactivity (in a BF-5000/300 type spectrometer, Berthold, Wildbad FRG, using Bray's scintillation mixture). The portion between the two ligatures of each intestinal sac was weighed. The results were expressed as disintegrations per minute (dpm) in the internal fluid of the sac per g of wet intestinal tissue.

Statistical processing of the results included the control of homogeneity of mean values by Chauvenet's criterion, aberrant values being eliminated, and comparison of the means (experimental versus C group) using Student's "t" test. The differences were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

Some data concerning the effects of Selenium and E vitamin deficiency showed important modifications at the intestinal level with the appearance of dispersed haemorrhages (14, 17). It has been shown that oral administered Selenium is absorbed in the propertion of 95—100% at the level of digestive way (9), and that both Selenium and E vitamin play an important role in maintaining the integrity of cell membranes, by controlling lipid peroxidases activity (13, 23).

In our experiment, Selenium and E vitamin were added to the fodder, but not as to be in excess. This supplementation modified the absorption of glucose and leucine in the jejunum : expressed as a decrease of glucose absorption on the 20-th day of posteclosional life (the second sacrification) in both Se and SeE groups, and as a decrease of leucine absorption on the 13-th day in the Se group, followed by an increase on the 56-th day ; in the SeE group, leucine absorption decreased significantly on the 20-th day (Table 1). It results from these data that during the treatment period both glucose and leucine absorptions were inhibited, but after this period the absorption process is directed in the opposite sense.

Table 1

^{14}C-D-glucose and ^{14}C-L-leucine intestinal absorption

	C	Se	SeE
IAG (dpm/g)	Day 13		
$\bar{x}\pm$SE	4091.28±677.88	4259.37±478.58	3739.42±492.24
D%	—	+4.10 SN	— 6.97 NS
n	7	8	7
IAL(dpm/g)	4767.00±455.26	3842.12±615.88	3633.62±779.07
	—	−19.41 $p<0.05$	−23.78 NS
	5	8	8
	Day 20		
IAG	4553.87±930.44	1410.25±139.53	1390.00±93.41
	—	−69.04 $p<0.001$	−69.46 $p<0.01$
	8	8	6

	C	Se	SeE
IAL	6305.12±1096.57	4739.75±572.24	2531.50±426.41
	—	−24.83 NS	−59.86 $p<0.001$
	8	8	8
	Day 56		
IAG	1281.87±369.64	1417.25±303.54	876.37±105.65
	—	+10.61 NS	−31.64 NS
	8	8	8
IAL	1387.00±418.69	2771.50±412.19	1658.37±361.34
	—	+99.81 $p<0.02$	+19.56 NS
	8	8	8

$\bar{x}\pm$SE = mean values±standard error ; D% = percent differences versus control ; n = number of individual data ; IAG = intestinal absorption of glucose ; IAL = intestinal absorption of leucine.

Data concerning the intestinal absorption of Selenium are scarce, so it is difficult to give an explanation for the dynamics of the absorption process. Nevertheless we suppose that Selenium and E vitamin modified the permeability for glucose and leucine of cell membranes (13, 23), thus inhibiting the absorption of these substances, and after the end of the treatment the properties of the intestinal wall were restored towards normal.

On the other hand, there is stated that Selenium influences the level of thyroid hormones (1, 2, 3, 4, 5) and it is well known that these hormones interfere with glucose intestinal absorption. Our previous data (10, 11, 12) showed that the inhibition of the thyroid function by thiourea produces a decrease of the glucose absorption in the jejunum.

We conclude that the Selenium or Selenium+E vitamin administration to the chickens influenced in a negative way glucose and leucine absorption in the jejunum, during the entire administration period (21 days). The cessation of the treatment restored the absorption of glucose and of leucine towards normal values, and in the Se group produced an important increase in the absorption of leucine, as an overshoot above the normal values.

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ULTRASTRUCTURAL EVIDENCE OF TRANSCRIPTION IN HEp-2 LINE CELLS

GR. MIHĂESCU

In thioacetamide treated HEp-2 line cells, a significant quantitative diminution of ribonucleoproteins takes place. Through the positive feed-back control the process of intranucleolar chromatin transcription is greatly increased. The nucleolar organizing region of chromatin activated in this way becomes visible as a granular network with an increased electrodensity in comparison to the nucleolar matrix.

The nucleolus is organized at the level of secondary chromosomal constrictions. Such secondary constrictions involved in nucleolus organization have been designated as nucleolar organizing regions (NOR). The chromosomes bearing such regions have been called nucleolar organizing chromosomes (NO chromosomes) (1—3).

A diploid cell contains as a rule two NO chromosomes, one for every haploid chromosomal complement, but in some instances the number of NO chromosomes involved in the organization of the same nucleolus is as much as five pairs in a human chromosomal set or an undefined number in radicular nodosities of *Trifolium pratense* (6). There is also a direct correspondence between the degree of ploidy and the number of NO chromosomes (4).

Numerous studies have demonstrated RNA synthesis in situ on nucleolar chromatin, but in majority of instances there was not possible to evidence the transcription situses, due both to inadequate autoradiographic resolution and to the highly contorted state of the genetic apparatus within living cells (8).

We have studied electronmicroscopically in situ RNA synthesis in Triturus oocytes (9). This phenomenon has also been studied in HeLa cells (8).

The present work is dealing with electronmicroscopic proofs of intranucleolar chromatin transcription in thioacetamide treated HEp-2 cells.

MATERIAL AND METHODS

Cells of the HEp-2 line from the cell cultures of the Virology Institute, grown on Eagle's medium supplemented with 10% calf serum, have been used in our experiment. When the monolayer was almost complete, the cells were treated with thioacetamide in NaCl 0.85% solution for 3 days, in a quantity equivalent to 150 mg/l medium/day. After the last treatment, the cells were infected with adenovirus 3, and 24h postinfection they were fixed in 2% glutaraldehyde and postfixed in 1% osmium tetraoxide in 0.15 M phosphate buffered solution. The thin sections were stained with uranyl acetate and lead citrate and examined in a Phillips 201 EM.

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RESULTS AND DISCUSSION

HEp-2 line cells of malign origin are heteroploid and generally have 1 or 2 nucleoli (Fig. 1).

Thioacetamide has an obvious effect on HEp-2 line cells. The result of its action is lysis of cytoplasmic components, specially a quantitative diminution of ribosomes. As a result of positive feed-back control, the nucleolus, a veritable deposit of ribosomal subunits, greatly increases its sizes (5), because r RNA synthesis is made with a much enhanced rate.

In some cells, after thioacetamide treatment, multiple nucleoli are seen (Fig. 2). These are the result of compensatory genes amplification of the nucleolus organizing region. In this region, cystrons for r RNA 18 S, 28 S, 5,8 S synthesis (2) are localized.

In some instances, the appearance of images which suggest the transcription course of r DNA genes is surprising (Figs. 3—6). The lines, sometimes ordered, with granular texture, are very probably genes for r RNA synthesis. Their granular appearance is given by RNA polymerase molecules which are arranged in a line on the length of the transcribed cystron. In the nucleolar matrix of granular consistence we cannot follow the lines of RNA fibrils which are in course of synthesis.

Our images suggest that at the level of nucleolar organizing region, the chromosome has a fibrillar unwinding structure, analogous to that observed in Balbiani rings in polytenic dipteran chromosomes. The nucleolar organizing region of chromosome is analogous to a loop from lampbrush chromosomes of amphibian oocytes (9).

The transcription process of nucleolar organizing chromatine becomes more visible after nucleolar segregation in its granular and fibrillar components (Figs. 7, 8). In this instance, the segregation is the result of adenovirus 3 replication (7). The image of a DNA region transcribed in rRNA is suggested by a double linear electrondense granular structure, which delineates an electron clear space. The granular structure is contiguous to r RNA molecule which goes on with a fibrillar nucleolus component.

CONCLUSIONS

1. r RNA synthesis is experimentally greatly increased after thioacetamide treatment.
2. Transcription of chromosomal nucleolar organizing region is electronoptic evidenced as a granular dense network, sometimes ordered, which follows the line of unwound DNA loops.
3. After nucleolar components segregation, images of intranucleolar chromatin transcription are rarely observed only in the fibrillar region.

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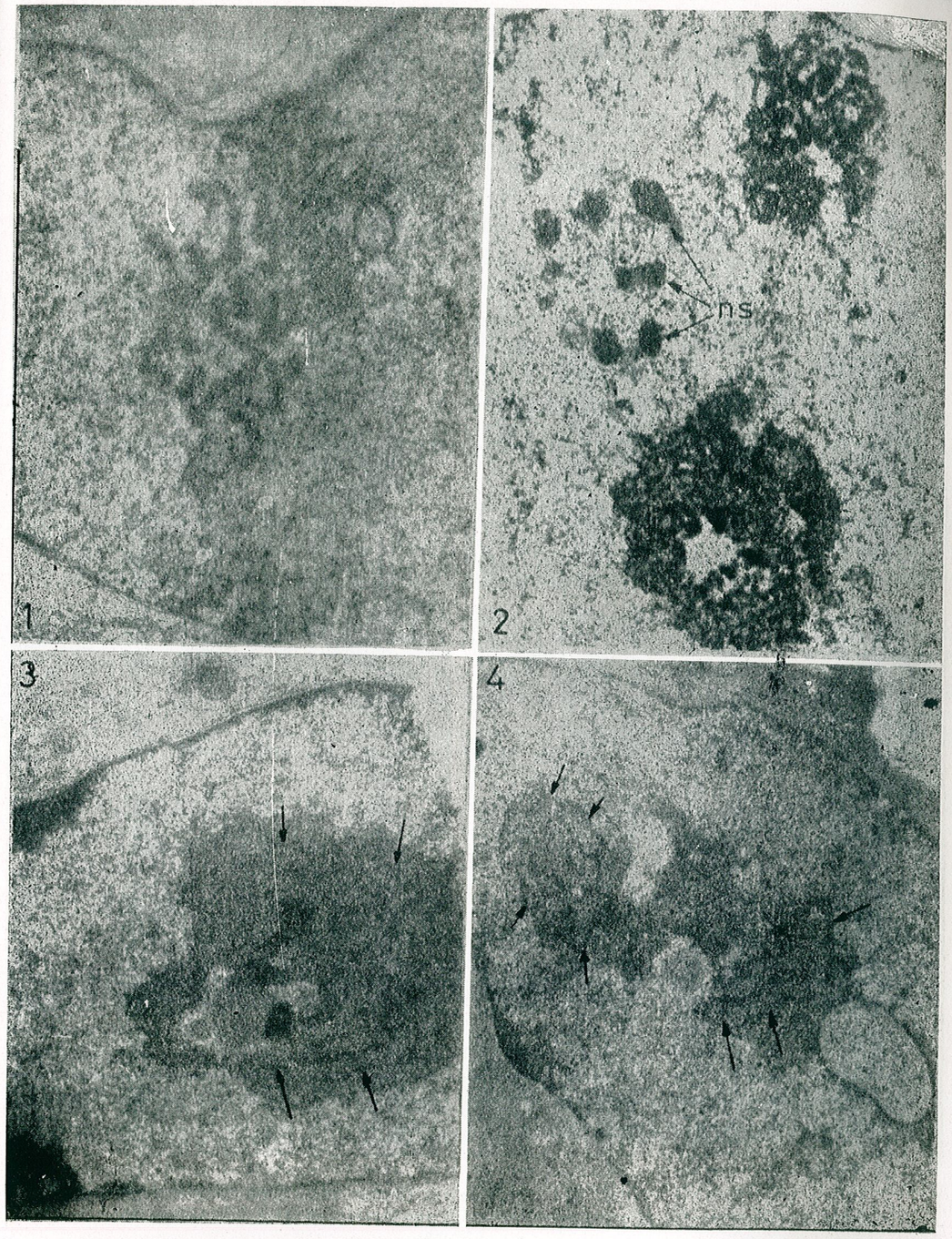
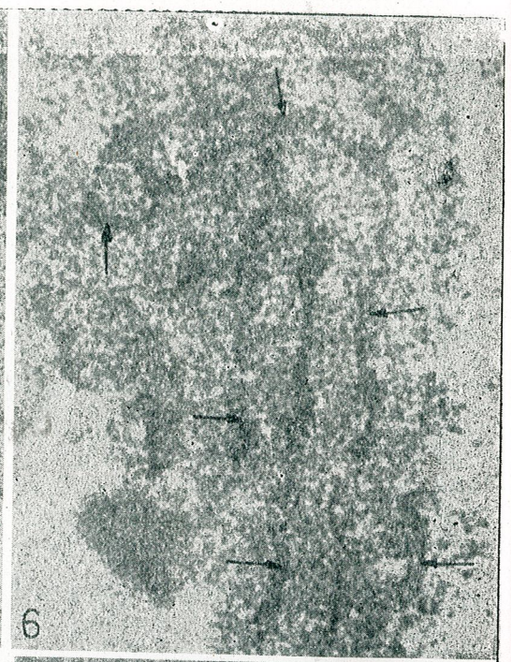
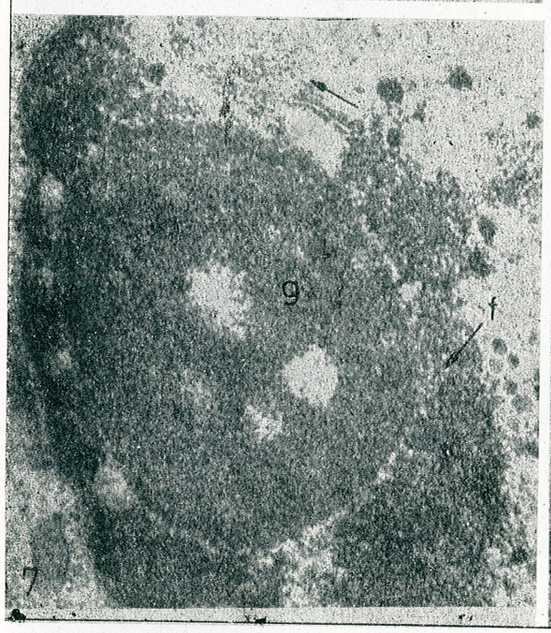
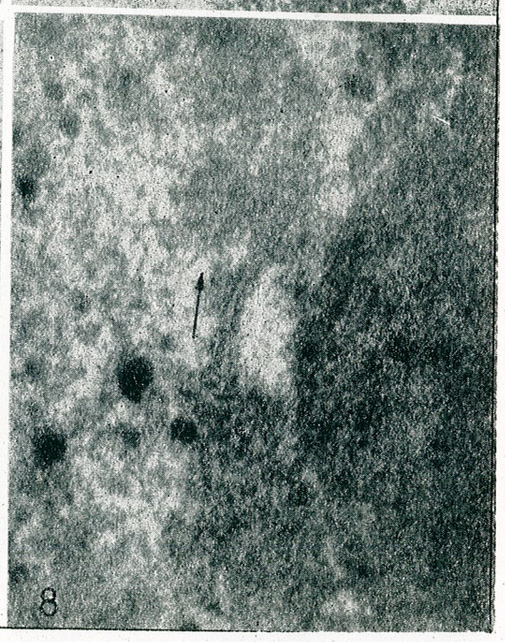


Fig. 1. — The nucleolus of an untreated HEp-2 line cell. The two nucleolar components (granular and fibrillar) are closely intermingled, ×30 000.
Fig. 2. — After thioacetamide treatment, the process of chromatin nucleolar transcription is greatly increased. The amplification of nucleolus organizing genes. Its morphological sign is the appearance of multiple satellite nucleoli, ×22 000.
Figs. 3, 4. — In the nucleolar area, a network of granular texture more electrondense than the nucleolar matrix is observed. The network results from unwinding of DNA fibers of chromatin nucleolus organizing region, ×30 000.







Figs. 5, 6. — Details of images 4 and respectively 3. Electrondense nucleolar network is superposed to DNA loops. The granules probably signify the presence of RNA polymerase molecules, ×59 000.

Fig. 7. — The nucleolus of thioacetamide and afterwards adenovirus 3 infected HEp-2 cell. The two nucleolar components are segregated. The fibrillar component is subdivided in two distinct zones, connected by a double granular structure which delimits an electron clear core, ×59 000.

Fig. 8. — A detail of Fig. 7. The double granular structure is the anchorage zone of some fibrillar structures, the length of which increases in the arrow sense, and are continued to nucleolar fibrils, ×90 000.

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ESTIMATION OF MUTAGENE EFFECT OF CHLORILATE

NICOLAE COMAN, MANUELA DORDEA and CORNELIA CRĂCIUNAȘ

The mutagene effect of chlorilate was investigated. The experiments were carried out on *Drosophila melanogaster* using the CyPm method. Two chlorilate concentrations were tested: a higher concentration equal with LD_{50} (concentration previously estimated to 1100 ppm) and a concentration equal to the dose most frequently used in agriculture (160 ppm). The results were compared with a control group raised on pesticide-lacking medium.
It has been found that both concentrations of chlorilate induce high frequencies of lethal recessive mutations (9.36% and 8.18%, respectively), while the increase ratio of the detrimental ones is low. This suggests that the tested herbicide acts upon the genetic material mainly through chromosomal breaks and not through point mutations. Taking into account the high percent of lethal recessive mutations induced by the agricultural dose of chlorilate, we conclude that the use of this herbicide should be limited only to the early stages of cultures, so that it may have the necessary time to clear away from plant tissues until harvesting.

Chlorilate, known under the commercial names of ramrod, propachlor or satecid, is a herbicide largely and successfully used in the protection of various plant crops. Chlorilate-based pesticides do not have negative effects upon soil [1, 3], but they seem to affect the proteosynthesis processes in germinated barley seeds [4] and in barley, wheat and pea adult shoots [2].

The present paper investigates the mutagenic effect of chlorilate upon a non-target species — *Drosophila melanogaster* — both for the LD_{50} concentration and the one used in agriculture.

MATERIAL AND METHOD

The Curly-Lobe-Plum (CyLPm) method, set up by Wallace [6] for testing the mutagenic effect of radiations, has been used with adequate adjustments for the study of pesticide effects. The method consists in homozygotizing a line of *Drosophila melanogaster* for the second pair of chromosomes, the treatment of homozygotized specimens with the analysed pesticide, and then re-homozygotizing the chromosomes resulting from pesticide-treated specimens. When a lethal recessive mutation is induced in one or both chromosomes of the second pair of treated specimens, the homozygotized descendants for the respective mutation are not viable. According to Vogel [5], the test of lethal recessive mutations is one of the most accurate means for estimating the mutations induced by various environmental factors.

The experiments were carried out with two chlorilate (2 chlor-N-(1-methyl-ethyl)-N-phenyl-acetomine) concentrations : a higher concentration, equal with LD_{50} in *Drosophila melanogaster* (concentration previously estimated to 1100 ppm), and a lower concentration, equal to the dose

most frequently used in agriculture (160 ppm). The schematic of the method is presented in Fig. 1.

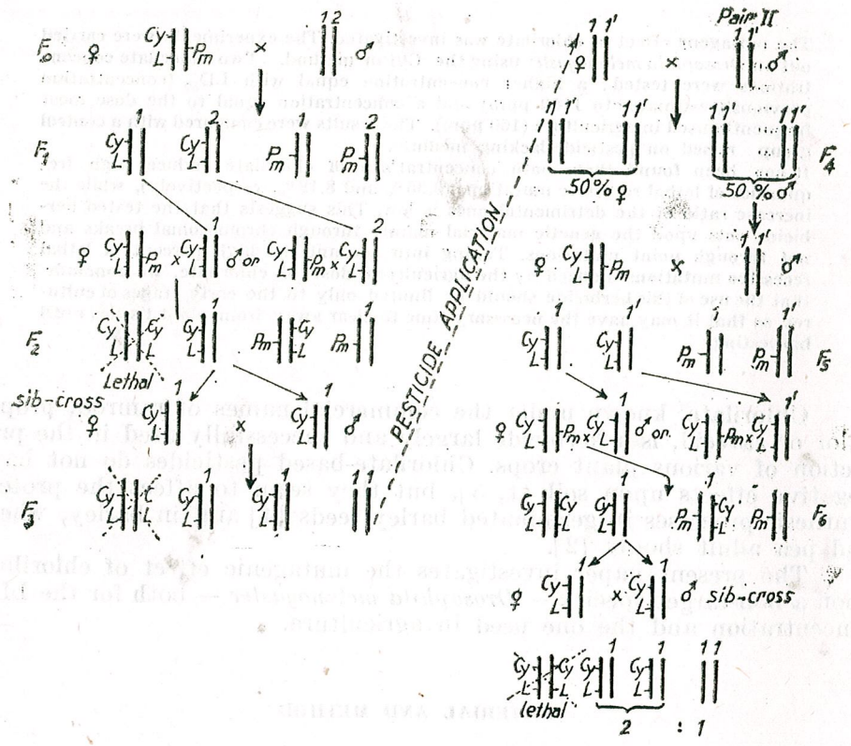

Fig. 1. — Diagram of the crossing for the CyLPm test.

A parallel control group was prepared, the homozygotized males for the second pair of chromosomes being raised on pesticide-lacking medium.

The ratio between CyL/+and +/+ specimens was estimated in each of the 200 culture vessels of generation F_7, so that the large number of specimens analysed assures the significance of the interpretation of the results. When the homozygotized form of the wild +/+ type displays a lethal recessive mutation on the second pair of chromosomes, the specimens will not be viable and, therefore, the whole generation F_7 will consist only of CyL/+ forms. But if the second pair of chromosomes in +/+ forms displays one or more detrimental mutations (subvital, substerile, subfertile), viability is somehow affected so that the ratio 2 : 1 in F_7 is disturbed due to the decreased occurrence of wild forms and the increased occurence of CyL/+ forms.

RESULTS AND DISCUSSIONS

In the control group (Fig. 2), only 0.99% of the specimens tested have been found to display lethal recessive mutations on their second pair of chromosomes, this value comparing favourably with the one (1%) estimated by Wallace [6] and Vogel [5].

The analysis of deviations from the ratio 2 : 1 (i.e. 66.66% CyL/+ : 33.33%+/+) has shown that 78.94% of the descendants of the specimens analysed range around the mean value, with an insignificant deviation of ±5%. 20.07% of these specimens display detrimental mutations, which affect to a various extent the viability of their descendents. Thus, 13.5% of the F_7 specimens deviate by 5—10% and 6.57% of the specimens by 10—15% in favour of the CyL/+ form.

This natural mutagenesis is a permanent factor for selection and, implicitly, for the modification of the population gene pool.

The chlorilate dose used in agriculture (160 ppm) induces a high percent (8.18%) of lethal recessive mutations in *Drosophila melanogaster* (Fig. 3), a figure which is 8 times larger than the one appearing in the control. Of all the specimens tested, 31.82% display detrimental mutations, i.e. almost 12% more than in the control. 21.82% of them deviate by 5—10%, 8.18% by 10—15% and 1.82% deviate by 25—30% in favour of the CyL/+ form.

It is, therefore, obvious that the chlorilate dose used in agriculture (160 ppm) leads to a significant increase both in detrimental mutations and in lethal recessive ones.

Chlorilate variant LD_{50} (concentration of 1100 ppm) brings also a high frequency of detrimental and lethal recessive mutations. The data in Fig. 4 show that 23.73% of the analysed specimens are affected by detrimental mutations: 17.8% with a deviation of 5—10% and 3.39% with a deviation of 10—15% as compared to the normal ratio of 2 : 1 in F_7. Mention should be made of the presence of 2 other categories of individuals (representing 1.69% and 0.85%), which display a segregation deviation of 15—20% and 20—25%, respectively.

Although the detrimental mutations induced by chlorilate dose LD_{50} are not more frequent than the ones induced by the agricultural dose, the frequency of the lethal recessive mutations is higher for the former dose (9.36%).

It is noteworthy that the frequency of lethal recessive mutations induced by chlorilate does not increase proportionally to the dose, reaching 8.18% for the agricultural dose (160 ppm), i.e. eight times larger than the one in the control, and 9.36% for the LD_{50} (1100ppm), i.e. nine times larger than the one in the control.

Since the occurrence of chlorilate-induced lethal recessive mutations is much more frequent than the occurrence of detrimental ones, it may be assumed that chlorilate acts mainly through chromosomal breaks.

CONCLUSIONS

— The chlorilate dose used in agriculture induces an increase in the frequ ency of lethal recessive mutatons eight times higher than in the control.

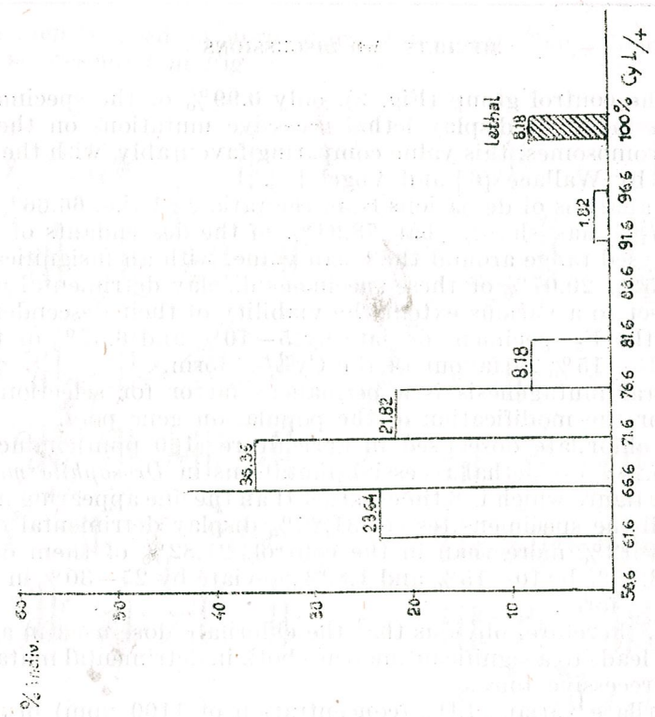

Fig. 3. — Frequency of deleterious and lethal recessive mutations after treatment with chlorilate at agricultural dose (160 ppm).

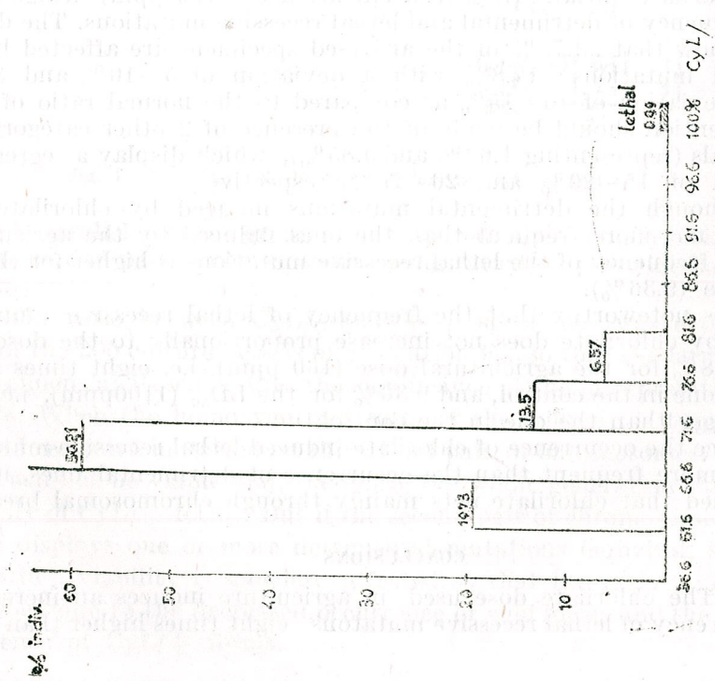

Fig. 2. — Frequency of deleterious and lethal recessive mutations in control, after the use of the CyLPm test.

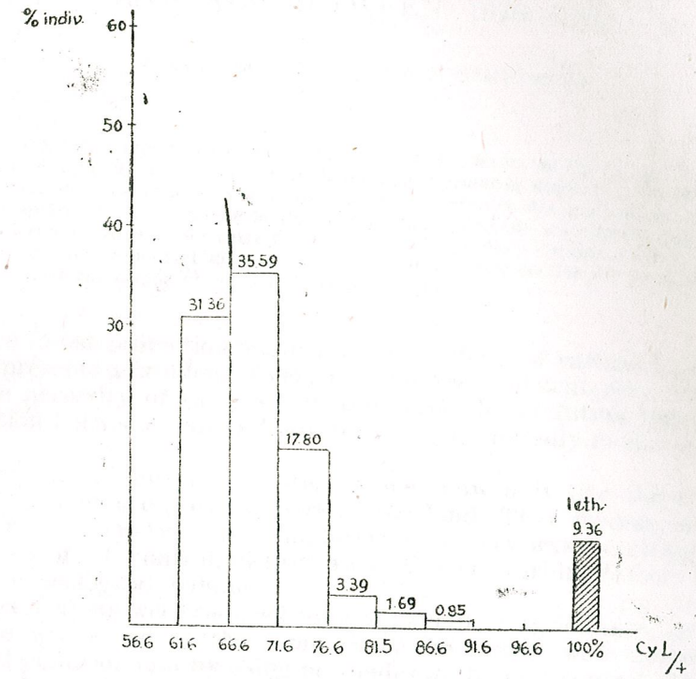

Fig. 4. — Frequency of deleterious and lethal recessive mutations after treatment with chlorilate at LD_{50} (0.11%).

— Chlorilate LD_{50} induces an increase in the frequency of lethal recessive mutations nine times higher than in the control.

— The frequency of chlorilate-induced lethal mutations is much higher than the frequency of detrimental ones, this suggesting that the tested herbicide acts upon the genetic material mainly through chromosomal breaks and not through point mutations.

— The high percent of lethal recessive mutations induced by the agricultural dose of chlorilate requires that the use of this herbicide should be limited only to the early stages of cultures, so that it may have the necessary time to clear away from plant tissues until harvesting.

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LYMANTRIA DISPAR ATTACK DYNAMICS IN ROMANIA BETWEEN 1976–1990

IRINA TEODORESCU and A. SIMIONESCU

Les recherches effectuées pendant 14 ans dans les forêts de *Quercineae* du sud de la Roumanie, attaquées par le défoliateur *Lymantria dispar* L., ont permis de tirer des conclusions concernant les caractéristiques des graduations de ce lepidoptère et les causes de la dynamique particulière de ses effectifs pendant ces dernières années. Sur cette base on été élaborées des solutions concernant la stratégie du moment et de perspective dans le contrôle corrélé des populations du *Lymantria dispar* et de ses ennemis naturels.

The forest protection against adverse effects of various human activities represents a problem of very high interest and acuteness, that arises from the necessity of the present and especially of future regulation of the relation between man and environment, profoundly modified by man himself.

The natural forest ecosystems cover now only one third of their initial area over the greatest part of the land. They include biocenoses with a great diversity, with complex trophic networks, resulting in high possibilities of self-control, of resistance to the disturbing factors that can affect the ecological balance.

Forest areas were reduced for the benefit of agriculture, by irrational exploitation and at the same time, the forests were destroyed by industrial pollution and by using pesticides in the pest control. Generally, the lack of knowledge or ignoring the present and future consequences of various human activities led to the alteration of the forest biocenoses structure, by reduction of their diversity and self-control capacity, with negative results upon the whole biosphere (7).

The negative direct effects of the human impact upon the forests, emphasized by the continuous and even more accelerated rate of their surface decrease are more conspicuous and easy to estimate. The indirect effects have as a result the destruction of the ecological balance, followed by the increase of the pests attack. These effects are more difficult to identify, to quantify and to anticipate, but they are not less important than the direct ones.

Among the world preoccupations for the knowledge of these effects, our research refers to one of the most studied insects, *Lymantria dispar* L. (gipsy moth). The larvae feed on more than 300 plant species, but the Quercineae are the most favoured food. Their attack produces great damages on large surfaces in Europe, North America, Asia and Africa.

This pest is present in all regions of Romania, its attack having economic consequences on surfaces of 61.9% in the Romanian Plain, 10.5% in Muntenia and Oltenia subcarpathian hills, 9.3% in Dobrogea, 8.65% in the West Plain of Transylvania, 8.04% in Banat and 1.5% in Moldova and Transylvania subcarpathian hills and plateau (Fig. 1). In these regions the pest develops frequent and highly intense graduations, with partial or total defoliations.

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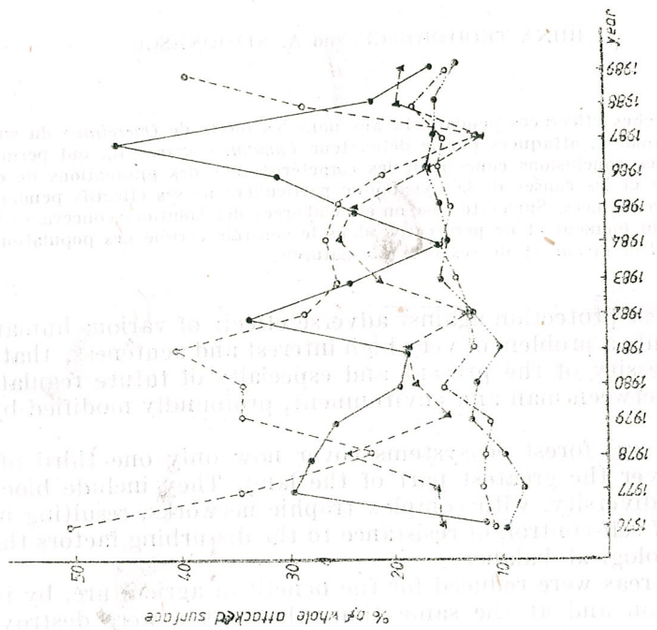

Fig. 2. — Areas weight dynamics with very weak (0...0), weak (0— —0), mean (0—.—0), strong (— . . .0) and very strong (0—0) attack between 1976—1989.

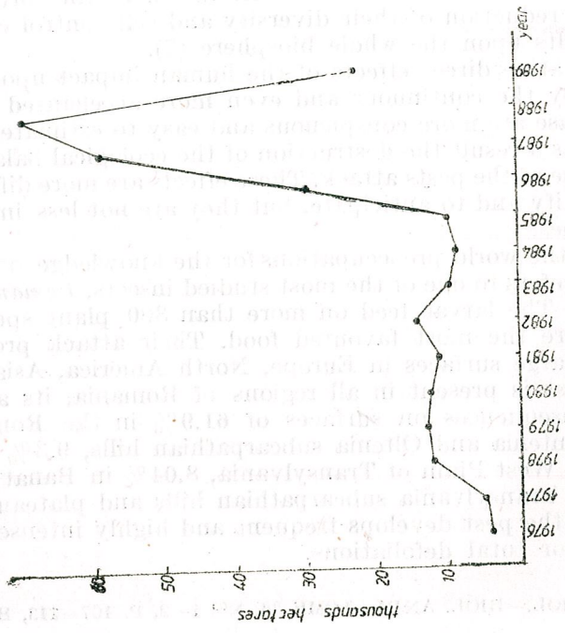

Fig. 1. — Dynamics of whole attacked areas by *Lymantria dispar* between 1976—1989.

Regarding the dynamics of forest surfaces attacked during the last 14 years, a little increase was recorded between 1976—1986, from 37,700 ha to approximatively 100,000 ha. In 1986 and especially in 1987, the attacked surfaces suddenly extended, realising 582,000 ha (15 times the value of 1976). The process continued in 1988, the attack covering 695.100 ha (Fig. 2). At the same time with the increase of all surfaces, the strongly attacked surfaces increased from 11.4% in 1976, to 45.5% in 1987. The surfaces with a very weak attack decreased 3.7 times from 50.1% in 1976, to 13.5% in 1987.

In the following years, the situation changed, the forest surfaces with a very weak attack increased from 27.4% in 1988 to 39.16% in 1989, while the surfaces with a very strong attack decreased from 22.38% in 1988 to 15.76% in 1989 (Fig. 2, Table 1).

Table 1

Areas weight dynamics with different degree of attack intensity between 1976—1989

Years	Attack intensity				
	very weak attack	weak attack	mean attack	strong attack	very strong attack
1976	50.1	15.7	12.2	10.6	11.4
1977	34.0	15.9	12.4	8.5	29.2
1978	23.8	24.9	12.5	10.2	28.6
1979	34.2	14.3	12.0	13.3	26.2
1980	34.85	17.82	14.59	12.96	19.77
1981	41.1	19.8	12.4	8.5	18.2
1982	28.4	13.4	13.0	12.0	33.2
1983	26.3	22.2	13.9	14.0	23.6
1984	28.3	26.3	15.2	13.9	16.3
1985	26.5	22.6	14.0	14.4	22.5
1986	22.0	17.0	15.0	15.0	31.0
1987	13.5	12.2	14.0	14.8	45.5
1988	27.40	19.99	16.69	13.59	22.38
1989	39.16	18.79	13.93	12.35	15.76

The increase of the attack level between 1976—1989 years was confirmed by the graduation phases of the pest, established according, to the mean fecundity values, obtained by examination of lepidopterous egg-masses, collected in attacked Quercineae forests in the South of Romania. (Table 2, Fig. 3). In more than 70% of the investigated forests (mean annual value), the pest was in the second phase, numerical increase (35.34%) and the third phase, eruption (35.16%), characterized by intense attacks, with defoliations on large surfaces. In 1979, 1981—1983 and 1987 years, on over 80% of all surfaces the pest was in the second and third phases. Between 1976—1989 years, the pest was in the prograduation period in 88.79% of the attacked surfaces and in the retrograduation (fourth phase, crisis) only in 11.21% of the same surfaces. The first incipient phase was represented on only 17.84% of all surfaces attacked.

The particular populations dynamic sunderlines the lack of efficiency of the natural control factors and especially of the biotic ones, responsi-

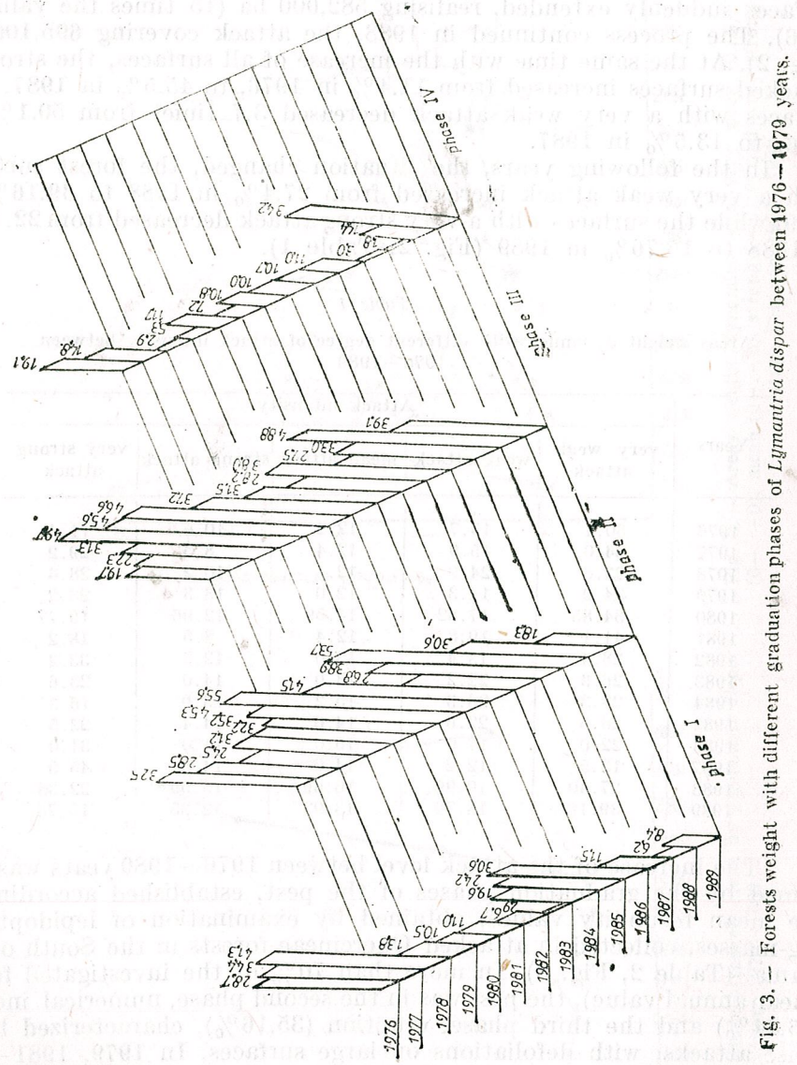

Fig. 3. — Forests weight with different graduation phases of *Lymantria dispar* between 1976—1979 years.

Table 2

Forest weight (%) with different graduation phases of *Lymantria dispar* in all attacked surfaces

Year	Graduation phases			
	Prograduation		Retrograduation	
	Incipient phase (I)	Numerical increase phase (II)	Eruption phase (III)	Crisis phase (IV)
1976	28.7	32.5	19.7	19.1
1977	34.4	28.5	22.3	14.8
1978	41.3	24.5	31.3	2.9
1979	13.8	31.2	49.7	5.3
1980	10.5	32.2	45.6	11.7
1981	11.0	35.2	46.6	7.2
1982	6.7	45.3	37.2	10.8
1983	2.9	55.6	31.5	10.0
1984	19.6	41.5	28.2	10.7
1985	24.2	26.8	38.9	11.0
1986	30.6	38.9	27.5	3.0
1987	11.5	53.7	33.0	11.8
1988	6.2	30.6	48.8	14.4
1989	8.4	18.3	39.1	34.2
	17.84	35.34	35.61	11.21

ble for the regulation of pest populations (1, 3, 4, 5). Moreover, in some forests surveyed many years consecutively, it was noticed a return of the small fecundity values (indicating the transition to eruption) to high levels, characteristic for the numerical increase phase, especially dangerous to forests (8, 9, 10).

All these facts underline also the inefficiency of the use of pesticides against pest populations. For many years, the organochlorine pesticides, with DDT as active ingredient (Detox-25, Defotox-16, Omicid-13 etc). They were substituted in 1985 by the organophosphoric insecticides were used, (Silvetox, Onefon, Carbetox etc.), pyrethroids (Decis ULV, Fastac ULV, Ripcord ULV, Nurrell etc.) and Dimilin (diflubenzuron) (2, 6).

The elimination of the natural enemies by the toxic action of pesticides led to the perpetuation of pest graduations with increasing the pest populations above the levels existing prior to treatments.

The decrease of oophagous parasites populations, emphasized by the very low degree of parasitation of egg-masses in the investigated forests, and also the reduction of other entomophagous (parasites and predators) represent the consequence of chemical control.

It is well known that the populations of natural enemies and their hosts represent the dynamic systems, resulted from their correlated evolution, by reciprocal adaptation. These systems have a reciprocal mechanisms for their effective regulations and maintained between certain limits compatible with their time/space persistence. Every subsystem of the whole system has the capacity to adapt its birth rate and mortality to the same parameters of the other subsystem, by negative feedback mechanisms. Each subsystem induces thus correlated changes to the other subsystem, keeping mutually under control the populations level.

When the natural enemies are represented by very small populations, they are unable to control the populations level of their host. In these circumstances the host multiplies excessively, for a long period of time, the possible fluctuations of pest populations effectives being induced only by the abiotic factors variations. This was the situation during the springs of 1989 and 1990, when the populations of many pests, including *Lymantria dispar*, were exposed to high temperatures in February (when the larvae eclosed), followed by a low temperature period, especially at night. This factor together with food shortage led to death of larvae. So, *Lymantria dispar* had very low populations in 1989, consisting of larvae eclosed after February. The analysis of egg-masses, laid in autumn, indicated low values of the mean fecundity (on 34.2% of surfaces) characteristic for the crisis phase. In 1990, the situation of 1989 appeared again due to the same climatic conditions in February and March.

Although *Lymantria dispar* dit not represent a problem in 1989 and 1990 in Quercineae forests in the South of Romania, the tendencies of its population dynamics must be permanently investigated. As a general rule, there is a natural tendency of populations increase in favourable environmental conditions. When the natural enemies are not efficient, this tendency may lead to the exceeding of the stability domain compatible with populations persistance. This fact may lead to a new and dangerous increase of gipsy-moth populations, during the next period of time.

The use of pesticides in an attempt to reduce the pest populations does not solve, but even aggravates the lack of balance between the natural enemies and pest populations, in favour of the latter.

The gipsy-moth is also favoured, as all phytophagous, by the existence of the chemical mechanisms for the annihilation of various toxical substances existing in plants. These mechanisms were acquired by natural selection for the detoxification of alkaloids and glycosides, contained by plants as a means of passive defence against the phytophagous. The latter employ these mechanisms to decompose the pesticides in unnoxious compounds.

The entomophagous have no such detoxifying mechanisms and they are more affected by pesticides. The decrease of their populations allows the pest to escape from their biological control.

CONCLUSIONS

For a long time, *Lymantria dispar* has developed large populations and massive defoliations were registered in the Quercineae forests in the South of Romania.

The use of the chemical method to control this pest generated a series of effects, which paradoxially favoured the pest populations. This method induced a particular dynamics of pest populations, with the progradation perpetuation, predominance of the numerical increase and eruption phases, return from the levels specific for the eruption phase, to those of the numerical increase, diminution or absence of the crisis and

latency periods, for a long time period. The esplanation consists in :

— the natural tendency of the populations to maintain their effectives at optimal levels, providing their perenniality, is in contradiction with man's attempt to lower these levels to economically acceptable values ;

— the possibility of phytophagous to decompose the pesticides into unnoxious compounds and, on this basis, to develop resistant races ; the parasites and predators have no such ability, this aspect leading to obviously adverse effects upon them ;

— numerical pest increase was facilitated by the low resistance of trees affected by pesticides, various industrial noxae and by very dry summers, with high temperatures, during the last years.

Essentially, the human activities alter the three functions of the forestry ecosystems, especially the self-control one.

The way to solve the problem of *Lymantria dispar* attack can be solved only using the cause-effect correlation : the implemented measures must act upon the effect (massive increase of pest populations) by elimination of the cause (the use of pesticides).

The use of microbial, hormonal and autocidal methods in the pests control allows restoration of the entomophagous populations and on this basis, the restoration of the dynamic equilibrium between their populations and that of their hosts.

The actual depression moment of the pest populations must be used to replace the chemical method by unpolluting means of pests control.

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DETERMINATION OF BACTERIAL OXYGEN CONSUMPTION IN THE WATER-BODY OF EUTROPHIC LAKE SYSTEMS

DORINA NICOLESCU

The estimation of bacterial oxygen consumption aroused much interest in the researches concerning the natural bacterial associations in the aquatic ecosystems especially owing to the impossibilities of separating the bacteria from the other components of plankton. The present paper suggests a method *in situ* able to bring a more precise determination by eliminating the respiratory consumption of algal nannoplancton.

It becomes more and more evident the fact that making evident and quantifying the functioning of the bacterial natural associations in the aquatic ecosystems can no longer be neglected; efforts are being made to surpass the difficulties in approaching the research methodology and technics.

Within the ecological practice, we consider the bacterial oxygen consumption determined "in situ" (aerobic respiration) as one of the most instructing parameters of the metabolic activity of bacterial natural associations in the lake systems.

The integrated researches upon the eutrophic lake systems in the Danube Delta, characterized by a high primary production (micro- and macrophytic), a rich input of organic substance, a high numerical density of heterotrophic bacteria, have aimed at the quantification of oxidative bacterial destruction of the organic substance in the water-body, taking as basis the bacterial oxygen consumption.

ANALYSIS OF THE EXISTING METHODOLOGY

The most used method of determining the oxygen bacterial consumption, by "in situ" experimentation, is a classic one. It deals with determining the dissolved oxygen (by the Winkler method) at the initial moment of the experiment, into a volume of filtered water and after 24 hours of incubation in the dark, the difference being conferred on the respiration of bacterial natural associations in the water-body. The filtration with the purpose of separating the bacteria from the other components of the plankton is achieved by different technics tested by us for the actual conditions in the Danube Delta lakes.

Thus, the filtration of the samples through cottonwool (1; 2) or through zooplanktonic net no. 25 (60—80 μm) (10) is recommended. The method proved to be unsatisfactory as components of the algal nannoplankton and some ciliates and rotifers penetrate the filtered matter and the value of the respiratory consumption assigned to the bacteria is distorted by their respiration. Another technique of excluding the phyto- and zooplankton is recommended by Y. Tezuka (9), it deals with the filtration through membrane filters of 5—8 μm or through filters with

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1.2 μm (8) porosity. This technique excludes the inconveniences previously shown, gives results appreciated as real in certain periods, but it presents a different factor of inaccuracy. In the ecosystems where a great quantity of organic substance is circulated, the planktonic bacteria are found preponderantly agglomerated in "detritus-bacterial aggregates" (3 ; 5) which do not penetrate the filters in their majority leading to their clogging.

THE PROPOSED METHOD

The testing of different technics under specific conditions of lake systems in the Danube Delta led us to the idea of a method composed of combined techniques through which we consider that most proxime to reality values of bacterial oxygen consumption are obtained (6).

Medium water samples from surface till bottom, directly filtered through zooplanktonic net no. 25, are gathered into glass bottles and an experiment of determining the primary productivity of nannoplankton is set by the Vinberg method ("of white and black bottles") with a double purpose :

— determining the gross primary productivity of algae in the filtered water-samples ;

— determining the oxygen consumption due to the components in the filtered water-samples (bacteria, algae, ciliates, rotifers), consumption assigned by the methods previously analysed to the bacteria.

The fact that the respiration of the phytoplankton represents 10—50% from the gross productivity, taking into account the environment conditions, is known from the specialised literature. The experts consider that 20% from the gross productivity represent the most probable medium value of the phytoplankton respiratory consumption (11 ; 3 ; 7). The algal nannoplankton respiration may be eliminated from the oxygen consumption experimentally determined.

A few examples resulted from the achieved experiments in the aquatic ecosystems in the Danube Delta will allow us to appreciate the performances of this proceeding in eliminating the interference of algal nannoplankton respiration with the bacterial oxygen consumption :

— *Roşu lake* (*Danube Delta*) — *July 1985* :

gross phytoplanktonic productivity (filtered water) = 3.420 mg O_2/l/24h
planktonic respiratory consumption = 1.300 mg O_2/l/24 h
phytoplanktonic respiration (20% from g.p.p.) = 0.684 mg O_2/l/24 h
oxygen bacterial consumption = (1.300—0.684) = 0.616 mg O_2/l/24 h

— *Matiţa lake* (*Danube Delta*) — *July 1982* :

gross phytoplanktonic productivity (filtered water) = 5.892 mg O_2/l/24 h
planktonic respiratory consumption = 2.946 mg O_2/l/24 h
phytoplanktonic respiration (20% from g.p.p.) = 1.078 mg O_2/l/24 h
oxygen bacterial consumption (2.946—1.078) = 1.868 mg O_2/l/24 h

— *Maliţa lake* (*Danube Delta*) — *July 1985* :

gross phytoplanktonic productivity (filtered water) = 1.470 mg O_2/l/24 h

planktonic respiratory consumption = 0.980 mg O_2/l/24 h
phytoplanktonic respiration (20% from g.p.p.) = 0.294 mg O_2/l/24 h
oxygen bacterial consumption (0.980—0.294) = 0.686 mg O_2/l/24 h

— *Matiţa lake* (*Danube Delta*) — *October 1985* :

gross phytoplanktonic productivity (filtered water) = 4.210 mg O_2/l/24 h
planktonic respiratory consumption = 1.180 mg O_2/l/24 h
phytoplanktonic respiration (20% from g.p.p.) = 0.842 mg O_2/l/24 h
oxygen bacterial consumption (1.180—0.842) = 0.338 mg O_2/l/24 h

It is worth mentioning that by the previously enunciated methods, at the best, the whole respiratory consumption in the filtered samples was assigned to the bacterial respiration (consumption resulted from the difference between the O_2 content dissolved the initial moment and after 24 hours of incubation in the dark).

The oxygen consumption due to the phytoplankton respiration in the filtered water (algal nannoplankton) is excluded by the described proceeding ; the above examples are relevant for the interference of this factor with the oxygen consumption values. In addition to the value alterations of the bacterial oxygen consumption, the fact that the development fluctuations of some or other algal populations during the year give a different quantum of influence of the respiratory consumption on the samples must be taken into account. These fluctuations bring so much more prejudices to the real curve of evolution in time of the bacterial oxygen consumption, using this method of determining the bacterial oxygen consumption, the image distortion of metabolic activity of the bacterial plankton is avoided.

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LA BIOMASSE BACTÉRIENNE PLANCTONIQUE DU DANUBE À L'EMBOUCHURE DANS LE DELTA (km 80 ET km 62)

DORINA NICOLESCU

Le travail a été présenté à la XXVIII[e] Conférence du Danube (Mamaia, 1988).
On a essayé de vérifier une méthodologie de calcul qui puisse compléter le tableau de la biomasse bactérienne du Danube pendant les années 1980—1985, en réalisant les déterminations seulement pour l'année 1984.

Au cours des années 1981—1985, dans le Danube au km 80 et au km 62 (respectivement — Ceatal Izmail et Ceatal Sf. Gheorghe) on a effectué des recherches portant sur la densité numérique du bactérioplancton dans les horizons de surface et à 10 m de profondeur (au centre) en cultures sur gélose nutritive, dans la période avril-octobre, annuellement. Les résultats ont mis en évidence des valeurs élevées, à grandes variations, en fonction de l'année et de la saison, ainsi que des différences entre Ceatal Izmail et Ceatal Sf. Gheorghe (3). En 1984, on a effectué parallèlement des déterminations sur la biomasse bactérienne, par la détermination de la concentration de l'ATP cellulaire, selon la méthode Holm-Hensen (2 ; 1) dans la manière de séparation et de détermination des différentes fractions du C.O.P. (5). Les résultats sont présentés dans le tableau n° 1.

En base des données sur les 2 variables (le nombre total de germes/l et la biomasse correspondante déterminée — mg/l), de l'année 1984, on a effectué une corrélation et on a obtenu un indice de corrélation r= —0,9893 et la suivante équation de régression :

$$\log y = -0{,}801579 - 0{,}930129 \log x$$

où :

x = nombre de germes/l ; y = biomasse/germe

Tableau 1

La biomasse bactérienne planctonique du Danube, en 1984 mg/l poids humide

Profil / mois	km 80 Ceatal Izmail centre	km 62 Ceatal Sf. Gheorghe centre
Avril	2.20	1,80
Mai	1,28	0,60
Juin	0,35	0,40
Juillet	2,90	1,10
Août	1,80	1,50
Septembre	2,70	1,90
Octobre	1,50	1,19
$\overline{X}_a$	1,82	1,19

Tableau 2

La biomase bactérienne planctonique du Danube (valeurs moyennes annuelles—par calcul de régression) mg/l poids humide

Profil / l'année	km 80 Ceatal Izmail	km 62 Ceatal Sf. Gheorghe
1981	0,80	0,78
1982	0,79	0,80
1983	1,53	1,62
1984*	1,38	1,28
1985	1,40	1,39

* Pour comparaison dans le tableau sont inscrites les données obtenues par le calcul de régression pour l'année 1984 aussi ; les valeurs réelles ont été : km 80 — —1,82 mg/l ; km 62—1,19 mg/l.

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A partir de cette équation de régression et de la densité numérique déterminée mensuellement au cours des années 1981—1985, on a déterminé la biomasse bactérienne annuelle, aux mois d'avril—octobre. Le tableau n° 2 présente les valeurs moyennes annuelles (IV—X) de la biomasse bactérienne planctonique, dont l'erreur des interpollations est de 5%.

Les données présentées mettent en évidence, d'une part, des valeurs élevées de la biomasse bactérienne pour l'année 1983 par rapport aux autres années (tableaux n° 1 et 2), fait signalé d'ailleurs aussi pour d'autres paramètres du bactérioplancton (densité numérique) dans d'autres secteurs du Danube (4); d'autre part, on remarque des valeurs plus élevées dans le profil de Ceatal Izmail par rapport à Ceatal Sf. Gheorghe (tableau n° 1), ce qui s'explique aussi par la contamination plus forte dans le Danube unique par rapport au bras de Tulcea, que par le processus plus accentué de sédimentation existant dans ce dernier.

A la suite de l'analyse des paramètres quantitatifs (densité numérique et biomasse) pour une période de 5 années, spécialement au sujet de la biomasse du bactérioplancton, dans le travail présent, on apprécie un accroissement valorique de 1981 vers l'année 1985, dont le maximum est atteint en 1983, valeurs qui nous permettent d'encadrer les eaux du Danube de ce secteur dans la catégorie de mésotrophie à tendance à eutrophie*.

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*Je tiens de remarcier le dr A. Vadineanu et le dr S. Cristofor pour l'aide accordée dans la série des déterminations, en vue de sélectionner l'ATP bactérien et le dr M. Oltean dans la détermination de l'équation de régression.

CALOSOMA (CHARMOSTA) INVESTIGATOR ILL., A NEW SPECIES FOR THE ROMANIAN FAUNA *(COLEOPTERA, CARABIDAE)*

E. NIȚU

During the research expedition in the "Biosphere Reservation Danube Delta", the author found 13 specimens of *Calosoma investigator* Ill., using the light traps catching method.

Calosoma (Charmosta) investigator Ill. was found by the author at Maliuc (7♂♂ and 6♀♀; 22.07.1991) (Fig. 1).

The recording is interesting both since it adds a new species in the fauna of the Danube Delta, and because it extends the fragmentary range of this species. *C. investigator* Ill. was until now recorded on the one hand from North Germany (uncommon), and on the other hand from southern Russia, Central Asia, Caspian region and from north-eastern Asia (26), more exactly from Arhanghelsk, Petchora, Tobolska, Baikal to Tunguska, Crimeea, Lower Don, Ural (13), Jaroslavl, Kaliningrad, Transcaspia to the Caucasus, Mongolia (5), Samara and Saratow (19), Iacutia, Priam, Sachalin, northeastern China and from the Irkutsk area (25). The species was mentioned by Miller and Zubrowski for Bessarabia, but later in 1938, it was invalidated, because that mention was based on misidentified specimens of *Calosoma denticolle* Gelb. (8) (Fig. 2).

C. investigator Ill. is considered a species living in forest or in forest steppes and rarely in steppes where it feeds especially on caterpillars. In North Siberian taiga, it is considered a natural enemy of caterpillars (15).

The ecological conditions in the new locality differ from those in which the species has been formerly recorded. Maliuc lies in a steppe with aridity index (i.a.) = 15 — 20. It is however worth mentioning that the species was found in association with *Pentodon bidens* Pall., which was considered by Savchenco as a characteristic species for arid steppes with *Artemisia* from the Ukraine. The present day steppe areas of Dobrudja were covered, about 3000 years ago, by a forest vegetation, the life conditions calling those present throughout most of the species range.

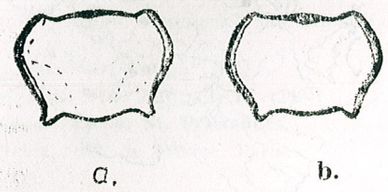

Fig. 1. — Pronotum contour of: a. *Calosoma denticolle* Gelb.; b. *Calosoma investigator* Ill.

The adaptation to the steppe conditions may be a recent event. It is also worth mentioning that Maliuc is the southeasternmost locality and at the same time with a low altitude (5 — 6 m to the sea level), from the range of the species.

Fig. 2. — The range of *Calosoma investigator* Ill.

The author believed that the species had, prior to the Ice Age, a wide and continuous range, that had been strongly affected by the Ice Age cooling (cca. 18,000 years ago), and the species could survive only in a few areas (glacial refuges). The climatic ameliorations in the postglacial times, enable the species to extend again northwards, without occupying its entire preglacial range. Its present-day fragmentary distribution is partially also a consequence of human activity. For example the small N. Germany population, had probably, until recent historical times a wiser extension.

The present theree subspecies of *C. investigator* Ill. evolved through isolation during Quaternary times. *C. investigator* Ill. (= *sibiricum* Mots.) in the Ussurian refuge; *C. investigator dauricum* Mots., in the Mongolian one; *C. investigator caspium* Dej., in the Caucasian (or arboreal Caspian) refuge (16).

The population from Maliuc seems closer to *C. investigator caspium* Dej., being known that in interglaciary and postglaciary, a faunistic and floristic exchange between this geographic district and Caspian or Transkaukasian zones. took place (4, 12, 16).

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— chlorophyll *a*: on the average 0.11—0.25 mg m^{-3} in the offshore or 0.2—0.9 mg m^{-3} in the western part (1.1—2.0 mg m^{-3} at the Bulgarian seashore);
— bacterioplankton: 111—126.10^{-3} cell.ml^{-1} —53—10 mg.m^{-3} offshore or 305—1 504.10^3 cell. ml^{-1} —158—704 mg.m^{-3} in the western part;
— protozoa: 10—18% from the total biomass of zooplankton in the oxygenated zones; the species *Mesodinium rubrum* (Lohman) spread in the whole sea has biomasees of 50—120 mg. m^{-3} in the offshore waters and 180—280 mg.m^{-3} in the western coastal zones where they form real "red-tides";
— ATP concentrations of the microplankton characterize the waters in the entrophic zone of the Black Sea pelagial as being mesotrophic, rarely eutrophic; at 1,800 m the ATP concentration of bacterioplankton is twice greater than that at 500—800 m.

In the chapter regarding the meso- and macroplankton is made an analysis of the results of the samples collected with a bottle of 150 liters (a device which should be generalized or with nets (the direct observations provided by the crew of the ARGUS submersible are also analyzed.)

We note some of the information and conclusions of these papers: — at the lower boundary of the oxycline (0.4—0.5 ml $O_2.l^{-1}$) the plankton forms in the day time a layer (from 2—5 m to 10—20 m in thickness) of high concentration (up to 38 g.m^{-3}) dominated by the *Pleurobrachia pileus* in the upper part (V—VI) by the *Calanus helgolandicus* in the middle part and *Sagitta setosa* in the lower part (situated between 150 m and 50 m depending on the position of the isooxygen surface);
— the average calorific value of the mesozooplankton diminished substantially (from 0.6—0.7 cal.mg to 0.2—0.3 cal.mg) because of the increase of Ctenophora and *Noctiluca*;
— the total biomass of the jelly-fish *Aurelia aurita* has been evaluated at about 400—900 million tons fresh weight or 1.1—2.5 million tons carbon;
— the population of *Aurelia* consumes 50—70% from the mean daily production of food for fishes.

Aspects regarding the pelagic ecosystem functioning are included in the 3 papers of the last chapter of the book.

On the basis of some in situ experimentations and of all data received in the expedition it is made an evaluation of the production and destruction of the main elements of the planktonic communities; also it is established a scheme of the trophic links between these elements and there are presented the structural-functional parameters which characterize the planktonic associations from the mesotrophic waters (Sushkina E.A. et al.).

Using a space-dynamic model through which the Black Sea pelagial is divided into 21 "cells", there is established the important role of the turbulent diffusion in the enriching of the sea water with nutrients and it is shown that the biological processes are prevalent in ecosystem in summer time but in winter the biological processes and the physical ones act equally (Lebedeva L.P.).

The paper ending the volume refers to the present structure of the Black Sea northern part ecosystem (Zaitsev Yu.P.) and discusses the main modifications occurred here in the last 20—30 years: the increase of the nutrient concentrations, the growth of the O_2 contents in the surface layer and the appearance under the thermocline of some large hypoxy zones, the growth of the biogasses from 52 to 800 g.m^{-3} for phytoplankton and from 54 to 175 g.m^{-3} for *Noctiluca*, the disappearance of the macrophytes from the Odessa zone etc.

These modifications have been registered also at the Romanian sea shore they being the result of the anthropic influences which increase more and more in the Black Sea ecosystems.

The volume "The Present State of the Black Sea Ecosystems" doubtlessly represents a work of great value, though it contains lacks among which we mention: some contributions seem to be written in haste, some conclusions are confusing, there are many bibliographical omissions (especially in the papers regarding the western part of the Black Sea), some data are not used sufficiently, analyses dominate instead of a compared integrative and correlative synthesis.

The volume presents interest for a wide range of specialists in hydrology, hydrochemistry, marine biology, ecology, marine fishing etc. containing valuable data which also serve to find solutions for limiting some negative anthropic factors and for the elaboration of the strategy necessary for the development of future researches in the Black sea.

M.-T. Gomoiu and V. Postaru

AVIS AUX AUTEURS

La « Revue roumaine de biologie — Série de biologie animale » publie des articles originaux d'un haut niveau scientifique de tous les domaines de la biologie animale : taxonomie, morphologie, physiologie, génétique, écologie, etc. Les sommaires des revues sont complétés par d'autres rubriques, comme : 1. La vie scientifique, qui traite des manifestations scientifiques du domaine de la biologie : symposiums, conférences, etc. : 2. Compte rendus des livres de spécialité.

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Les travaux seront accompagnés d'un court résumé de 10 lignes au maximum, en anglais. Les textes de travaux ne doivent par dépasser 7 pages (y compris les tableaux, la bibliographie et l'explication des figures).

La responsabilité concernant le contenu des articles revient exclusivement aux auteurs.

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