La « Revue roumaine de Biologie — Série de Zoologie » paraît six fois par an.
Toute commande de l'étranger (fascicules ou abonnements) sera adressée à ROMPRESFILATELIA, Boîte postale 2001 — telex 011631 — Bucarest, Roumanie, ou à ses représentants à l'étranger.

Les manuscrits, les livres et les revues proposés en échange, ainsi que toute correspondance, seront envoyés à la rédaction : 296, Splaiul Independenței, Bucarest, Roumanie.

EDITURA ACADEMIEI REPUBLICII SOCIALISTE ROMÂNIA
str. Gutenberg, 3 bis, București, România

REVUE ROUMAINE DE BIOLOGIE

— SÉRIE DE ZOOLOGIE —

TOME 18 1973 Nº 2

SOMMAIRE

REV. ROUM. BIOL.—ZOOLOGIE, TOME 18, Nº 2, p. 89—170, BUCAREST, 1973

UN NOUVEAU GENRE D'ISOPODE TERRESTRE, *LEPTOTRICHUS* B.-L. DANS LA FAUNE DE LA ROUMANIE

PAR

V. GH. RADU

Genus *Leptotrichus* comprises several species and subspecies, exclusively circum-mediterranean. Most of them were insufficiently defined by the authors, thus creating difficulties for the determination of new species and even of the known forms. The author of this paper endeavours to specify some taxonomic characters already used and to introduce others, too, in order to determine the exact place in the taxonomic system, of a new form of genus *Leptotrichus*, found by him in Dobrogea (Romania).

Parmi les nombreux genres d'isopodes terrestres de la faune de Roumanie, le genre *Leptotrichus* constitue non seulement une nouveauté, mais aussi une rareté, autant par l'aréal restreint qu'il habite que par le nombre très réduit d'individus. En effet, nous ne l'avons trouvé que dans trois endroits, en Dobroudja, et nous n'avons pu recueillir, dans chaque endroit, que quelques exemplaires appartenant à l'espèce *Leptotrichus pilosus* Dollf. 1905.

Etant donné qu'il est l'un des porcellionides les plus difficiles à déterminer, dans la bibliographie existante les données sur ce genre sont assez souvent imprécises ou même erronées.

Cette difficulté est augmentée aussi par le fait que dans la présentation des espèces, les auteurs n'ont pas utilisé, le plus souvent, que quelques caractères, parfois mal définis, en laissant de côté d'autres, pourtant strictement nécessaires pour une suffisante comparaison et une précise délimitation des espèces. Cette méthode simpliste de travail taxonomique, utilisée autrefois par méconnaissance des exigences de la taxonomie et par le petit nombre des espèces découvertes, combattue par les taxonomistes rigoureux, comme par exemple Emile Racovitza, est trop souvent

REV. ROUM. BIOL.—ZOOLOGIE, TOME 18, Nº 2, p. 91—102, BUCAREST, 1973

reprise aujourd'hui par commodité et quelquefois par économie d'espace graphique. Mais elle ne tarde pas à montrer ses insuffisances et ses conséquences nuisibles. Au fur et à mesure que le nombre des espèces décrites d'un même genre augmente, on doit approfondir l'analyse taxonomique des espèces, on doit recourir à de nouveaux caractères distinctifs, caractères qui n'ont pas été considérés par les auteurs précédents, les pères des espèces déjà décrites et à ce moment, le travail devient extrêmement difficile, assez souvent impossible. Il faut reconsidérer l'ancienne description si l'on peut disposer du matériel respectif. Le plus souvent on ne le retrouve plus et alors on est forcé de prendre une décision dans les circonstances d'imprécision, ou de refuter tout le travail. Dans les cas les plus heureux, on pourrait arriver à des synonymies.

L'espèce que nous présentons ici a été décrite et illustrée par quelques figures, sous les noms de trois sous-espèces : *Leptotrichus* (*Leptotrichus*) *pilosus pilosus* Dollf. 1905 [2] [3] [10][15], *L.* (*L.*) *pilosus mesopotamicus* Frankenb. 1939 [4] et *L.* (*L.*) *pilosus medius* Strouh. 1960 [10] [14]. Nous avons trouvé en Roumanie une nouvelle sous-espèce, que nous avons dénommée *Leptotrichus* (*L.*) *pilosus dobrogicus*.

Pour contribuer à une systématisation plus précise du genre Leptotrichus, représenté jusqu'ici par 17 espèces et sous-espèces, localisées toutes dans des territoires circumméditerranéens (Dollfus [2] [3] Verhoeff [12] [13], Arcangeli [1], Strouhal [6] [7] [8] [9], Vandel), [11]), surtout à la délimitation plus exacte des sous-espèces du *Leptotrichus* (*L.*) *pilosus*, nous ajouterons, à l'occasion de la description de la nouvelle sous-espèce, quelques caractères qui n'ont pas été pris en considération par les auteurs précédents et nous apporterons des précisions sur d'autres caractères déjà envisagés.

Leptotrichus (*Leptotrichus*) *pilosus dobrogicus* [1] n. subsp.

Localités. Agigea, Eforie Sud (localités situées à quelques km. au sud de Constanţa), la colline Dénis-Tépé, près de la ville de Babadag et la colline Chervant, non loin de la ville de Măcin.

Biotope : Sous des pierres, dans des endroits arides, des pâturages pierreux, inondés par les rayons du soleil.

Dimensions du corps. Femelle : 10 mm longueur — 4 mm largeur ; mâle : 7,5/ 3,5 mm.

Forme générale du corps. Très bombée, comme chez les espèces d'*Armadillidium*.

Couleur du corps. En général très faible, de nuance brune très claire, un peu plus accentuée chez les individus collectés sur les collines Chervant et Dénis-Tépé (Babadag). La couleur est due à un réseau pigmentaire très fin, de teinte marron sur la tête, brune violacée plus accentuée sur les lobes latéraux, sauf les bords marginaux qui n'ont pas de pigment. Sur tout le reste du corps (péréion, pléon, pléotelson) le même réseau brun

[1] Nom dérivé de *Dobroudja*, province du sud-est de la Roumanie, comprise entre le Danube et la mer Noire.

clair-violacé. Les bords des épimères et du pléotelson et les bords postérieurs des tergites sont clairs, exempts de pigment.

Les tergites sont lisses, parsemés de poils dont la constitution varie d'après l'endroit considéré du tergite. Tous sont sensitifs et ont une constitution écailleuse à la base, mais la constitution de la tige en diffère. Le bord antérieur des tergites est pourvu d'une rangée transversale régulière, sous-marginale de poils dont la tige, conique, est constituée par un faisceau de très fins filaments (fig. 1, A, *a*). On trouve de tels poils, rarement dispersés, aussi sur la moitié antérieure de la surface des tergites (fig. 1, *A*, *b*), entremêlés avec des poils de la même dimension, mais dont la tige a la forme d'une languette très allongée, quelquefois un peu dilatée à l'extrémité terminale (fig. 1, A, *c*). Ce dernier type de poils reste presque seul représenté sur la moitié postérieure des tergites, sur les épimères et sur le pléotelson. Le bord postérieur des tergites possède une rangée de poils sensitifs de forme assez caractéristique, dont la tige principale, pointue, est encadrée par deux écailles très élargies dans le même plan, à la façon de deux ailes (fig. 1, A, *d*).

Le bord des épimères est garni d'une rangée de poils beaucoup plus fins et simples, donc d'un autre type que ceux de la surface des tergites, dépourvus d'écailles à leur base (fig. 1, B_1), mais qui n'ont pas tous la même dimension. Parmi eux, à des distances à peu près égales, se trouvent intercalés des poils bien plus gros que ceux de la surface des tergites et toujours simples.

A l'aide d'un objectif suffisamment fort, on peut observer :

1. Les poils qui se trouvent à la surface des épimères (et en général à la surface des tergites) sont constitués (comme nous l'avons montré plus haut), par une forte tige entourée, à la base, par quelques écailles (fig. 1, B_{2a}).

2. Parmi les poils situés vers le bord des épimères, au vosinage de la rangée des pores glandulaires, on observe des poils du même type, mais plus ou moins dissociés, dans le sens que leurs écailles basales sont plus nombreuses et groupées irrégulièrement, le plus souvent en une rangée disposée longitudinalement par rapport à la longueur de l'animal. On peut observer toutes les transitions de cette dissociation (que nous avons notées sur la figure 1, B_2 dans l'ordre des lettres *a*, *b*, *b'*, *b''*, *c*, *c'*, *c''*), jusqu'à la réalisation de groupes linéaires de petits écailles-poils, avec la disparition de la tige centrale.

Par ces observations, nous nous croyons justifiés de penser que les poils qui garnissent le bord des épimères, représentent un produit adaptatif similaire à celui que nous avons décrit ci-dessus, c'est-à-dire que les gros poils représentent la tige et les petits poils les écailles d'un certain nombre de poils sensitifs alignés et modifiés au bord des épimères.

Les pores glandulaires ne sont pas amassés dans un seul champ glandulaire, comme par exemple chez les Porcellionides, mais ils sont dispersés dans une rangée linéaire, parallèle au bord des épimères (fig.1, B), ainsi qu'ils sont représentés par *Strouhal* dans sa figure 5 [10].

La tête. La forme et les rapports dimensionnels de la tête, de ses lobes latéraux et de la proéminence frontale sont représentés par la fig.

2, *A* et par la formule : $\frac{b}{a} - 0{,}62$; $\frac{g}{a} - 0{,}12$; $\frac{d}{a} - 0{,}11 - 0{,}12$; $\frac{d}{c} - 0{,}50 - 0{,}54$; $\frac{f}{a} - 0{,}59$; $\frac{c}{f} - 0{,}34$.[1])

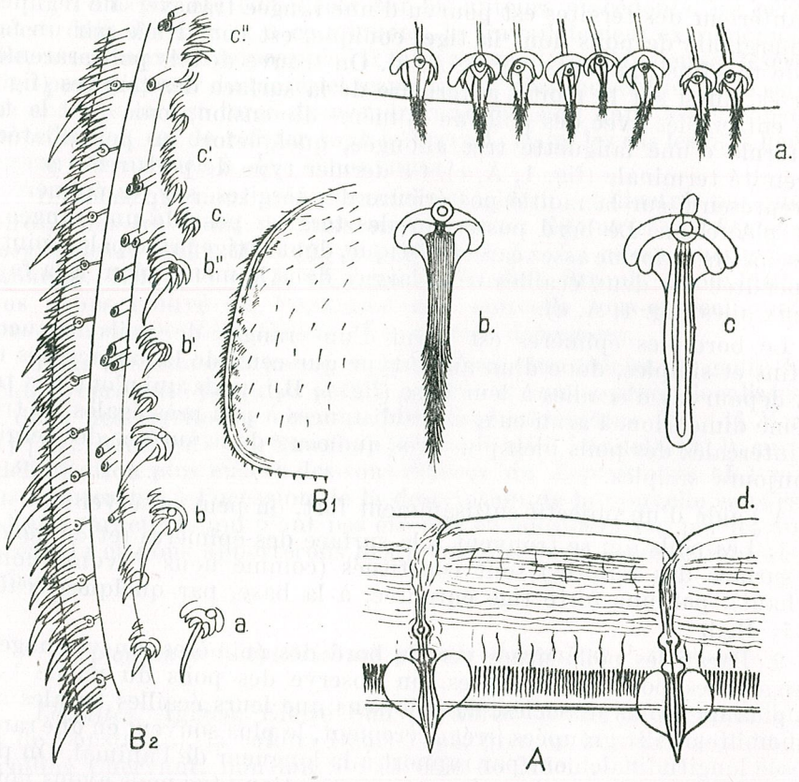


Fig. 1. — *Leptotrichus* (*Leptotrichus*) *pilosus dobrogicus*, n. subsp. A. Poils du tergite III.*a*, poils du bord antérieur du tergite ; *b*, poil de la moitié antérieure de la surface du tergite ; *c*, poil de la moitié postérieure de la surface du tergite ; *d*, poils du bord postérieur du tergite. B_1. L'épimère gauche du péréionite II. B_2. Une portion beaucoup plus grossie du bord du même épimère ; *a*, *b*, *b'*, *b''*, *c*, *c'*, *c''*, degrés de dissociation de plus en plus accentuée des poils sensitifs. (D'autres explications dans le texte).

Les yeux (fig. 2, A, B) sont constitués par 12 — 14 ocelles disposées plus ou moins régulièrement en 4 rangées.

Les antennules. Prenant comme unité dimensionnelle l'article moyen, le plus court (fig. 2, C, *a*), l'article basal est 2 1/2 fois et l'article terminal environ 4 fois plus long que l'article moyen. L'article terminal porte à son extrémité un nombreux groupe d'aesthétasques (environ 28), disposées

[1] dans un travail déjà ancien [5] nous avons motivé le but et indiqué la manière d'utiliser la méthode de la biométrie dans la taxonomie des isopodes.

en 3 — 4 rangées irrégulières, à côté d'une forte épine, prolongement pointu de l'article (fig. 2, C, *a*—*b*).

Les antennes. L'article terminal du fouet est deux fois plus long que l'article proximal (fig. 2, D, *a*). Sur l'article terminal existe une rangée transversale d'environ 10 aesthétasques, situées approximativement à la

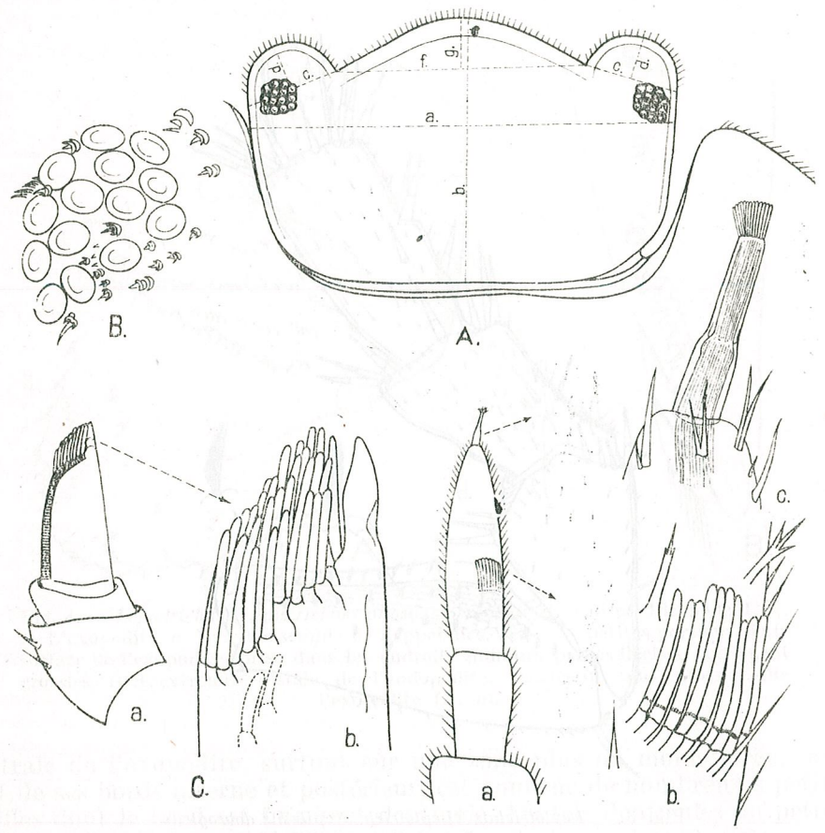


Fig. 2. — *Leptotrichus* (*Leptotrichus*) *pilosus dobrogicus* n. subsp.
A. La tête. Vue dorsale (explications dans le texte) B. L'œil droit de la figure A plus fortement grossi. C. L'antennule *a* : vue d'ensemble ; *b* : l'extrémité de l'article terminal plus fortement grossie. D. L'antenne. *a* : les deux articles du fouet de l'antenne. *b* : le groupe des aesthétasques et *c* : le bâtonnet terminal du fouet de l'antenne, plus fortement grossi.

moitié de sa longueur (fig. 2, D, *a* — *b*). Le bâtonnet sensitif qui prolonge l'extrémité de l'article terminal du fouet est constitué par une partie basale plus épaisse et une partie terminale plus mince, surmontée par un pinceau de poils sensitifs très fins (fig. D, *a*, *c*).

Le péréiopode VII ♂, représenté dans la figure 3, ne diffère pas essentiellement de celui présenté par Strouhal [10] dans sa figure 17 pour *Leptotrichus* (*L.*) *pilosus medius*. On peut y saisir pourtant quelques

différences que nous croyons significatives, étant donnée l'importance de cet appendice dans la détermination des espèces. Nous mentionnons en premier lieu que les épines de l'extrémité distale des articles sont évidemment plus nombreuses dans la figure donnée par Strouhal que dans la nôtre. L'ischiopodite diffère sensiblement de celui donné par Strouhal

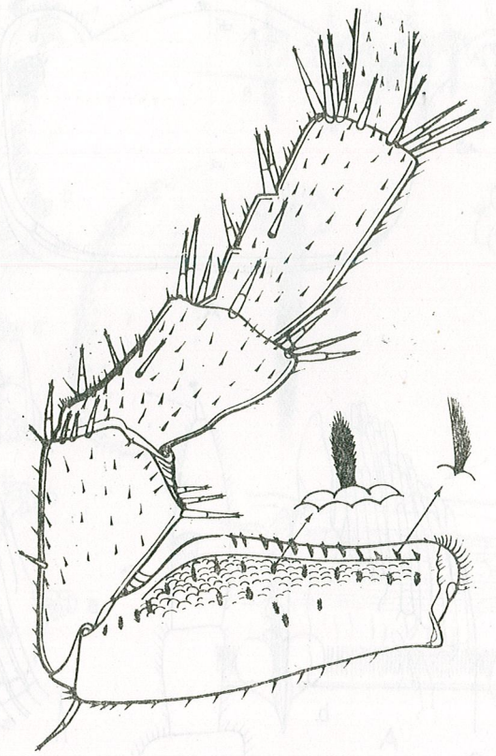


Fig. 3. — *Leptotrichus* (*Leptotrichus*) *pilosus dobrogicus* n. subsp. Le péréiopode VII ♂.

dans le sens que ses bords sont parfaitement droits. La partie distale du bord externe n'est pas marquée par une saillie qui est assez prononcée dans le cas de Strouhal. En outre, sur la face supérieure du carpopodite nous n'avons pas pu retrouver l'arête longitudinale mentionnée et figurée par Strouhal. De même, sur le basipodite nous signalons la présence de nombreux poils touffus, la plupart disposés en 2 — 3 rangées longitudinales régulières. Ils manquent dans la figure de Strouhal.

Le pléopode 1 ♂. L'exopodite (fig. 4, A) ressemble à celui décrit chez les trois sous-espèces de *Leptotrichus* (*L.*) *pilosus*, plus rapproché de celui de *Leptotrichus* (*L.*) *pilosus medius* [10]. Pourtant il diffère de celui-ci dans les petits détails, surtout par le manque du petit talon figuré et mentionné dans le texte par Strouhal ([10], p. 107), situé à l'angle constitué par le

bord médial (interne) de l'appendice et le bord postérieur, oblique et concave, de son prolongement distal (postérieur). Cette différence a la même valeur que les différences constituées, dans cet endroit de l'exopodite, entre les trois sous-espèces décrites. Certaines différences existent aussi dans la conformation de la partie basale de cet appendice. La face

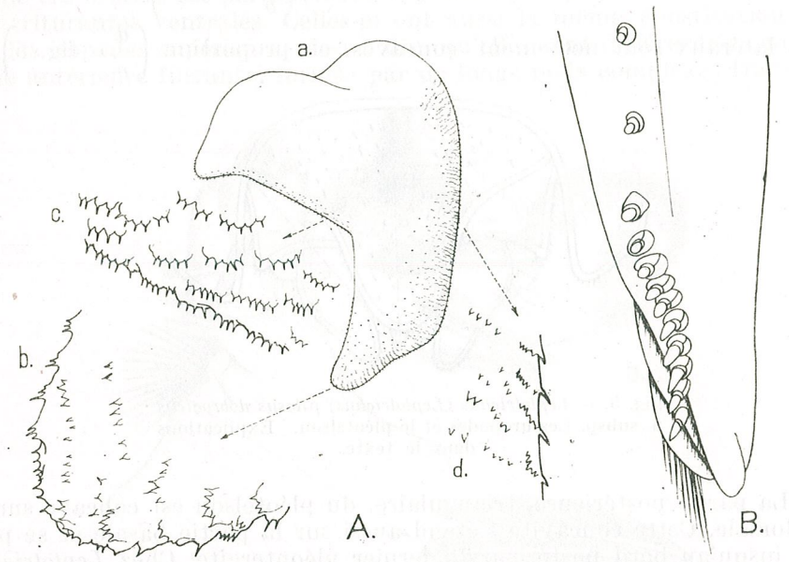


Fig. 4. — *Leptotrichus* (*Leptotrichus*) *pilosus dobrogicus* n. subsp. Pléopode 1. ♂. A. L'exopodite. *a*. Vue d'ensemble de l'appendice. *b*, *c*, *d* : petites portions de la surface de l'exopodite, prise dans les endroits indiqués par les flèches, fortement grossies. B. L'extrémité distale de l'endopodite, beaucoup plus grossie que l'exopodite (A, *a*).

ventrale de l'exopodite, surtout sur une zone plus ou moins large, partant de ses bords interne et postérieur, est pourvue de nombreuses petites écailles dont le bord est finement denticulé. De tels denticules ou petites épines se trouvent aussi isolés ou groupés en petit nombre (2 — 4).

L'endopodite diffère aussi, sensiblement, de celui décrit par Strouhal chez la sous-espèce *medius* ([10], fig. 20). L'extrémité de cet appendice se termine, d'après nos observations (fig. 4, B) par trois plis inégaux, l'externe étant le plus proéminent, et par une houppe de poils située sur le pli interne qui est subterminal. Strouhal représente fidèlement ces plis, mais il trouve que la houppe terminale de poils est située sur le pli externe. De même Strouhal ne présente pas la rangée longitudinale de forts poils sensitives, observée par nous (fig. 5), dont les premiers 10, situés vers l'extrémité de l'appendice, sont étroitement accolés l'un à l'autre ou très rapprochés entre eux, puis, en commençant par l'onzième, se trouvent bien distancés entre eux.

Les uropodes (fig. 5). Le bord postérieur de l'article basal est fortement concave. L'exopodite court, avec le bord externe très bombé dans

sa moitié basale. L'endopodite, assez court, dépasse très peu l'extrémité du pléotelson.

Le pléotelson (fig. 5) a une forme triangulaire, avec l'extrémité largement arrondie, dépassant un peu, en arrière, l'extrémité des derniers épimères. Le rapport longueur/largeur $\left(\frac{a}{b}, \text{ fig. } 5\right)$ est de 0,7. Les bords latéraux sont nettement concaves, en proportion $\left(\frac{d}{c}, \text{ fig. } 5\right)$ de

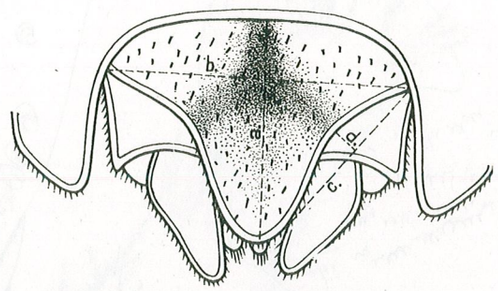


Fig. 5. — *Leptotrichus* (*Leptotrichus*) *pilosus dobrogicus* n. subsp. Les uropodes et le pléotelson. Explications dans le texte.

0,14. La partie postérieure, triangulaire, du pléotelson est concave sur la face dorsale. Cette concavité s'étend aussi sur la partie basale et se prolonge jusqu'au bord postérieur du dernier pléontergite. Chez *Leptotrichus* (*L.*) *pilosus medius*, cette concavité ne s'étend pas sur la partie basale du pléotelson [10].

L'appareil buccal. Nous présentons ici seulement la mandibule droite, la plus caractéristique du point de vue spécifique. Il est à remarquer surtout la disposition particulière des poils sur l'apophyse mobile (fig. 6, *a*, *b*) et la disposition des pénicilles, l'un bipectiné, inséré directement sur l'apophyse mobile, trois, également bipectinés, à la base et à côté de cette apophyse et un groupe de nombreuses autres pénicilles, monopectinés, disposés en éventail à l'extrémité d'un tubercule situé à une petite distance sous l'apophyse mobile (fig. 6, *a*, *c*).

L'estomac présente, en lignes générales, la constitution caractéristique aux isopodes supérieurs (fig. 7)[1].

Les pièces latérales (lateralia) sont couvertes par des poils sur toute leur surface, mais pas uniformément, leurs dimensions, densité et orientation variant d'après certaines zones. Toute la surface médiale de ces pièces (fig. 7, *a*) est pourvue de petits poils, un peu rares. Sur la zone dorsale, dans sa partie antérieure (fig. 7, *b*), les poils sont courts, plus nombreux et disposés en rangées transversales. Mais, au fur et à mesure

[1] Comparaison d'après un manuscrit de feue M^me^ Varvara V. Radu : *Structura intestinului anterior la isopode* (La structure de l'intestin antérieur chez les isopodes), où elle décrit minutieusement la structure de cet organe dans la série des isopodes terrestres, manuscrit qui, malheureusement, est resté jusqu'à présent non publié.

qu'on s'approche de la partie postérieure, les rangées s'altèrent et les poils deviennent de plus en plus longs et moins nombreux (fig. 7, *c*). Sur la face ventrale des lateralia, dans leur tiers antérieur, il y a une véritable brosse, un champ très dense de poils plus longs et pourvus de longues racines enfoncées profondément dans les tissus sous-jacents (fig. 7, *d*). La position de ces brosses est parfaitement opposée à la région filtrante des plaques triturantes ventrales. Celles-ci ont aussi la même constitution que chez les isopodes supérieurs, c'est-à-dire qu'elles sont différenciées en une partie antérieure filtrante, formée par de longs poils complexes transver-

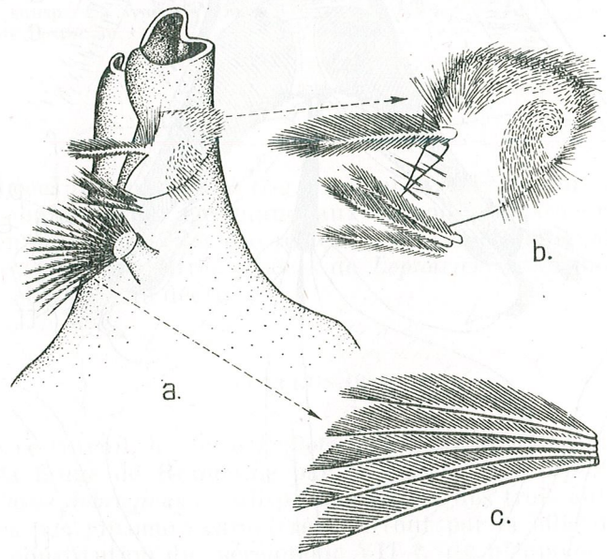


Fig. 6. — *Leptotrichus* (*Leptotrichus*) *pilosus dobrogicus* n. subsp. *a* : l'extrémité triturante de la mandibule droite ; *b* et *c* : parties plus grossies de la mandibule (*b* — apophyse mobile, *c* — pénicilles).

saux (fig. 7, *f*) et une partie postérieure (un talon triturant), plus petite $\left(\text{environ } \frac{1}{4} \text{ de la longueur totale des plaques}\right)$, constituée à la façon d'une lime par des poils complexes, très courts et juxtaposés (fig. 7, *t.*) Au dessus de ce talon, sur la partie postérieure de la face ventrale des lateralia, on voit une plaque triturante dorsale (fig. 7, *p. t.*) de la même dimension que le talon triturant et parfaitement opposée à celui-ci. Le bord médial des plaques triturantes ventrales est limité par un double pli, dont le plus externe est pourvu d'une forte bordure de poils.

Parmi les détails non signalés encore, en ce qui concerne la structure de l'estomac chez les isopodes terrestres, nous remarquons encore la présence d'une touffe de longs et fins poils située derrière l'extrémité postérieure (le talon) des plaques triturantes ventrales (fig. 7, *tf*) ; plus en arrière et un peu latéralement, il y a encore une rangée d'épines courtes et épaisses,

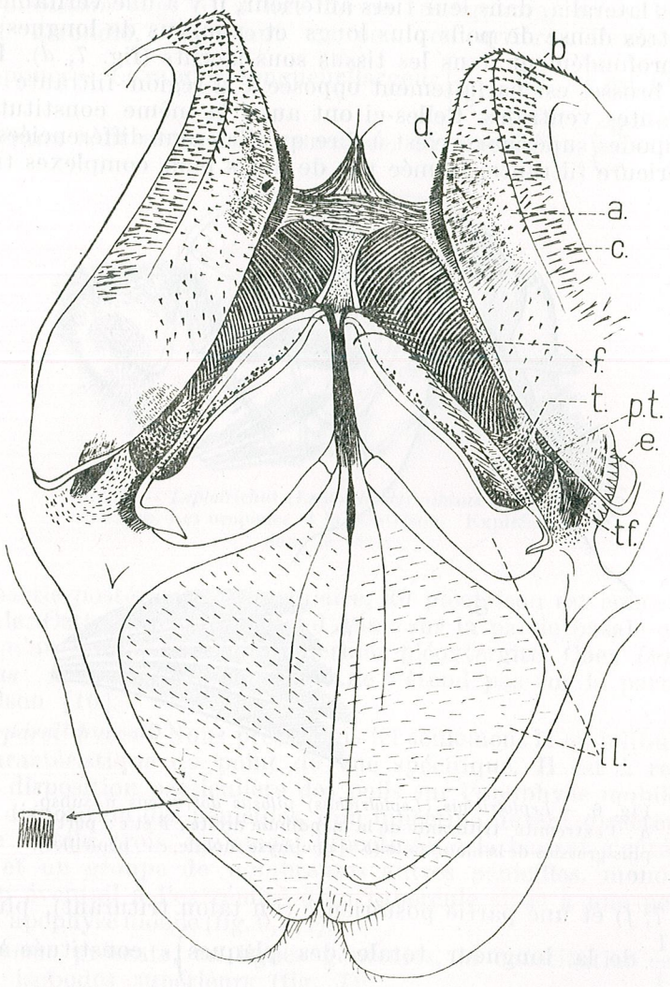


Fig. 7. — *Leptotrichus* (*Leptotrichus*) *pilosus dobrogicus* n. subsp. L'estomac, ouvert, vu par dessus. *a* : face médiale des latéralia ; *b*, *c* : portion antérieure et postérieure de la face dorsale des latéralia ; *d* : brosse ventrale des latéralia ; *e* : rangée d'épines ; *f* : partie filtrante des plaques triturantes ventrales ; *il* : inférolatéralia ; *pt* : plaque triturante dorsale ; *t* : le talon triturant de la plaque triturante ventrale ; *tf* : touffe de poils.

très pointues (fig. 7, *e'*). Nous devons remarquer que toutes ces conformations (lamelles, rangées et touffes de poils, épines, etc.) sont autant de dispositifs fonctionnels situés sur le trajet des particules alimentaires triturées et jouent certainement un rôle important dans la physiologie de la nutrition de ces animaux.

Fig. 8. — *Leptotrichus* (*Leptotrichus*) *pilosus dobrogicus* n. subsp. La valvule dorsale de l'estomac.

Les pièces inférolatérales (fig. 7, *il*) et la valvule dorsale (fig. 8) ont aussi une conformation commune aux isopodes supérieurs avec, bien entendu, leurs particularités qui restent à être mises en évidence et comparées avec celles des autres espèces de *Leptotrichus*, au moment où l'estomac de celles-ci sera décrit.

CONCLUSIONS

Dans ce travail, le genre *Leptotrichus* a été signalé pour la première fois dans la faune de Roumanie par la sous-espèce *Leptotrichus* (*Leptotrichus*) *pilosus dobrogicus* n. subsp., qui diffère des trois autres sous-espèces connues par plusieurs caractères, surtout par la ciliature des épimères, par la constitution du péréiopode VII ♂, du pléopode 1 et du pléotelson. De nouveaux caractères ont été envisagés (rapports biométriques de la tête et du pléotelson, l'origine de la coloration du corps, la structure de la mandibule et de l'estomac), d'autres ont été étudiés avec plus de minutiosité (ciliature du bord des épimères, pilosité des tergites, concavité du pléotelson, etc.), afin d'élargir la gamme des caractères taxonomiques à comparer, pour permettre une délimitation plus précise des taxons.

BIBLIOGRAPHIE

1. Arcangeli Alceste, Atti della Societa Italiana di Science Naturali, 1913, **52**, 477 — 478.
2. Dollfus A., 1896, Mém. Soc. Zool. de France.
3. — 1905, Feuille du Jeune Natural., Série IV, **35**.
4. Frankenberger Z., 1939, Sbornik entom., odd. Nar. Musea v Prage, **165**, 23 — 31.
5. Radu Gh. V., 1950, Lucrăr. Ses. Șt. Acad. R.S.R., **2**.
6. Strouhal Hans, 1936, Sitz. Ber. der Akad. der Wissensch. in Wien, Abt. Math.—Naturw., **145**, 154.
7. — 1936. Acta Inst. et Mus. Zool. Univ. Atheniensis, **1**, *4 — 5*, 53.

8. Strouhal Hans 1937, Sitz. Ber. der Akad. der Wissensch. in Wien, Abt. Math.—Naturw.
9. — 1937, Zool. Anz., **117**.
10. — 1960, Zool. Anz., **165**, *3/4*, 90 — 115.
11. Vandel Albert, 1962, *Isopodes terrestres*, in : *Faune de la France*, Ed. P. Lechevalier, Paris, **66**
12. Verhoeff, Karl W., 1901, Zool. Anz., **24**.
13. — 1923, Arch. f. Naturgesch., **89**, *5*.
14. — 1941, Rev. Fac. Sci., Univ. Istambul., S. B., **6**.
15. Verhoeff Karl W. u. Strouhal H., 1967, Zool. Jb. Syst., **93**, 465 — 506.

Reçu le 7 novembre 1972

Université « Babeș-Bolyai »
Chaire de Zoologie
Cluj, str. Clinicilor 5 — 7

NOTES ON THE EAST ASIAN GENUS *SINILABEO* RENDAHL, 1932 (PISCES, CYPRINIDAE)

BY

PETRU BĂNĂRESCU

Sinilabeo Rendahl, 1932 (= *Altigena* Lin, 1933) is accepted as valid genus; it is closer to the African *Varicorhinus* and to the East and South-East Asian *Cirrhinus*. It includes only two species : *tungting* and *discognathoides*. *Varicorhinus lemassoni* and *V. brevis* are considered subspecies of *S. tungting*, *V. tonkinensis* a subspecies of *S. discognathoides*; *Labeo xanthogenys* is considered a synonym of *S. t. lemassoni*, *Varicorhinus pogonifer* a synonym of *S. d. discognathoides* and *V. graffeuili* a synonym of *S. discognathoides tonkinensis*.

The generic name *Sinilabeo* was proposed by Rendahl [16] in 1932 for the Barbine minnow *Varicorhinus tungting*; one year later, Lin [9] proposed *Altigena* as a subgenus of *Osteochilus*, ascribing to it four species of Barbinae from South China (including the middle Yangtze drainage and Hainan Island) : *Varicorhinus discognathoides*, *V. brevis*, *V. tungting* and *V. pogonifer*; he does not mention any of them as generotype; the first-listed one, *discognathoides*, is considered generotype by Hensel [5]. Nichols [11] recognizes both *Sinilabeo* and *Altigena* as valid subgenera of *Varicorhinus*, ascribing *tungting* to the first, the three other species to the second subgenus. Chang [4] recognizes *Sinilabeo* as genus. Pellegrin and Chevey [13] [14] [15] described from the Song Koi drainage, Vietnam D. R., four species close to *tungting* and *discognathoides* : *Varicorhinus tonkinensis*, *V. lemassoni*, *V. graffeuili* and *Labeo xanthogenys*.

MATERIAL

The 20 specimens examined (which include 6 holotypes) belong to the following collections : American Museum of Natural History, New York, Muséum National d'Histoire Naturelle, Paris, Naturhistorisches Museum, Vienna and United States National Museum, Washington.

VRE. ROUM. BIOL. – ZOOLOGIE, TOME 18, Nº 2, p. 103–110, BUCAREST, 1973

DELIMITATION AND RELATIONS OF THE GENUS

Sinilabeo (=*Altigena*) includes rather large-sized East Asian Barbine minnows, whose mouth structure is essentially the same as in the Chinese species of *Cirrhinus* which I recently revised [3]: both lips are continuous round the mouth; the upper lip is smooth or slightly furrowed laterally and separated from the snout by a deep but narrow groove; the post-labial groove is developed only on the sides behind the lower lip, but its two ends are connected by a shallower groove, which delimits the lower lip from the mentum; this groove appears to be quite slight when the inner papillose face of the lower lip is applied on the lower jaw and, on the contrary, rather deep when the lower lip is reflected; there is a rather deep groove between lower lip and lower jaw. The mouth of *Sinilabeo* differs from that of the Chinese *Cirrhinus* only in having a rather long posterior prolongation of the postlabial groove (Figs 1, 4 — 6, 10, 11). Practically the same structure of the mouth as in *Sinilabeo* occurs in the only available African species of *Varicorhinus*: *V. mariae*.

The scales are of *Tor*-type as defined by Karaman [7], being characterized by very numerous and more or less parallel striae; those of *Cirrhinus* are of the same type, but the striae are more closely set in *Sinilabeo*, slightly more spaced in *Cirrhinus*. The pharyngeal teeth are identical in *Sinilabeo* and *Cirrhinus*, having long and oblique grinding surfaces, the grinding surfaces of the teeth of all the three rows being disposed in the same plane.

Sinilabeo can thus hardly be separated from *Cirrhinus*, differing from it only in the larger size of the specimens, darker coloration and longer posterior prolongations of the postlabial groove. A comparison is necessary also with *Varicorhinus*. This nominal genus, as accepted until recently, is a heterogeneous assemblage. The Anatolian and West Asian species were separated by Karaman [6] as *Capoeta*; the North African *V.* (*Pterocapoeta*) *maroccanus* was included by Karaman [7] within *Varicorhinus*; yet, according to the illustration of the mouth published by him, this species seems closer to *Capoeta*. Most species included by Nichols [11] within *Varicorhinus* belong to a distinct genus, *Onychostoma*, which I reviewed recently [1] [2]; a few of them belong to *Sinilabeo*, while two others, *posehensis* and *mutabilis*, must be ascribed to monotypic genera: *Rectoris* Lin, 1933 and *Parasinilabeo* Wu, 1939. From these four genera, *Sinilabeo* comes closer to the African *Varicorhinus*; I pointed out the identity of mouth structure in *Sinilabeo* and in *Varicorhinus mariae* (yet I had no specimens from the generotype: *V. beso*). In spite of this identity, I hesitate in synonymizing these two genera, because of the very great distance between Africa and East Asia: no representatives of the *Varicorhinus* — *Sinilabeo* group is known to occur in West Asia, India, Indonesia or the Indochinese Peninsula (except North Vietnam whose fish fauna is, zoogeographically, East Asian). A definitive solution requires the examination of all or most species of *Varicorhinus*, *Sinilabeo*, *Cirrhinus* and *Labeo*. In the present state of knowledge of the East and South-East Asian Barbinae, it is

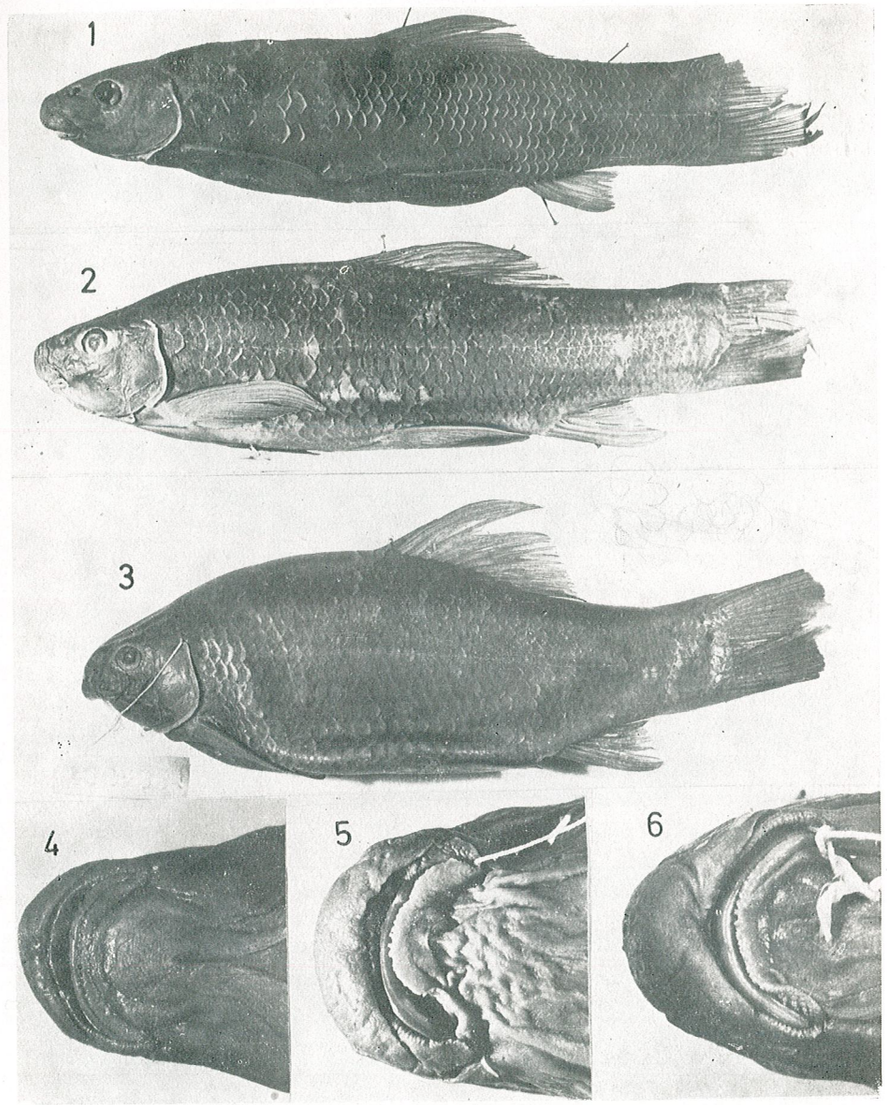


Fig. 1. — *Sinilabeo lungling lungling* Nichols. U.S.N.M. 130073.
Fig. 2. — *Sinilabeo lungling lemassoni* (Pellegrin et Chevey). Holotype, M.N.H.N. 35 — 319.
Fig. 3. — *Sinilabeo lungling lemassoni*; holotype of *Labeo xanthogenys*. M.N.H.N 35—331.
Fig. 4. — *Sinilabeo lungling lungling*, ventral view of mouth.
Fig. 5. — *Sinilabeo lungling lemassoni*, holotype, ventral view of mouth.
Fig. 6. — *Sinilabeo lungling lemassoni*, holotype of *Labeo xanthogenys*, ventral view. of mouth.

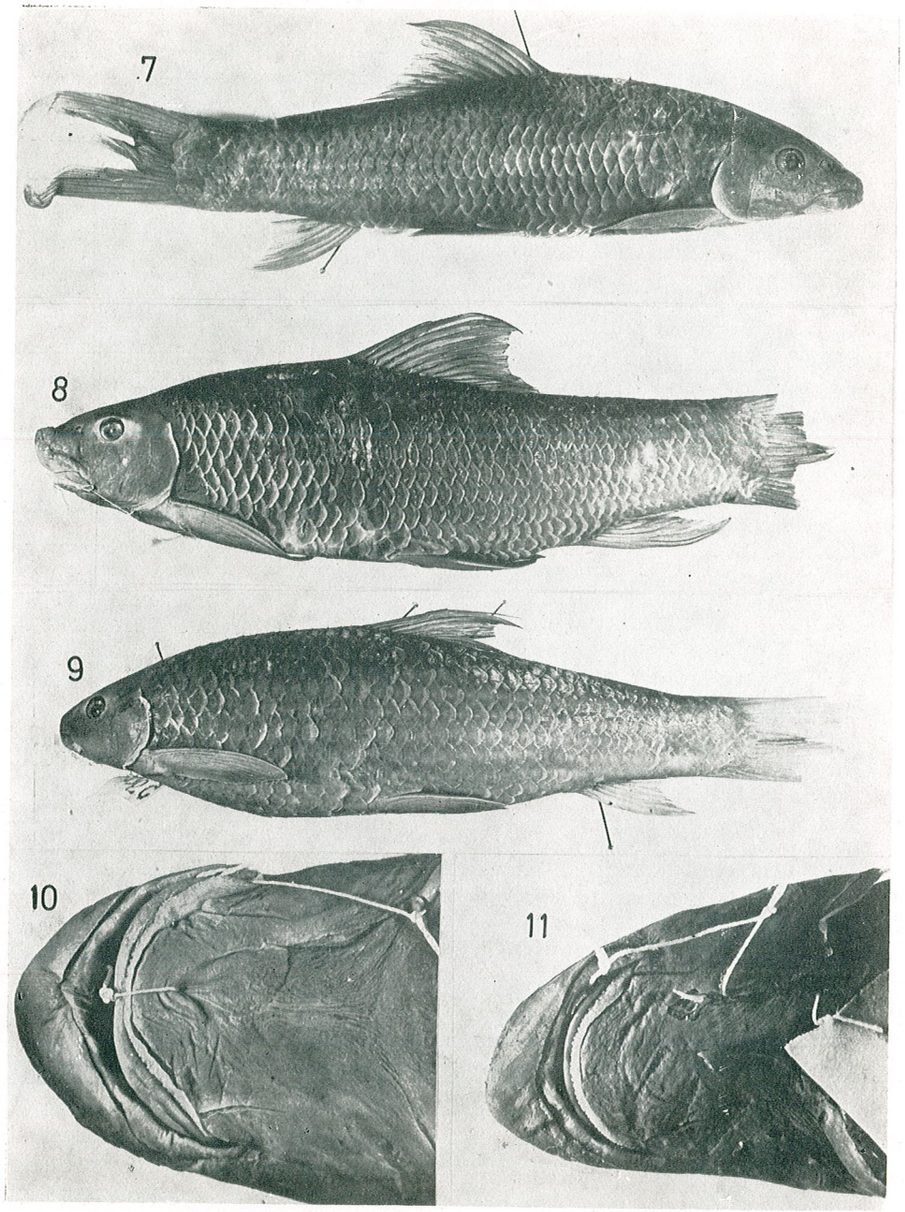


Fig. 7. — *Sinilabeo discognathoides discognathoides* (Nichols et Pope). N.M.W. 10205.
Fig. 8. — *Sinilabeo discognathoides tonkinensis* (Pellegrin et Chevey). Holotype, M.N.H.N. 34 — 257.
Fig. 9. — *Sinilabeo discognathoides tonkinensis*, holotype of *Varicorhinus graffeuili*. M.N.H.N. 35 — 321.
Fig. 10. — *Sinilabeo discognathoides tonkinensis*, holotype, ventral view of mouth.
Fig. 11. — *Sinilabeo discognathoides tonkinensis*, holotype of *Varicorhinus graffeuili*, ventral view of mouth.

preferable to recognize small genera, including only few but evidently closely related species.

I cannot accept Lin's [9] opinion that *Altigena* (i. e. *Sinilabeo*) is close to *Osteochilus*; in the last-named genus the upper lip is strongly papillose and furrowed and the papillae on the median part of the lower lip are much longer than in *Sinilabeo* and *Cirrhinus*, practically transformed into fimbriae.

SYSTEMATIC ACCOUNT

Genus **Sinilabeo** Rendahl, 1932
(= **Altigena** Lin, 1933)

1 a. **Sinilabeo tungting** (Nichols, 1925)

Figs 1, 4

Synonyms: *Varicorhinus tungting* Nichols, 1925 (Lake Tungting, middle Yangtze drainage; Wu, 1930 (Luchow, Szechwan); *Sinilabeo tungting*, Rendahl, 1933 (Chungking, Szechwan, upper Yangtze); *Osteochilus* (*Altigena*) *tungting*, Lin, 1933 (reference); *Sinilabeo tungting*, Chang, 1944 (Loshan and Omei, Szechwan).

Specimens examined: Holotype of *V. tungting*, A. M. N. H. 8426, lake Tungting, 235.0 mm. st. length; paratypes of the same, A.M.N.H. 10694, two specimens, 80.0 and 81.0 mm.; U. S. N. M. 87464, Suifu, Szechwan, four specimens, 189.0 — 249.0 mm.; U. S. N. M. 130073, "China" (surely Szechwan), two specimens, 198.0 and 200.0 mm.; U. S. N. M. 130085, "China" (surely Szechwan), four specimens, 215.0 — 272.0 mm.; M. N. H. N. 34 — 15, upper Yangtze, one specimen, 328.0 mm. (originally labelled *Labeo diplostomus*).

D 3 /10 — 11; L. lat. 42 $\frac{6^1/_2 - 8}{6 - 6^1/_2}$ 46; Sp. br. 34 — 45; D. phar. 5. 4. 2 — 2.4.5.

Edge of dorsal fin convex; 6 to 6 1/2 scales between lateral line and ventral origin; mouth semi-circular, horse-shoe shaped, its size variable; in some specimens the cleft of the mouth reaches under or behind nostrils, in other specimens almost under the front of the eye. In all specimens examined both pairs of barbels are present, but the barbels may eventually lack in other specimens. Body depth 22.4 — 25.2 % of standard length, caudal peduncle 19.4 — 21.6 %, etc. (for other body proportions see table 1).

Range: upper and middle Yangtze drainage, but apparently not lower Yangtze.

Table 1

Body proportions in *Sinilabeo* (available specimens)

Species		*tungting tungting*	*tungting lemassoni*	*discognath. discognath.*	*discognath. tonkinensis*
st. length, mm.		80.0—249.0	95.0—256.0	166.0—235	230.0—315.0
in % of standard length	max. depth	22.4—25.2	27.6—34.2	25.3—28.1	26.6—29.2
	caudal peduncle	19.4—21.6	21.4—23.6	18.4—20.4	18.4—20.6
	least depth	14.2—15.8	14.5—16.1	13.0—13.8	11.7—15.3
	predorsal dist.	44.0—42.7	45.2—49.7	45.1—48.3	44.0—47.0
	preventral dist.	50.0—57.5	50.3—54.5	49.0—51.0	45.0—50.3
	P—V distance	29.6—35.8	28.3—33.8	29.8—31.4	26.2—32.9
	V—A distance	18.6—24.2	22.1—23.8	23.8—25.6	23.8—26.5
	head	22.0—23.6	20.8—23.2	20.5—23.6	19.5—23.0
	snout	7.0—10.3	7.8— 9.3	8.5—10.4	8.3—10.7
	eye diameter	3.8— 5.4	3.9— 5.3	3.7— 4.2	3.4— 3.6
	pectoral	18.6—22.2	20.2—23.0	18.7—20.2	20.1—21.2
	ventral	16.1—19.8	22.1—25.0	18.3—19.9	20.1—21.2
	dorsal, height	18.7—21.2	22.1—25.0	20.4—22.3	22.6—23.4
	dorsal, base	17.1—19.4	18.1—21.4	17.0—19.3	16.1—19.2
	anal, height.	13.7—17.1	16.9—18.3	13.5—18.1	18.7—21.4
in % of head	snout	31.8—43.5	34.4—42.2	41.8—43.7	42.2—46.2
	eye diameter	15.6—24.4	17.3—23.7	18.0—18.7	15.1—18.1
eye diameter in % of interorbital		31.2—44.5	37.0—50.0	31.6—43.5	34.1—39.2

1 b. **Sinilabeo tungting lemassoni** (Pellegrin & Chevey, 1936)

Figs 2, 3, 5, 6

Synonyms: *Varicorhinus lemassoni* Pellegrin&Chevey, 1936 (Lai-Chau =Black River, Song Koi drainage; Back-Mé, in a tributary to Claire River, same drainage; *Labeo xanthogenys* Pellegrin&Chevey, 1936 (Thau Son, a tributary to Claire River).

Specimens examined: Holotype of *V. lemassoni*, M.N.H.N. 35 — 319 Lai-Chau, 256.0 mm.; paratype of the same, M.N.H.N. 35 — 320, Bac-Mé, 95.0 mm.; holotype of *L. xanthogenys* M.N.H.N. 35 — 331, Than Son, 217.0 mm.; M.N. H. N. 35—361, Song-Koi drainage, 1 specimen, 190.0 mm. (labelled *L. xanthogenys*).

D 3/10—11; L. lat. 45 $\frac{7}{6-7}$ 48; Sp. br. ±35—43; D. phar. 5.4.2—2.4.5.

The four specimens examined approach *S. tungting* in having the edge of the dorsal fin convex and 6 or 7 scales between lateral line and ventral insertion; they differ from *tungting* only in the higher number of scales in lateral line (yet the extreme values overlap). In describing *V. lemassoni*, Pellegrin and Chevey [14] compare it with *V. tonkinensis*, mentioning rather minor differences between them (and not the main ones: dorsal convex versus notched, 6 or 7 scales between lateral line and ventral as against only 4) and with *V. shansiensis* and *V. barbatus*, which both

actually belong to *Onychostoma*. When describing *Labeo xanthogenys*, Pellegrin and Chevey [16] compare it only with the Indian *L. diplostomus* and with the Chinese *L. jordani* (a synonym of *Cirrhinus chinensis*) and not with *V. lemassoni*, described by themselves only two months earlier. Actually the holotype and the second specimen of *xanthogenys* represent a *Sinilabeo*, identical to *lemassoni* in respect of the main characters (dorsal convex, number of scales). The holotype of *xanthogenys* differs from *lemassoni* in having a much deeper body (depth 34.2 % of st. length, as against 27.6 — 28.4 % in *lemassoni*, but 27.4 % in the second *xanthogenys* specimen) and larger mouth. In the holo- and paratype of *lemassoni* the cleft of the mouth reaches under nostrils, the end of maxillary between nostrils and eye, the insertion of mandible in front of the vertical from eye centre; in "*xanthogenys*", the cleft of the mouth reaches under the vertical from half-way nostrils-eye, the insertion of the mandible beyond the vertical from middle of eye. Since the size of the mouth is subject to similar individual variations also in *S. tungting tungting*, I think one is justified to synonymize *xanthogenys* with *lemassoni*. The body depth too can undergo individual variation. All the four specimens examined have two pairs of barbels.

Range: Song Koi drainage, Vietnam D.R.

1 c. **Sinilabeo tungting brevis** (Lin, 1931)

Synonyms: *Varicorhinus brevis* Lin, 1931 (Canton, Kwantung); *Osteochilus* (*Altigena*) *brevis*, Lin, 1933 (Canton, Kwantung; Winshon, Kwangsi).

No specimen available.

D 3/12; L. lat. 42 $\frac{7\frac{1}{2}}{7\frac{1}{2}}$ 44.

According to Lin's description [9], this fish approaches *tungting* and *lemassoni* in having $7\frac{1}{2}$ scales between lateral line and ventrals, differing from them only slightly, having one more branched dorsal ray (yet this character may vary) and a somewhat different number of scales in lateral line (in this character *brevis* is intermediate between the two other subspecies). Quite probably, the dorsal fin is convex, but Lin does not mention this character. Apparently no barbels (yet some specimens may have). Mouth, according to Lin, as small as in the holo- and paratype of *lemassoni*, but this character may be subject to individual variation, as in the two other subspecies.

Range: Hsi-kiang drainage in Kwangsi and Kwantung provinces, South-East China.

2 a. **Sinilabeo discognathoides discognathoides** (Nichols & Pope, 1927).

Fig. 7

Synonyms: *Varicorhinus discognathoides* Nichols & Pope, 1927 (Nodoa, Hainan Island); *Labeo pangusia* (non Hamilton Buchanan), Koller, 1927 (Wutchi, Hainan Island); *Osteochilus* (*Altigena*) *discogna-*

thoides, Lin, 1933 (reference); *Varicorhinus pogonifer* Lin, 1931 (Hainan Island); *Osteochilus* (*Altigena*) *pogonifer*, Lin, 1933 (Hainan Island).

Specimens examined: Holotype of *V. discognathoides*, A. M. N. H. 8389, Nodoa, 235.0 mm. st. length; paratype of the same, A.M.N.H. 10678, Nodoa, 176.0 mm.; N.M.W. 10205, Wutchi, Hainan, 1 specimen, 166.0 mm. (determined *Labeo pangusia* by Koller [8]).

D 3/10; L. lat. 39 $\frac{6}{4}$ 40; Sp. br. 38 — 44; D. phar. 5.4.2—2.4.5.

This species differs from *S. tungting* in having the upper edge of the dorsal fin notched and only 4 scales between lateral line and ventral fins (as against 6 — $7^1/_2$). Two pairs of barbels present in the specimens examined, although Nichols & Pope [12] mention "no barbels". Mouth rather large, end of maxillary under vertical from anterior margin of eye, insertion of mandible beyond vertical from middle of eye. The body proportions are mentioned in table 1.

According to Lin [9], "*Osteochilus*" *pogonifer* differs from *discognathoides* in having two pairs of barbels, a smaller mouth (the maxillary not reaching to the vertical from the anterior margin of eye) and 39 — 42 scales; it has 4 scales between lateral line and ventrals, as in *discognathoides*. Since the barbels are present also in the holo- and paratype of *discognathoides*, and the size of the mouth undergoes, in *S. tungting*, a wide individual variability, I cannot accept *pogonifer* as distinct species.

Range: Hainan Island.

2 b. **Sinilabeo discognathoides tonkinensis** (Pellegrin & Chevey, 1934).

Figs 8—11

Synonyms: *Varicorhinus tonkinensis* Pellegrin & Chevey, 1934 (Nghia Lô, Song Koi drainage); Pellegrin & Chevey, 1936 (Lai Chau = Black River); *V. graffeuili* Pellegrin & Chevey, 1936 (Nam Lung, tributary to Black River).

Specimens examined: holotype of *V. tonkinensis*, M.N.H.N. 34 — 257, Nghia Lô, 265.0mm.; M.N.H.N. 35 — 318, Lai Chau, 1 specimen, 315.0 mm.; holotype of *V. graffeuili*, M. N. H. N., 35 — 321, Nam Lung, 230.0 mm.

D 3/10 — 11; L. lat. 41 $\frac{6}{4}$ 45; Sp. br. ± 44 — 46; D. phar. 5.4.2 — 2.4.5.

As in *S. discognathoides*, the upper edge of the dorsal fin is notched and there are 4 scales between lateral line and ventrals; practically the only difference between the two subspecies is the number of scales in lateral line: 39 — 42 in *discognathoides*, 41 — 45 in *tonkinensis*. In all the three specimens examined the mouth is large and semi-circular, the end

of the lower lip lying under the vertical from the anterior margin of the eye, the insertion of mandible behind the vertical from middle of eye; yet this character may vary in this subspecies, too. A single (maxillar) pair of barbels in the holotype of *tonkinensis* and in the Lai Chau specimen, two pairs in the holotype of *graffeuili*.

In the original description of *graffeuili*, Pellegrin & Chevey [14] mention a few differences between it and *tungting* but make no comparison with *tonkinensis* described by themselves [13]. The small differences in body proportions (e. g. depth 28.2—29.2 % of st. length in "*tonkinensis*", 26.6 % in the type of *graffeuili*) are comprised within the normal intrasubspecific variability. Also the presence and number of barbel pairs is subject, in *Sinilabeo* as well as in *Cirrhinus*, to individual variability.

Range: Song Koi drainage, Vietnam D.R.

★

This tentative revision of the genus *Sinilabeo* is based but on few specimens, representatives of this genus being, probably because of their large size, poorly represented in museum collections, as compared to other East Asian genera of minnows. A major difficulty raises from the fact that the size of the mouth, a character which is constant in other genera of Barbinae, seems to be subject, in *Sinilabeo*, to a strong individual variability. This conclusion, based on the comparison of several *S. tungting tungting*, was here generalized to the other species and subspecies. Future investigations, based on large series, may prove that this conclusion is wrong, in which case *pogonifer*, perhaps also *xanthogenys*, would be raised to specific rank.

Acknowledgements: Mrs. Prof. M.-L. Bauchot, Paris and Drs E. A. Lachner, Washington, P. Kähsbauer, Vienna and D. E. Rosen, New York, lent specimens or facilitated the study of specimens under their care. A visit to the museums in the U.S.A. was financed by the Smithsonian Institution's T.F.H. Fund, thanks to the recommendation of Dr. E. A. Lachner.

REFERENCES

1. Bănărescu P., 1971, Rev. roum. Biol. — Zool., **16**, *4*, 241 — 248.
2. — 1971, Rev. roum. Biol. — Zool., **16**, *6*, 357 — 364.
3. — 1972, Rev. roum. Biol. — Zool., **17**, *4*, 251 — 256.
4. Chang H. W., 1944, Sinensia, **15**, *1 — 6*, 27 — 60.
5. Hensel K., 1970, Annotat. zool. botan., Bratislava, **57**, 1 — 45.
6. Karaman M., 1969, Mitteil. Hamburg Zool. Mus. Inst., **66**, 17 — 54.
7. — 1971, Mitteil. Hamburg. Zool. Mus. Inst., **67**, 175 — 254.
8. Koller O., 1927, Ann. Naturhist. Mus. Wien, **41**, 25 — 49.
9. Lin S. Y., 1933, Lingnan Sci. J., Canton, **12**, 337 — 348.
10. Nichols J. T., 1925, Amer. Mus. Novit., **182**, 1 — 8.
11. — 1943, *The fresh-water fishes of China*. The American Museum of Natural History, New York.
12. Nichols J. T., Pope C. H., 1927, Bull. Amer. Mus. Nat. Hist., **44**, 321 — 394.

13. Pellegrin J., Chevey P., 1934, Bull. Soc. zool. France, **59**, *4*, 337 — 343.
14. — 1936, Bull. Soc. zool. France, **61**, *1*, 18 — 27.
15. — 1936, Bull. Soc. zool. France, **61**, *3*, 220 — 232.
16. Rendahl H., 1932, Ark. för Zool., **24 A**, *16*, 1 — 134.
17. Wu H.-W., 1930, Sinensia, **1**, *6*, 65 — 86.
18. — 1939, Sinensia, **10**, *1 — 6*, 92 — 142.

Received October 11, 1972

The "Traian Săvulescu" Institute of Biology
Department of Animal Systematics and Evolution

QUELQUES CONSIDÉRATIONS ZOOGÉOGRAPHIQUES SUR LES PUCES (*SIPHONAPTERA*) DE ROUMANIE EN FONCTION DE LEURS HÔTES

PAR

MARIA SUCIU

In this paper there are discussed some aspects of the *Siphonaptera* geographical distribution depending on the hosts area and the paleogeographical conditions of this region.
These problems are integrated in the European and Paleartic faunistic context, Romania being situated, from the geographical point of view, at a crossing of the dispersion direction.

La position géographique de la Roumanie dans le sud-est de l'Europe, son évolution géologique et paléogéographique, les formes variées du relief, du climat et de la végétation ont déterminé la composition spécifique de sa faune ainsi que sa distribution. Il ne faut jamais perdre de vue qu'il existe dans la nature une interdépendance continuelle entre les facteurs biotiques et abiotiques.

Etant ainsi encadrée dans la sous-région européenne du paléarctique, la Roumanie présente une faune très complexe. Nous y trouvons des éléments cosmopolites, holarctiques, européens, euro-sibériens, orientaux méditerranéens, boréaux et endémiques; pour certains d'entre eux, le territoire de notre pays représente la limite méridionale ou nordique, orientale ou occidentale.

Les divers aspects et les implications des relations parasite-hôte nous obligent à faire dans ce cadre une analyse zoogéographique des espèces-hôtes les plus représentatives appartenant aux oiseaux et aux mammifères.

La faune des oiseaux de Roumanie comprend 326 espèces y compris les migrateurs. Elles appartiennent à sept types d'éléments faunistiques [11]: éléments arctiques; éléments sibériens (*Nucifraga caryocatactes*,

Pyrrhula pyrrhula) ; éléments européens (*Turdus merula, Erithacus rubecula, Muscicapa albicolis, Parus major major, Phoenicurus phoenicurus*, etc.) ; éléments méridionaux, comprenant les espèces de steppe et de buissons xérophiles (*Streptopelia decaocto, Netta rufina*, etc.) ; éléments mongoliques (steppe) (*Oenanthe isabellina, Monticola saxatilis*) ; éléments thibétains qu'on trouve dans les Carpates à côté des formes européennes et mongoles, eurybiontes et eurythermes (*Tichodroma muraria, Anthus spinoletta*) ; éléments chinois (*Egretta alba*) ; éléments transpalépus colybita) [9].

Pour ce qui est des rongeurs, les hôtes les plus importants des Siphonaptères, on peut affirmer que l'origine géographique des espèces habitant notre pays est multiple. Dans la composition de notre faune de rongeurs, il y a des éléments européens (*Apodemus, Dryomys nitedula, Eliomys quercinus, Glis glis, Muscardinus avellanarius, Clethrionomys glareolus, Pitymys subterraneus, Citellus citellus, Arvicola terrestris scherman*) ; des éléments euro-sibériens (*Arvicola terrestris terrestris, Apodemus agrarius, Cricetus cricetus, Sciurus vulgaris, Micromys minutus, Microtus arvalis, M. agrestis*) ; des éléments pontiques (*Sicista subtilis, Mus musculus spicilegus*) ; des éléments est-méditerranéens (*Mesocricetus auratus, M. newtoni, Spalax leucodon*) ; des éléments méditerranéens *Lepus europaeus* [2] [4 — 5].

En Roumanie, les rongeurs sont répandus surtout dans la steppe et la sylvo-steppe : *Citellus citellus* et *Mesocricetus newtoni* sont des espèces caractéristiques de la steppe, le premier est répandu dans tout le pays à l'exception du Plateau de Transylvanie, tandis que l'aire du second est limitée à la Dobroudja. Le campagnol des champs (*Microtus arvalis*) et e mulot sylvestre (*Apodemus sylvaticus*) sont largement répandus. Certaines espèces préfèrent les biotopes des fôrêts de montagne, d'autres les fôrêts à feuilles caduques de la plaine, ou les endroits très humides (*Apodemus agrarius*). On remarque toutefois une interpénétration des espèces d'une zone de végétation dans une autre.

Selon notre opinion, la distribution des Siphonaptères doit être continuellement envisagée par rapport à la distribution et à l'origine géographique des hôtes, surtout celle des mammifères. Néanmoins ce n'est pas toujours l'hôte qui détermine l'aire des espèces de puces ; par leurs stades larvaires, qui dépendent des nids des hôtes, elles sont soumises aux mêmes lois naturelles que les insectes terricoles.

On considère, aussi, que les transformations géologiques, spécialement durant les périodes glaciaires, ont profondément affecté les relations des puces [13] avec leurs hôtes.

La méthode qui procède par une confrontation des zones de distribution des puces avec celles des hôtes montre qu'il y a plusieurs types de zones de distribution [10].

En Roumanie ont été signalées jusqu'à présent 72 espèces et sous-espèces de Siphonaptères, appartenant à six familles et 27 genres, infestant 84 espèces et sous-espèces de mammifères et d'oiseaux [18].

L'analyse de la distribution géographique des espèces de puces de notre pays a prouvé qu'il y a des cas où l'aire des parasites coïncide

avec celle de l'hôte. Par exemple : *Monopsyllus sciurorum sciurorum* (Sch.) chez l'écureuil (*Sciurus vulgaris* L.) ; *Paraceras melsis* (Walker) chez le blaireau (*Meles meles* L.) ; *Archaeopsylla erinacei erinacei* (Bouche) chez le hérisson (*Erinaceus europaeus* L.) ; *Nycteridopsylla eusarca* Dampf chez *Nyctalus noctula* Schreber ; *Rhinolophopsylla unipectinata unipectinata* (Tasch.) parasite des espèces de *Rhinolophus*. La zone de distribution des puces peut être plus vaste que celle des hôtes principaux, comme par exemple *Ctenophthalmus orientalis* (Wagner) chez *Citellus citellus* L, *C. assimilis assimilis* (Tasch.) chez *Microtidae* et *Muridae*. L'aire d'une espèces de puce peut couvrir l'aire de plusieurs espèces d'hôtes se trouvant dans les mêmes biotopes ; *Hystrichopsylla talpae orientalis* Smit parasite de *Talpa*, mais aussi de beaucoup d'espèces de *Muridae* et *Microtidae*. *Palaeopsylla soricis* (Dale) et *Doratopsylla dasycnema cuspis* Roths., se trouvent sur d'espèces de *Soricidae*. Dans certains cas l'aire d'une espèce de Siphonaptères n'empiète pas sur celle de l'hôte principal, apparaissant sur un autre territoire chez un autre hôte, qui devient le seul hôte, comme c'est le cas de *Rhadinopsylla sobrina* Peus signalée par nous chez *Spalax microphthalmus* Güld. [19].

Nos observations concernant la distribution des puces concorde à certains points de vue avec celles de B. Rosický [10] en Tchécoslovaquie ; selon cet auteur, il existe en Europe six zones de distribution des Siphonaptères en rapport avec la distribution des hôtes. En se basant sur la distribution de la superfamille *Ceratophyloidea*, B. Rosický [11] distingue quatre complexes faunistiques : 1) *le complexe des forêts à feuilles caduques* ; 2) *le complexe de steppe*, avec trois régions ; *pannonienne, macédonienne* et *de la mer Noire* ; 3) *le complexe de la faune méditerranéenne de la Péninsule Balkanique* ; 4) *le complexe de la faune de montagne.*

Nous considérons qu'il faut adopter cette opinion de Rosický si on veut arriver à une conception unitaire de la zoogéographie des Siphonaptères au moins pour le centre et le sud-est de l'Europe, afin que sur cette base on puisse tirer plus tard plus aisément des conclusions au sujet de l'origine des puces de cette partie du paléarctique. Nous avons cependant apporté quelques modifications aux zones établies par cet auteur et qui se sont imposées comme la conséquence des caractéristiques du milieu en Roumanie.

1) Le *complexe de steppe* comprend une grande partie de la Dobroudja, l'est de la plaine du Baragan et le sud-est de la Moldavie. Nous avons considéré qu'à côté du complexe de steppe, il faut reconnaître l'existence d'une région de steppe au voisinage de la mer Noire. En effet, la steppe pontique présente des caractères à contours précis qui se reflètent dans la composition de la flore et de la faune. On y trouve des espèces de puces qui manquent aux autres types de steppe, par exemple : *Ctenophthalmus congener vicarius* Jordan & Rothschild ; *C. rettigi rettigi* Roths ; *Ctenophthalmus transmiti* Suciu. Aussi, parmi les Siphonaptères qui montrent une préférence pour les hôtes des steppes arides, on peut citer : *Stenoponia tripectinata tripectinata* (Tirab.) *Ctenophthalmus ruris* Jordan, *Citellophilus simplex* (Wagner), *Nosopsyllus consimilis* Wagn.

2) Le *complexe de sylvo-steppe* s'étend dans la Plaine roumaine et en Dobroudja. Dans les forêts de cette zone, domine *Ctenophthalmus*

agyrtes, espèce typiquement européenne. Dans ses conditions de sylvo-steppe il se produit une interférence entre les espèces de steppe et celles de sylvo-steppe.

Dans le nord-est de la Roumanie, on trouve une province très caractéristique qui occupe de la dépression de Jijia-Bahlui ayant un caractère de sylvo-steppe et beaucoup d'éléments sarmatiques. C'est ici que pourrait exister *Ctenophthalmus congener secundus* Wagner signalé par Taskayeva et Hamar [24] en Dobroudja et au Lovrin, comme une conséquence de son expansion à partir des steppes d'Ukraïne.

De même, la végétation de la Plaine Occidentale (continuation de la Plaine Pannonienne) de Roumanie a en général un caractère de sylvo-steppe et, à un moindre degré, un caractère de forêt. La faune de Siphonaptères de cette région est pour la plupart commune avec celle de Hongrie [20 — 23]. Une espèce caractéristique est *Ctenophthalmus jeanneli*, qui n'est pas connu des territoires voisins.

3) Le *complexe des forêts sous-montagneuses* (*sous-carpatiques*), ayant comme espèces caractéristiques de puces : *Malaraeus penicilliger* (Grube), *Megabothris turbidus* (Roths.). *Ctenophthalmus agyrtes* (Heller) et *Doratopsylla dasycnema cuspis* (Roths.).

4) Le *complexe des forêts de montagne*, qui comprend les forêts des Carpates et des monts de Transylvanie (Monts Apuseni). Les espèces de puces qu'on trouve fréquemment le long de la chaîne carpatique, quoique en un petit nombre d'exemplaires, sont : *Rhadinopsylla mesoides* Smit, *Ctenophthalmus obtusus* Jordan & Roths., *C. uncinatus uncinatus* (Wagner), *C. agyrtes kleinschmitianus* Peus, *Peromyscopsylla silvatica silvatica* (Meinert), *P. bidentata* (Kol.), *Palaeopsylla soricis starki* (Wagner), *Ornithophaga mikulini* Ros. & Smit.

5) *Le complexe alpin*, s'étend au-delà de la zone des forêts de sapin et d'épicéa, arrivant jusqu'aux hauts sommets couverts d'une végétation alpine (praires, Juniperus). En cette région les petits mammifères sont rares et ont comme parasites des puces du genre *Amphipsylla* (*A. sibirica* (Wagner)) et *Rhadinopsylla* (*R. mesoides* Smit.).

6) *Le complexe méditerranéen*, qui s'étend dans le Banat et la Dobroudja du sud-ouest. Ici règne un climat sous-méditerranéen qui exerce son influence sur la faune et la flore. Si d'autres ordres d'insectes sont bien représentés dans ces régions, il existe une seule espèce méditerranéenne de puces : *Ctenophthalmus agyrtes serbicus* Wagner.

Certaines espèces de puces possèdent une large valence écologique, se trouvant tout aussi souvent dans les forêts de sylvo-steppe, subcarpatique ou de montagne (*Ctenophthalmus agyrtes*, *Hystrichopsylla talpae orientalis* Smit). L'espèce européenne *Leptopsylla taschenbergi* (Wagner) se trouve en Roumanie, dans des biotopes extrêmement variés, dans la forêt de Letea, située dans le Delta danubien, dans des forêts à feuilles caduques (Ieşelniţa—Banat et Babadag—Dobroudja), dans des forêts de conifères et d'arbres feuillus (Gura Apei — Monts Retezat, 990 m) et dans la zone sub-alpine (sommet Gemeni, 1770 m).

Espèce circumméditerranéenne [1], *Stenoponia tripectinata tripectinata* (Tiraboschi) avance vers l'intérieur du continent jusqu'en Roumanie. Nous croyons néanmoins que la distribution de cette espèce est continue

depuis le sud de la Péninsule Balkanique jusqu'en Roumanie. Actuellement, l'aire de cette espèce en Europe est discontinue. I. Szabo [21] suggère que *Stenoponia tripectinata* existe en Hongrie.

Rhadinopsylla a des formes paléarctiques et néarctiques. Une des espèces de ce genre, *R. sobrina* Peus, décrit dans les Monts Oiti (Grèce) à grande altitude sur *Spalax leucodon*, à été retrouvée par nous chez *Spalax microphthalmus* dans le nord de la Roumanie (Suceava) à une altitude de 500 m. Notre pays constitue la limite septentrionale de sa distribution.

Le genre *Palaeopsylla* comprend des puces parasites d'Insectivores, répandus dans les régions paléarctique et orientale. Deux des trois groupes phylogénétiques [14] existent en Roumanie : 1) Le groupe *soricis* avec *P. soricis starki* Wagner, caractéristique de l'Europe de l'Est et très fréquente dans les Carpates ; 2) le groupe *minor*, répandu dans les sous-régions européenne et méditerranéenne, dont font partie *P. similis similis* Dampf et *P. steini* Jordan.

Ctenophthalmus agyrtes (Heller) est parasite des Murides, plus particulièrement des *Apodemus* et ses sous-espèces présentent une variabilité géographique accentuée. Nous discuterons ce problème dans un travail consacré spécialement à *Ctenophthalmus agyrtes* en Roumanie.

Ctenophthalmus capriciosus Smit, caractérisé par la structure des sternites VIII et IX, qui possèdent à leur extrémité deux soies sub-spiniformes, présente deux sous-espèces européennes : *Ctenophthalmus capriciosus capriciosus* Smit, forme méditerranéenne décrite en Yougoslavie et *C. capriciosus bychowskyi* (Vysotkaya), forme carpatique, signalée dans les Carpates d'Ukraine [25] et les Carpates méridionales de Roumanie [17].

Ctenophthalmus fransmiti Suciu, rencontré jusqu'à présent dans la steppe de Dobroudja [16] et l'Asie mineure [7], doit se trouver à notre avis aussi en Bulgarie et peut-être en d'autres points de la steppe pontique.

Ctenophthalmus assimilis assimilis (Tachenberg) a une large distribution en Europe ; dans notre pays on le trouve sur des hôtes à partir des régions de steppe jusqu'aux zones des hautes montagnes.

Ctenophthalmus rettigi rettigi Rothschild sur les *Muridae* et les *Microtidae*, ainsi que *Ctenophthalmus jeanneli* Jordan sur *Spalax* n'ont été signalés jusqu'à présent que sur le territoire roumain. Jusqu'à l'obtention de nouvelles données nous considérons ces formes comme ayant une aire restreinte.

La famille *Ischnopsyllidae* est représentée dans la faune de Roumanie par neuf espèces parasitant des chauves-souris des familles *Rhinolophidae* et *Vespertilionidae*. Le genre *Nycteridopsylla* possède deux espèces : *N. eusarca* Dampf et *N. dictena* (Kolenati). *Ischnopsyllus* est répandu dans la région paléarctique, avec quelques espèces dans la région orientale et éthiopienne, ayant en Roumanie les espèces suivantes : *I. obscurus* (Wagner) ; *I. elongatus* (Curtis), avec une large distribution en Europe, mais se trouvant aussi en Asie (Liban), Asie centrale et dans les Iles Nipponnes ; *I. variabilis* (Wagner) est largement répandue en Europe continentale, en Asie occidentale et dans le Caucase, mais n'a été signalée en Roumanie qu'en Dobroudja ; *I. octactenus* (Kolenati), fréquente en Roumanie aussi bien que dans toute l'Europe et en Asie occidentale ; *I. inter-*

medius (Rothschild), connue partout en Europe, mais rare dans notre pays, parce que son hôte, la chauve-souris *Eptesicus serotinus* est, elle aussi, assez rare ; *I. hexactenus* (Kolenati) est une espèce européenne. *Rhinolophopsylla unipectinata unipectinata* Taschenberg est une sous-espèce européenne et asiatique qu'on trouve sur les chauves-souris du genre *Rhinolophus* qui habitent de nombreuses cavernes de Roumanie.

Les puces de la famille *Lepiopsyllidae* se trouvent dans les régions paléarctique et néarctique comme parasites des Rongeurs, notamment de la famille des *Muridae*.

Le genre *Peromyscopsylla* a quatre espèce en Europe : *P. silvatica*, caractéristique de l'Europe boréale, fréquente en Roumanie à des altitudes dépassant 700 m ; *P. spectabilis* en Angleterre (Ecosse), France, Espagne ; *P. falax* dans les Alpes et *P. bidentata* espèce européenne et asiatique (Sibérie, Tian-san, Altaï), fréquente au long de la chaîne carpatique roumaine.

Amphipsylla, avec une distribution néarctique et paléarctique, est représentée par *A. sibirica* (Wagner), qui présente des formes géographiques se trouvant dans les massifs montagneux d'Europe centrale, les Ourals, la Sibérie, au Canada, mais extrêmement rare en Roumanie.

Malgré le fait que les données sur le genre *Ornithophaga* (Mikulin), sont rares dans la littérature, on peut toutefois esquisser quelques-unes de ses caractéristiques. On connaît trois espèces paléarctiques et une espèce néarctique, parasites d'oiseaux. *O. anomala* Mikulin, forme asiatique, décrite tout d'abord sur *Picoides tridactylus* puis sur *Dendrocopus leucotus* ; *O. mikulini* Rosický & Smit, chez *Parus major* en Tchécoslovaquie et *Picoides tridactylus* en Roumanie [12] [15] ; *O. sinaia* Ros. & Smit sur *Parus major* (Roumanie) ; *O. nearctica* Holland & Loshbaugh sur *Dendrocopus pubescens leucurus* des Monts Stansbury [6].

La famille *Ceratophyllidae* est abondamment représentée dans la faune de Roumanie (19 espèces et sous-espèces). Nous insistons seulement sur quelques formes qui présentent une distribution intéressante.

Parmi les 60 espèces et sous-espèces du genre *Nosopsyllus* des régions paléarctique, éthiopienne et orientale, on n'en trouve que cinq en Europe, dont trois existent en Roumanie : *N. fasciatus*, *N. consimilis* et *N. mokrzeckyi*.

Nosopsyllus consimilis (Wagner), largement répandue dans les steppe asiatique et dans le sud de l'Ukraine soviétique, a suivi une ligne de dispersion par l'est et le sud de la Roumanie, atteignant sa limite occidentale dans le Banat. Dans le nord de la Dobroudja on remarque une interpénétration de l'aire de *N. fasciatus* et *N. consimilis*, que nous pouvons considérer comme une zone de transgression des deux espèces [18].

Le genre *Ceratophyllus*, holarctique, est parasite d'oiseaux. Parmi les espèces ayant une aire spéciale en Roumanie, nous pouvons citer *C. borealis* Rothschild, comme parasite des oiseaux de haute montagne et *C. pullatus* Jordan & Rothschild, limitée au Delta du Danube.

Nos conclusions zoogéographiques exposent l'état actuel de nos connaissances concernant la distribution des Siphonaptères, suscitent de nouvelles investigations.

CONCLUSIONS

Pour comprendre la distribution des Siphonaptères en Roumanie, ainsi que dans le contexte européen et paléarctique, nous sommes d'accord avec l'opinion selon laquelle les problèmes de distribution géographique des puces devraient aussi être envisagés par rapport à la distribution et l'origine géographique des hôtes, et tout spécialement des mammifères.

En analysant de ce point de vue les parasites et les hôtes, nous avons constaté qu'en Roumanie il y a de nombreuses situations de coïncidence de leur aire ou non.

On a distingué six complexes faunistiques correspondant aux associations de végétation et au climat.

L'origine géographique des Siphonaptères de Roumanie est beaucoup plus complexe que celle indiquée par Taskayeva et Hamar [24]. Ces auteurs ont inclus les 27 espèces qu'ils analysent dans quatre groupements principaux : 1) formes cosmopolites ; 2) formes eurasiatiques des forêts ; 3) formes de prairie dans la zone des forêts eurasiatiques et 4) formes paléarctiques.

La Roumanie se trouve au croisement de plusieurs voies de dispersion des formes venant de l'est (Asie du Sud, Sibérie), de l'ouest (Europe centrale), du sud (régions méditerranéennes du sud de la Péninsule Balkanique) et du nord (Europe boréale). C'est ce qui explique les multiples affinités des Siphonaptères de Roumanie, parmi lesquelles dominent les espèces européennes et euro-sibériennes.

BIBLIOGRAPHIE

1. Beaucournu J. C., 1968, Ann. Soc. Ent. Fr. (N. S.). *4*, *3*, 615 — 635.
2. Calinescu R., et collab., 1969, *Biogeografia României*, Ed. Ştiinţifică, Bucureşti.
3. Christov L., 1964, *Die Fauna Thrakiens*, **1**, Bulgarska Akad. na Naukite Zool. Ins. a Muzei.
4. Hamar M., 1960, *Biogeografia*, **48**, 135 — 150.
5. — 1967, *Din viaţa rozătoarelor*, Ed. Ştiinţifică, Bucarest.
6. Holland G. P. and Loshbugh G. jr., 1958, Canadian Entomologist, **90**, *8*, 486 — 493.
7. Lewis Robert E., 1970, Jur. Kanasas Ent. Soc., **43**, *2*, 184 — 197.
8. — 1960, *Monografia geografică a Republicii Populare Române*, Acad. R.P.R., Bucarest.
9. Radu D., 1967, *Păsările din Carpaţi*. Ed. Acad., Bucarest.
10. Rosický B., 1957, *Fauna C.S.R.*, **10**, *Blechy-Aphaniptera*. Nauk Ces. Akad. Ved., Praha.
11. — 1966, *Folia Parasitologica*, **13**, 64 — 72.
12. Rosický B. and Smit F. G.A.M., 1965, Ceskoslovenská parasitologie, **12**, 245 — 253.
13. Sakaguti K., 1962, *A monograph of the Siphonaptera of Japan*, Nippon Printing and Publishing Co., Ltd. Osaka.
14. Smit F. G. A. M., 1960, Bull. Brit. Mus. (Nat. Hist)., Ent. **9**, *7*, 357 — 367, 369 — 388.
15. Suciu Maria, 1968, Anal. Univ. Buc. Seria St. Biol. **XVII**, 45 — 58.
16. — 1969, Folia Parasitologica, Praha, **16**, 159 — 163.
17. — 1969, Acta Entomologica bohemoslovaca, **66**, *4*, 240 — 242.
18. Suciu Maria, 1970, *Contribuţiuni la studiul sistematic ecologic şi zoogeografic al sifonapterelor* (*Siphonaptera*) *din România* (thèse), Bucarest.

19. Suciu Maria et Hamar M., 1968, Ex. Trav. Mus. Hist. Nat. « Gr. Antipa » **8**, 927 — 938.
20. Szabo I., 1964, Mus. Hist. Nat. Hung., **6**, *1 — 2*, 121 — 129.
21. — 1965, Mus. Hist. Nat. Hung., **7**, *1 — 2*, 177 — 188.
22. — 1966, Mus. Hist. Nat. Hung., **8**, *1 — 2*, 167 — 180.
23. — 1969, Parasit. Hung., **2**, 79 — 118.
24. Taskayeva E. Z. i Hamar M., 1962, Nauk. Dok. Vysh, Skol., **1**, 12 — 16.
25. Visotskaya S. O., 1968, Parazitologuia, **2**, *4*, 344 — 346.

Reçu le 15 mai 1972

Faculté de Biologie, Bucarest
Laboratoire de Zoologie des invertébrés
Splaiul Independenței, 91 — 95.

A RE-EXAMINATION OF GASTRULATION IN TELEOSTS

BY

W. W. BALLARD

I. INTRODUCTION

Until the recent development of techniques for exploring problems of heredity and differentiation at the cellular and ultrastructural levels, the principal advance in embryology in the twentieth century was coming from the study of cellular interactions and cellular affinities by means of embryonic transplantation, and separation of parts of embryos by extirpations and tissue culture. Embryonic induction, discovered more than fifty years ago, is still a mystery, though hundreds of examples are known. How is it that cells which could have been capable of many sorts of differentiation are led into just one path of differentiation by some external influence, usually from their neighboring cells? The real nature and the control of differentiation are a continuing challenge to future research.

In the 1930's and 1940's, when the influence of Spemann in Germany and Harrison in the U.S.A. was spreading most rapidly, the favorite material for such research was amphibian embryos during the gastrulation period. While it is now quite clear that it is fruitless to make a definition in general morphological terms of the *gastrula stage*, one can pursue very precise inquiries into the period or the process of gastrulation. In all vertebrates, and in many other metazoan animals, there is a time when the fertilized egg undergoes mitotic cleavages producing a mass of small cells, none of which show differentiation. Embryos during this process assume various forms, called blastula, morula, blastodisc, etc. Then comes a burst of cellular rearrangement and differentiation, with the transitory appearance of germ layers (ectoderm, mesoderm, endoderm), and a baffling array of temporary structures in different phyla and classes. During the gastrulation process of vertebrates, for instance, the embryos of amphibia show a blastopore and a gastrocoel but those of birds and teleosts do not, and the birds uniquely show a primitive streak, and the teleosts show uniquely a periblast. These temporary structures merely complicate

REV. ROUM. BIOL. – ZOOLOGIE. TOME 18, No 2, p. 119–135, BUCAREST, 1973

the understanding of the general underlying process by which each of these embryos becomes a neurula, consisting of the same organs in similar arrangements. Yet inquiry into this burst of differentiation must concern itself with all these evolving structures, if only for correlating the dynamic processes in time and space.

It was W. Vogt (1925—1929) who made this possible for amphibian embryos by the technique of printing spots of color on the living cells of the blastula and following the drift of the colored spots during gastrulation. He was able, from hundreds of individual experiments, to construct a series of maps of the "morphogenetic movements" of groups of cells as they moved from the surface of the blastula to the interior, and from the animal hemisphere to the vegetal, and from the ventral to the dorsal side. He could reconstruct from the same evidence a fate map of the blastula, showing which parts would normally become incorporated into, and differentiate properly within, the various organs and tissues of the neurula.

Without this normative guide, it would have been impossible to design and evaluate the hundreds of transplantation, extirpation, and culture experiments that followed in the next decades. Similar fate maps and charts of morphogenetic movements were soon made for other species, including the chick. Experiments with amphibia and chicks have yielded interestingly different results, helping very much to distinguish between special and general phenomena.

Teleost fishes represent the most numerous class and one of the most highly specialized groups of vertebrates. One might expect that study of their gastrulation would again yield new and different insights into the general problem of differentiation. Curiously there have been only two attempts to make the prerequisite normative studies of teleost gastrulation by experimental methods. Pasteels [7] published in 1936 both a fate map and charts of the morphogenetic movements of *Salmo*, and Oppenheimer [6] in the same year published a fate map for *Fundulus*, both studies being made with the vital dye technique of Vogt. The question before us is whether these studies provide an adequate basis for planning further experiments on the gastrulation process in teleosts.

II. THE 1936 DESCRIPTION OF *SALMO* GASTRULATION

Vogt and his followers convincingly demonstrated four components of the coordinated "morphogenetic" cellular movements in amphibian embryos: (1) *Epiboly* or movement down over the surface from the animal pole, (2) *Convergence* from either side toward the future or actual body axis, (3) *Invagination* of cells from the surface into the interior, and (4) *Extension* along the body axis.

The teleost embryo presents structural features different from those shown in any of the amniote vertebrates, or in cyclostomes or dipnoi or elasmobranchs, and different even from other bony fishes such as

the chondrostei or the holostei. For instance at the late pregastrula stage, a *Salmo* embryo consists of a high dome of undifferentiated cells, the outermost of which have formed a simple epithelium and the lowest of which rest upon a syncytial layer of cytoplasm containing many huge amitotic nuclei. The syncytium continues over the surface of the egg sphere, enclosing the liquid non-cellular yolk.

Figure 1 reproduces Pasteels' 1936 charts of the morphogenetic movements which appear in this dome of cells, this blastodisc, as it gradually spreads out over the yolk (which is not shown). He indicates the

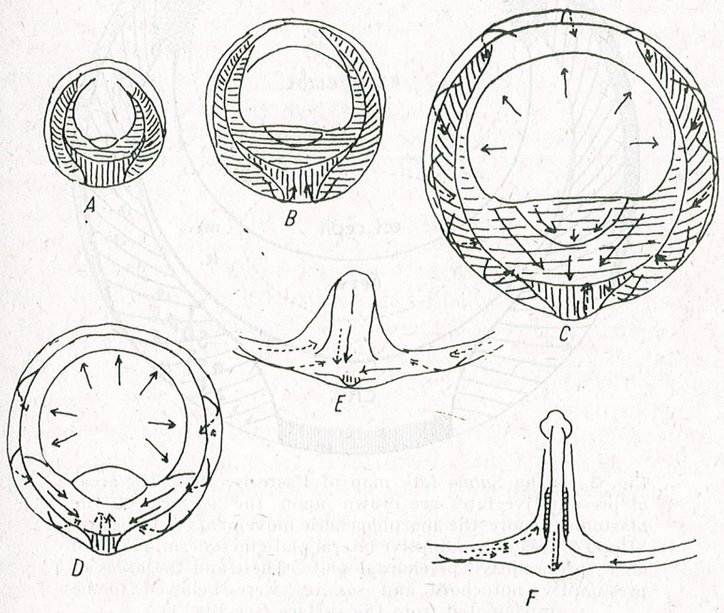


Fig. 1. — The charts of morphogenetic movements in *Salmo*, from Pasteels, 1936. Top views of blastodisc, yolk sphere not shown. *A*, pregastrular stage; *B*, invagination starting at embryonic sector; *C* and *D*, epiboly, convergence and invagination become more and more active; *E* and *F*, germ ring and embryonic axis, showing elongation as well as continuation of the other movements. At stage *F*, the blastodisc has covered the upper half of the yolk.

four types of movement by arrows. Note particularly that, whereas in amphibia and some other vertebrates there is a blastopore through which invagination takes place, there is no blastopore in the teleost embryo, yet he shows invagination taking place from the surface to the interior all around the rim of the blastodisc. Figure 2 shows his fate map for the *Salmo* blastodisc before gastrulation, drawn upon the surface. It implies that cells in the midline are drawn inward from the surface to form the notochord, and that cells from the margins also invaginate to form the somites. This analysis was well received a generation ago and

has acquired a degree of immortality by incorporation in the new *Traité de Zoologie* and various textbooks. I shall show that it rests on some serious misconceptions.

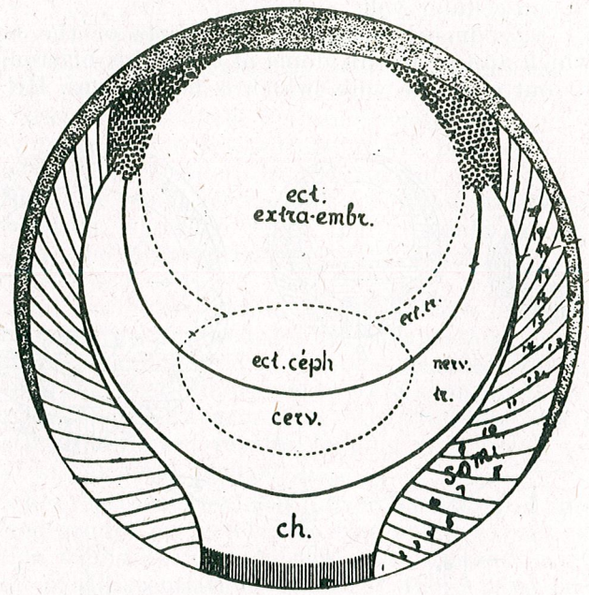


Fig. 2. — The *Salmo* fate map of Pasteels, 1936. The areas of presumptive fates are drawn upon the surface of the blastodisc before the morphogenetic movements have begun. Stippled area : presumptive lateral plate mesoderm. Hatched area : presumptive prechordal plate. These and the areas of presumptive notochord and somites were believed to be invaginated from the surface (see Fig. 1).

III. TESTS OF THE RELIABILITY OF THE 1936 STUDY OF *SALMO*

The map shown in figure 2 is drawn on the outer surface of the pregastrular stage. It predicts that these surface cells, in postero-anterior order along the midline, will form respectively the prechordal plate (vertical hatching), notochord (ch), spinal cord (nerv. tr.), brain (cerv.), and general epidermis. Pasteels could not get clear evidence as to the location of the future endoderm cells, but supposed that they must be superimposed in a very thin layer on the notochordal area.

But it can be shown [1] that none of these surface cells take part in the formation of any internal organs at all. One needs only to expose a batch of *Salmo* pregastrulas to a weak solution of the vital dye, Nile blue sulfate, exactly long enough so that only the cells of the cellular envelope have absorbed the blue color. This has to be controlled very carefully by dissecting samples of the batch at intervals of time, after

fixation in $HgCl_2$ solution. This fixative does not destroy the color, but enhances its contrasting effect by turning the unstained cells pure white. After the stained but living embryos are returned to running water, the dye becomes fixed in these superficial cells and does not penetrate to deeper layers. Gastrulation proceeds normally and the organ systems appear. Only the epidermal cells are blue. There is no blue color in somites, central nervous system, notochord, or intestine. These organs must then have formed from unstained cells of the interior.

With the embryos of other teleosts, *Salvelinus* and *Gobius*, I have been able to go a step farther [1]. The stain, confined to the exterior cells, can be bright enough so that identifiable groups of cells exactly at the margin of the blastodisc can be watched and photographed every few hours during gastrulation. They do not invaginate. They remain on the surface and contribute only to epidermis.

What was Pasteels' evidence that in addition to epiboly there is invagination going on all around the rim of the blastodisc? He reported that, after dyed spots had been printed on the surface of the pregastrula exactly at the rim, blue cells began to appear in the interior, moving in a different direction from those remaining on the surface, in fact drawing closer to the embryonic axis. The three drawings to the left of figure 3, incorporate both his own record of this observation, and his interpretation of it [7]. The observation is correct. I have confirmed it hundreds of times. But his interpretation is wrong. He incorrectly assumed that when the dye carrier was pressed against the surface cells only these became colored. In fact, if enough dye is transferred by this contact method so that it can be seen a day or two later in the living embryo (as in his experiments) one can show by fixation in $HgCl_2$ and prompt dissection immediately after staining, that the dye has penetrated to a depth of several cells, often even through the entire thickness of the blastodisc near the rim. The blue cells which he really did see moving in the interior were not invaginated cells but cells which has originated in the interior and received their dye by diffusion. As suggested on the right side of figure 3, the surface cells were moving in simple epiboly while the internal cells, dyed at the same time, were moving obliquely, adding a component of convergence to their epiboly [2].

Having strong reason to suspect that all the arrows around the edge of the 1936 chart represent false conclusions, I began to wonder about the accuracy of the fate map. Even if no cells were moving from the surface to the interior to become prechordal plate, notochord, somites, lateral plate, etc., it still seemed possible that the areas on the map represented presumptive fates of cells lying deeper inside. The Vogt technique of printing spots of vital dye on the embryo is inadequate for such an inquiry, but I found a better way by using ordinary chalk of various colors, such as one uses on the schoolroom blackboard [3] [4]. With a very delicate needle one can pick up finely powdered chalk and push it among the internal cells, where it lies in intercellular spaces and is carried along as the cells themselves move. The location of the marks can be recorded in daily drawings of the same nearly transparent individual at successive embryonic stages. As much as a week later one can dissect the young fish and find the particles embedded in brain, muscle, notochord or other

tissues, all of which have nevertheless achieved normal structure and function. Since the chalk has no power of movement itself, the changes in its position must correspond to the movement of the cells which surround it.

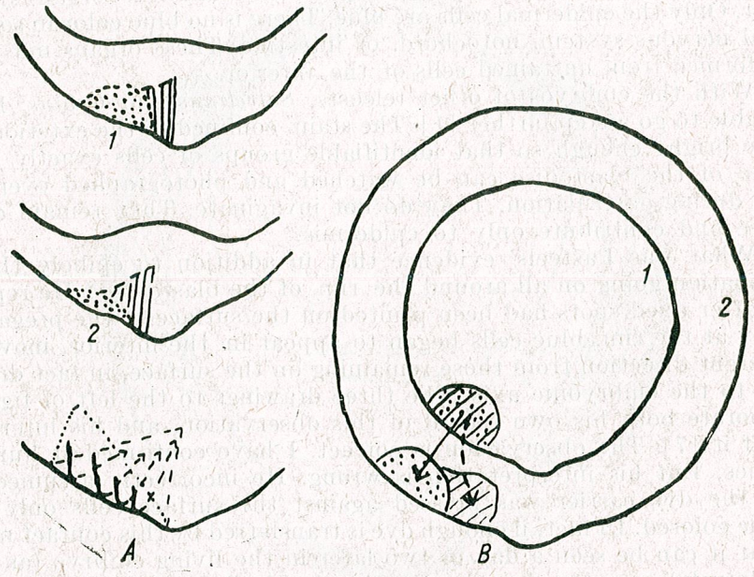


Fig. 3. — Separation of deep cells from superficial cells after a spot of Nile blue sulfate dye has been placed on the surface at the edge of the blastodisc. On the left, drawings by Pasteels which imply invagination. On the right, younger and older stages are superimposed, showing colored cells of both the surface and the interior undergoing epiboly, the latter separating from the former by a convergence movement, without invagination.

Figure 4 assembles sixteen cases specially chosen from over 1200 *Salmo* embryos which had received chalk implants among their cells at the late pregastrular stage. Each case represents one embryo's partial answer to the question, "what is the fate of the cells lying under this exact spot on the 1936 fate map?", after it had received an implant of chalk there by vertical thrust of the needle. (Two separate embryos received implants at sites 7, 10, 11 and 12). The particles of chalk were not usually distributed evenly along the path of the needle. By luck most of them may rest among deep cells, or among superficial cells. The cases are deliberately selected to show the full range of discordance of my results with the predictions of the 1936 map. Many of my experiments did in fact yield results in agreement with those of Pasteels. Nevertheless, I have literally hundreds of cases which show discordances similar to the ones chosen, though of lesser degree.

The chalk of implant 1, instead of being carried into the seventh left somite as the map predicts, appeared in the hindbrain, in left somites 1 — 4, in lateral plate mesoderm outside somites 10 to 20, and farther

out on the yolk sac, and in the tail bud. Chalk from spot 2 did not go into the spinal cord at all but it scattered along most of the somites 10 to 40 of the left side, and farther out on the yolk sac. Chalk from site 3 lodged in the spinal cord at the level of the 20th somite, but also within somite 14 of the left side, in the lateral plate at the level of the 10th left somite, and in pharyngeal endoderm, at the transverse level of the ears.

Chalk from point 4 did not reach the notochord, but lodged deep in the posterior part of the hindbrain and in the skin over the anterior hind-

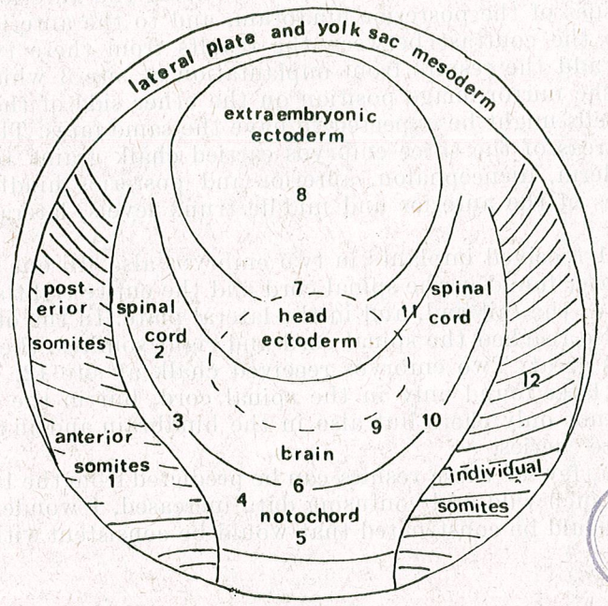


Fig. 4. — Numbers placed on the 1936 *Salmo* fate map, representing sites where chalk grains were implanted in vertical lines among the interior cells at the pregastrular stage, to test the validity of the map. (See text).

brain. Also some of it was embedded in the anterior left somites. From point 5, some of the chalk grains were carried into the notochord as predicted, but some of them also appeared in the skin of the head, within the midbrain and hindbrain — on the right side anteriorly and on the left side posteriorly — and in head mesoderm of the right side, right lateral plate, heart, and pharynx endoderm. Chalk from point 6 turned up in the floor of the forebrain, and in the spinal cord and the skin covering it at the level of somites 1 to 9, and it was also liberally scattered in the pharyngeal endoderm.

Two separate implants, made at the very center of the blastodisc in different embryos (spot 7) both went to head mesoderm, but some

chalk from one of them also went to the yolk sac, while some from the other appeared also in the right eye and in the diencephalon. Chalk implanted at spot 8 went to the yolk sac, but also to somites beyond the 35th on the left side, i.e. within the tailbud. Chalk from spot 9 entered the brain but not the spinal cord, and was also found in the skin over the forebrain, both in the notochord and in the head mesoderm of the right side, and in the posterior part of the right somite band.

Site 10 received implants in two different embryos. In the first, the chalk went to dorsal diencephalon, to the hindbrain — in which the more posterior grains lay deeper than the anterior ones — and to head mesoderm. In the other, it went to the skin covering the anterior hindbrain, to deep tissues of the posterior hindbrain, and to the anterior few right somites. To the contrast between the results from these two embryos one should add the results from implantation at site 3 which is almost exactly in the mirror-image position on the other side of the blastodisc, where the cells might be expected to have the same fates. The cells from these two areas of the three embryos carried chalk grains to head skin, head mesoderm, diencephalon, anterior and posterior hindbrain, spinal cord, somites of the anterior and middle trunk levels, lateral plate, and pharynx.

Site 11 received implants in two embryos also. In one of them the chalk was later found in the spinal cord and the entire right somite series as far back as the tail bud, and in the lateral plate. In the other, though the chalk also reached the spinal cord and right somites, the scatter was only half as great. Two embryos received chalk at site 12. In the first, grains were later found only in the spinal cord, but in the second they were found not only there but also in the hindbrain and in the posterior right somite series.

Clearly, few of these results can be predicted from the 1936 map. As the mass of such wild and confusing data increased, I wondered whether a new map could be constructed that would be consistent with them. (See below).

IV. NEW CHARTS OF THE MORPHOGENETIC MOVEMENTS IN *SALMO*

Grouped in a wide variety of special experiments, many hundreds of cases have been collected showing what happened when chalk grains were implanted at particular points on the pregastrular blastodisc. The record for each embryo contains a drawing of where the chalk had been implanted, both in relation to the center of the blastodisc and to the axis of bilateral symmetry. A series of drawings shows the course of migration of the particles, and also a full record of the regions or tissues or organs in which they were finally found. Records from many embryos are assembled in figure 5, 6 and 7 to show the types of movement revealed during the fourth, fifth and sixth days of culture at 10° C. In each chart the younger blastodisc is superimposed on the older one, and arrows connect the earlier and later positions of the same cloud of implanted particles. In many cases, the particles separated into two or more groups, the deeper ones making the greater degree of convergence toward the axis.

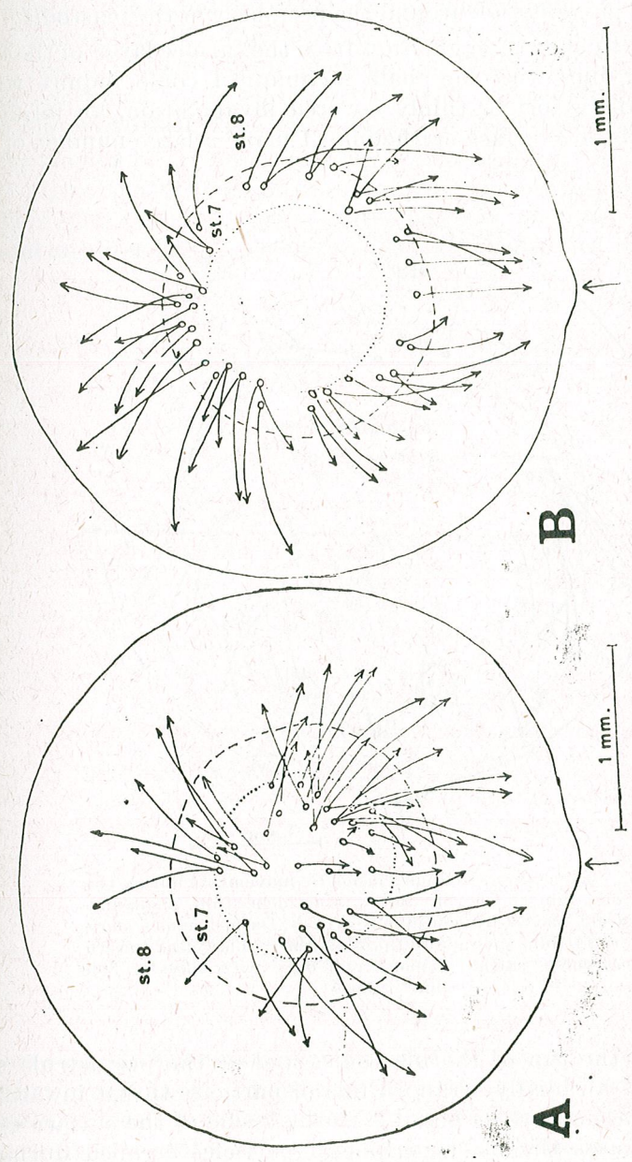


Fig. 5. — Directions of the morphogenetic movements of interior cells of *Salmo* during the fourth day of incubation at 10°C. The blastodisc of the younger stage (dashed line) is superimposed on that of the older stage (continuous line). Each small circle represents a site of implantation of chalk particles in a separate embryo, the arrow showing the movement of the particles during 24 hours. *A* represents sites closer to center of blastodisc, *B* those near the periphery. Outside arrow indicates plane of bilateral symmetry.

The arrows show movements of epiboly, convergence and extension quite like those of the 1936 chart, with the striking difference that there is no evidence of invagination around the periphery of the blastodisc.

My efforts to show invagination from the surface layer of the blastodisc had failed, but with the chalk technique I could inquire whether there was perhaps a sort of thinly covered invagination, an inrolling of deeper cells inside the surface epithelium. I made a large number of chalk

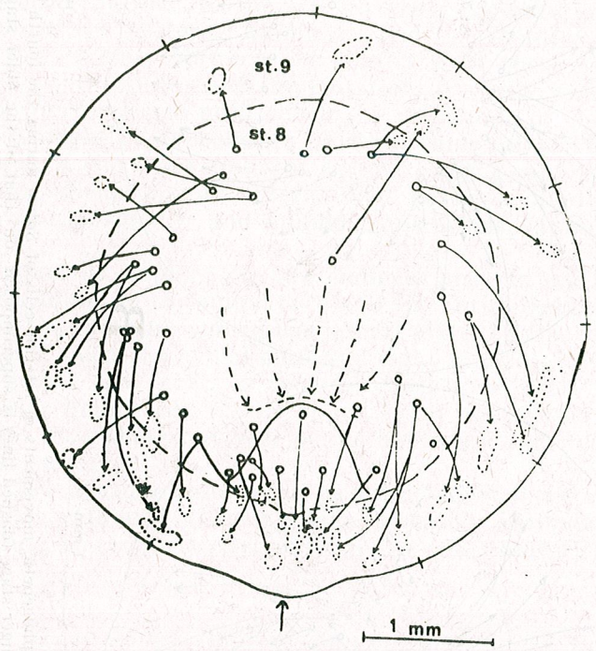


Fig. 6. — Direction of the morphogenetic movements during the fifth day, shown as in figure 5. Evidence from chalk implantations shown by continuous arrows. Dashed arrows show movements from the rapidly thinning central blastodisc toward the embryonic shield, demonstrable only with spots of Nile blue sulfate.

implantations at the rim of the blastodisc and at the pregastrula stage, each consisting of a radially arranged line of particles thrust inward from the very edge, and as close as possible to the inside of the surface epithelium. In many cases the most peripheral particles escaped during the closure of the wound, and of course it could not be determined until the following day where the implant would lie in relation to the axis of bilateral symmetry. Nevertheless enough embryos were treated in this way so that evidence became available as to what was happening in all sectors of the

blastodisc during the most active period of the morphogenetic movements (Fig. 8). If an inrolling of cells, a "thinly covered invagination" had in fact occurred, the radial lines of chalk particles should have been bent into V lines, reorienting like the bent arrows of supposed invagination at the edge of the 1936 chart. None of them did. The chalk particles closest to the rim continued to be closest to the rim. The lines drifted somewhat toward the axis during the next days, but remained relatively straight.

By stage 9 of my series (see Fig. 7 and *E* of Fig. 1) epiboly has reached full vigor and the rim of the blastodisc has been thickened as a germ ring. This soon becomes partly separated into an inner layer, the *hypoblast*, and an outer layer, the *epiblast* [5], and thickened in one sector, the

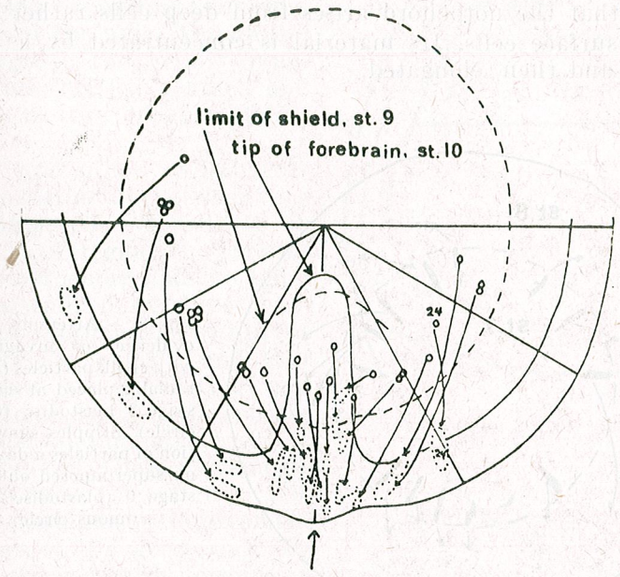


Fig. 7. — Direction of the morphogenetic movements during the sixth day, shown as in figure 5. Rapid consolidation and elongation of the embryonic axis.

embryonic shield, where the embryonic axis develops [4]. Many investigators in the past have assumed that this hypoblast, and most of the embryonic shield, arises by invagination from the surface. However, one can lift up a small sector of the edge of the blastodisc and push in a very small piece of material impregnated with Nile blue sulfate, locating it under the center of the blastodisc. During the next day and a half, the deep central cells which have become blue by contact with it can be seen to migrate centrifugally and join the hypoblast and embryonic shield.

Their emigration leaves the center of the blastodisc very much thinned out, floating on a fluid-filled cavity. Many illustrations of this can be found in the older literature and cited as evidence of invagination. The observations (and the drawings and photographs of serial sections) are correct, but the interpretations were incorrect.

If such a dye carrier is implanted beneath the blastodisc of the pregastrula but rather close to the edge, it may later be found — by chance in about one of a dozen trials — in the sector where the embryonic axis is forming. In the cases which are thus achieved by good luck, the dye becomes brilliantly concentrated in all of the notochord except its most posterior part, and continues to be separated from the rim of the blastodisc by a short section of unstained notochord (Fig. 9) which finally comes to lie in the tail [3]. This result, confirmed in numerous cases, is taken as evidence that the notochord arises from deep cells rather than from invaginated surface cells. Its material is concentrated by a very early convergence and then elongated.

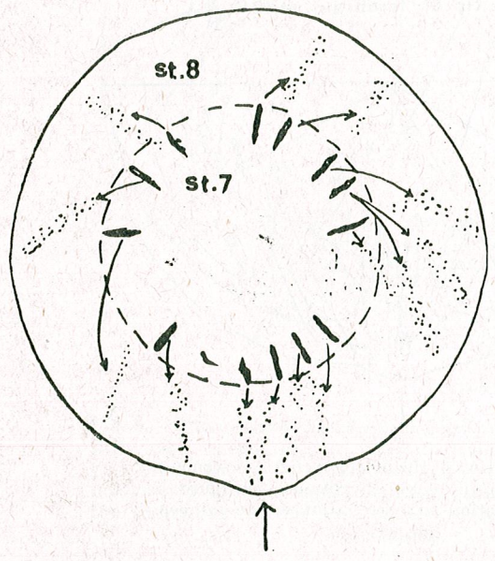


Fig. 8. — Attempts to get evidence of invagination with chalk particles (black), radially placed at edge of stage 8 blastodisc (dashed circle). Stipples show location of particles a day later, on superimposed outline of stage 9 blastodisc (continuous circle).

One can wait until the position of the axis of symmetry is detectable by a faintly swollen point on the rim of the blastodisc, and then lift up this sector only, and wipe chalk particles upon the very deepest cells. Later on, most of these chalk grains are found in the endoderm, only a few of them occasionally in the notochord, and very rarely in the somites. Thus, the future endoderm cells are also originally internal cells,

lying deep to the future notochord cells. They are not invaginated, but perform early movements of convergence and then elongation.

The deep surface of the germ ring can be marked with chalk particles by similar but later operations. Because the cells of the germ ring are rather loosely assembled and the hypoblast and epiblast layers are thin, the chalk grains not uncommonly become incorporated into both layers. I had two spectacular cases in which one implant of black chalk was made at 30° from the plane of bilateral symmetry, and another of yellow chalk was made at 60°. In both cases the granules in the epiblast continued their epibolic drift, while those in the hypoblast, though remaining in the expanding germ ring, drifted toward the axis. And during this convergence, the yellow hypoblast chalk was carried beneath and then medial to the black epiblast chalk.

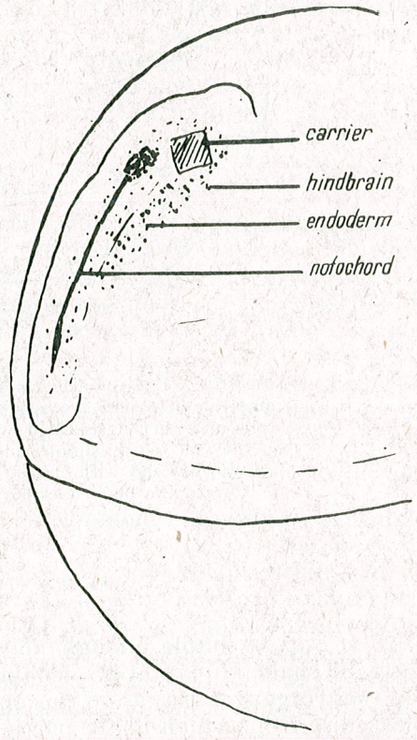


Fig. 9. — Notochord stained by placing a carrier of Nile blue sulfate dye under the blastodisc of the 3-day-old *Salmo*, near the edge and in the plane of bilateral symmetry. The blue notochord cells are not invaginating in an anterior direction, they are extending posteriorly.

These and other experiments have led to a distinction between very *early morphogenetic movements* in which the head region, the nervous system, the notochord and the endoderm are assembled by direct condensations of internal cells within the early embryonic shield; and *later movements* into the germ ring and through it to positions in the somites and other mesodermal structures of the trunk and tail. These movements are summarized in figure 10, which also distinguishes between the two functions of the germ ring. Its epiblast is charged with forming a yolk sac of ectoderm and mesoderm, which remains as the belly skin of the young fish after the yolk supply has been used up. Its hypoblast collects cells from the deeper parts of most sectors of the blastodisc, and feeds them gradually toward the mesodermal organs and tissues of the more and more posterior parts of the embryonic axis. Note that this figure contains no suggestion of invagination. However, there is ample evidence that cells from the germ ring are carried into mesodermal structures of the tail, and no evidence that the germ ring contributes to nervous, notochordal, or endodermal structures.

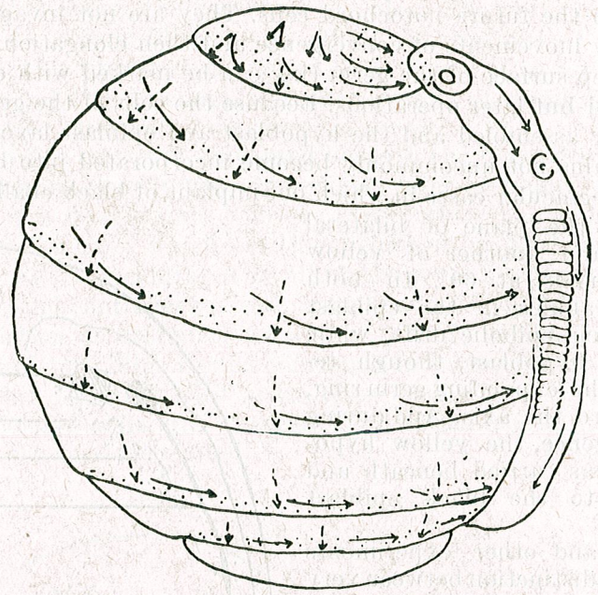


Fig. 10. — Late morphogenetic movements in *Salmo* diagrammed in side view, successive stages superimposed. Epiblast cells of germ ring (dashed arrows) accomplish the completion of the yolk sac by simple epiboly. Hypoblast cells (continuous arrows) descend with the germ ring but continually converge toward the body axis, forming somites and lateral plate.

V. A NEW FATE MAP FOR *SALMO*

It was a simple enough matter, as the records from hundreds of cases of chalk implantation accumulated, to make a chart that showed, for each organ or tissue of the embryo, the regions of the pregastrular blastodisc from which chalk particles had been drawn into it. If the new fate map were to be anything like that of 1936, all these special regions — for the nervous system, for the somites, for the notochord, etc. — could be cut out from the charts and assembled like a flat picture-puzzle of fifteen or twenty pieces. Results such as those recited in relation to figure 4 quickly ruled out this possibility. The regions from which the moving cells carry the chalk into the different mesodermal structures — head mesoderm, anterior and posterior somites, lateral plate — overlap broadly, and when they are viewed collectively they reach into every part of the blastodisc. In the half of the blastodisc which is later to contain the embryonic shield, the areas from which chalk was drawn into forebrain, hindbrain, spinal cord, notochord, endoderm and heart all have a common center on the future axis of symmetry and they all overlap very broadly. The only way to bring this information together is in a three-dimensional fate map, the first proposed for any vertebrate.

Such a concept is at once supported by the experiments described above relating to the notochord and the endoderm. One can print blue spots upon the area already associated with the brain and spinal cord, both in the 1936 map and in my charts derived from chalk implants, and the color becomes concentrated in nervous tissue, but not in the notochord or endoderm — presumably because the dye has not reached far enough down during the time of application at the pregastrula stage to reach these cells at lower levels of the blastodisc.

Many times it has also been noted that chalk particles, originally lined up vertically where they had been thrust by the needle, soon begin to scatter in groups (Figs 5 — 7). Generally, where they are being carried toward the germ ring the deeper ones move faster and undergo more pronounced convergence than the superficial ones. Thus, particles from one vertical thrust can be carried to widely separated points along the body axis. Only very deeply planted particles finally come to rest in the notochord or the intestine.

The evidence falls into place when combined with the results of an extension of the first experiment which I have described. Bathing the pregastrular stages of *Salmo* in Nile blue sulfate dye as before, but using different concentrations and exposure times, it was possible to obtain large numbers of individuals of two uniform types. In one, moderately exposed, the dye penetrated to the depth of several cells below the surface epithelium, but was fixed there and did not spread deeper after the embryos were returned to running water. In the other, strong exposure allowed the dye to penetrate deeper, half the way to the bottom of the central part of the blastodisc, before the spreading was stopped by removal from the dye solution.

Gastrulation and anatomical development proceeded normally. During and after the neurula stage, many embryos of both batches were dissected in $HgCl_2$ solution, with this highly significant result: in the moderately stained embryos, the dye was later found in all the central nervous system except the ventral keel of the brain, and in somites posterior to the tenth pair, but *not in more anterior somites*, or notochord, or endoderm. In the more heavily stained embryos, the dye was found in all parts of the central nervous system, and in *all* the somites, but not in notochord or intestine [3].

The precision of these results made it unnecessary to think, as seemed permissible at an earlier stage in my research, that it followed from the high degree of overlap of areas contributing cells to very different fates, that such cells were randomly mixed. It pointed to the conclusion that these areas are stacked up at the pregastrular stage, layer upon layer. It would naturally follow from such an arrangement that a string of chalk particles, thrust down in a vertical line through several such layers, would be subsequently distributed to a variety of tissues and regions, the several layers moving with their own graded velocities and directions. One suspects a sorting out of cells to different regions and organs by morphogenetic movements of maximum simplicity and efficiency.

The new *Salmo* fate map, built to be consistent with all this evidence, has to be three-dimensional, and may be shown in a series of transverse slices seen obliquely from above (Fig. 11). The half of the circular blasto-

disc on which the embryonic shield becomes centered may be called the axial half, the other one the abaxial half. The latter contains chiefly prospective mesodermal cells, with a thin layer of prospective epiderms covering them. There are a few prospective forebrain cells toward the center, highly placed. In the more external parts of the axial half, there lie many cells of the prospective nervous system. They are covered only by the superficial epithelium and their mass thins out laterally but projects more deeply as it approaches the future axis of symmetry, where it descends below middle depths. In the lowest part of the axial half there is a very thin layer of prospective endoderm, overlaid by a layer of prospective notochord. Between the upper prospective neural material and the notochordal layer there are bilateral intrusions of the mesodermal sheet of the abaxial half of the blastodisc. How the multitudes of cells in this continuous mesodermal stratum are apportioned among the many organs

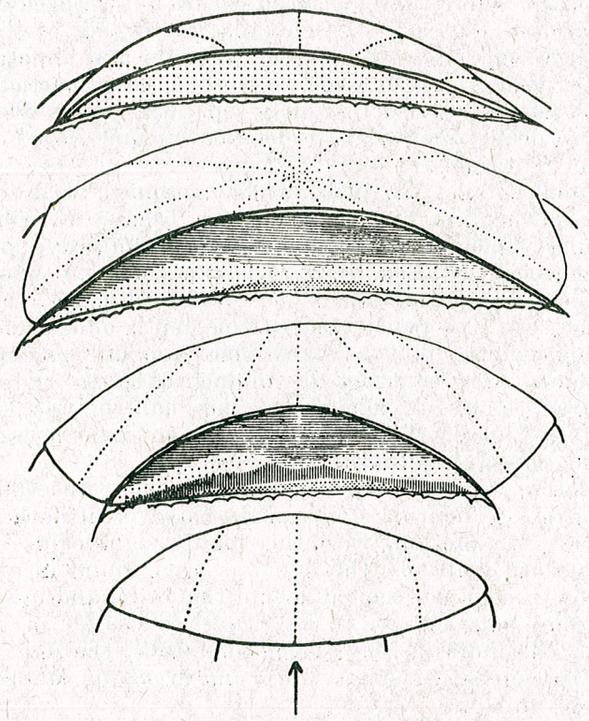


Fig. 11. — Three-dimensional fate map of the pregastrular blastodisc of *Salmo*, incorporating the results of experiments described in this paper. In the transverse dissections, the horizontal lines represent prospective nervous system, the vertical lines represent prospective notochord, the coarse stipple represents prospective mesoderm (not resolved into layers), and the fine stipple represents prospective endoderm.

of primarily mesodermal origin is not revealed by the present experiments, but one may guess that the most likely fate for each cell is related in some way to the depth at which it lies and its original distance from the point where the embryonic axis is first seen. The experiment described last also indicates that cells destined for the anterior ten or so pairs of somites lie closer to the future midline, covered over by prospective neural cells, while those destined for more posterior somites lie farther laterally and at greater distance, and therefore closer to the surface.

If this fate map is not in its turn found defective, and if fate maps for representatives of other orders of teleosts — which are now in preparation — show the same peculiarities, then it will be necessary to change some ideas about the uniformity of the major aspects of gastrulation in vertebrates, which have stood unquestioned for thirty years.

Also, if this fate map is found reliable, the door is opened to more precise analytical experiments on the factors which produce and guide the processes of gastrulation in this very large and very important group of vertebrates.

Acknowledgment

For the opportunity to work in Romania I am indebted to the Exchange Program of the Academy of the Socialist Republic of Romania and the National Academy of Sciences of the United States of America. I wish to thank the staff of the Institute of Biology "Traian Săvulescu" for many kindnesses.

REFERENCES

1. Ballard W. W., 1966, *The role of the cellular envelope in the morphogenetic movements of teleost embryos.* J. exp. Zool., **161**, 193 — 200.
2. — 1966, *Origin of the hypoblast in Salmo.* I. *Does the blastodisc edge turn inward?* J. exp. Zool., **161**, 201 — 210.
3. — 1966, *Origin of the hypoblast in Salmo.* II. *Outward movement of deep central cells.* J. exp. Zool., **161**, 211 — 220.
4. — 1968, *History of the hypoblast in Salmo.* J. exp. Zool., **168**, 257 — 272.
5. Ballard W. W., Dodes L. M., 1960, *The morphogenetic movements at the lower surface of the blastodisc in Salmonid embryos.* J. exp. Zool., **166**, 67 — 84.
6. Oppenheimer J. M., 1936, *Processes of localization in developing Fundulus.* J. exp. Zool., **73**, 405 — 444.
7. Pasteels J., 1936, *Études sur la gastrulation des vertébrés méroblastiques.* I. *Téléostéens.* Arch. Biol., **47**, 205 — 308.

Received June 28, 1972

Dartmouth College
Department of Biology
Hanover, New Hampshire, U.S.A.

COMPARATIVE STUDY OF THE BUCCO-PHARYNGEAL CAVITY IN SOME SPECIES OF FISHES BELONGING TO THE COBITIDAE FAMILY

BY

LOTUS MEŞTER

In this paper there are examined four species of Romanian Cobitidae, giving a special attention to buco-pharyngeal cavity, which was less analysed till now. Using different research methods (anatomical, histological and histochemical ones), the author mentions for the first time in literature the presence of two horny formations in the posterior part of the bucco-pharyngeal cavity. These formations, the structure of which is similar to that of the bitter bone in carp, have probably a masticatory function. It is pointed out also the comparative structure of the papillae from the roof and of the palatal organ, in correlation with the species ecology.

The structure of the alimentary canal in teleost fishes has made the object of many papers, the authors frequently correlating the anatomo-histological data with the ecological ones. A synthesis and a revision of older papers may be found in the works of Oppel [13] [14] [15] and Bolk et al. [2]

Călugăreanu [4] [5], Lupu [8] [9] [10] and Bușniță [3] have particularly analysed the medium and posterior segment of the alimentary tract in *Misgurnus fossilis* and *Cobitis taenia*, as related to intestinal breathing. Interesting papers upon some families related with the Cobitidae belong to the following authors : Curry [6] on carp ; McVay and Kaan [11] on crucian ; Al-Hussaini [1] on Cyprinidae — the latter also giving cytological and physiological data.

In the present paper we describe the structure of the bucco-pharyngeal cavity in some Cobitidae species from Romanian waters, which have not retained, so far, the attention of the researchers.

REV. ROUM. BIOL.– ZOOLOGIE, TOME 18, Nº 2, p. 137–144, BUCAREST. 1973

MATERIAL AND METHODS

The following Cobitidae species have been comparatively examined :

1. *Misgurnus fossilis* (L.)—Sîrba pool (Oltenia).
2. *Cobitis taenia* L. — Colentina, Olteţ.
3. *Cobitis elongata* Heck. & Kner — Nera.
4. *Sabanejewia romanica* (Băc.)—Olteţ.

For fixation we utilized : Bouin, Bouin-Hollande, Susa and Zenker. After fixation, the whole pieces were decalcified with trichloracetic acid 5% and embedded in paraffin. The sections thickness varied between 7 and 10 μ ; they were stained with mucicarmine Mayer, azan, May-Grünwald-Giemsa, alcian blue and P.A.S.

MACROSCOPIC ANATOMY

The analysed fishes are benthonic, with a large spectrum of food composition.

Misgurnus fossilis is found in ponds and in slow-flowing waters, with preference for muddy bottoms. Its food consists of insect larvae, worms, crustaceans, molluscs and aquatic vegetation.

Cobitis taenia is found in ponds or in slow-flowing waters with muddy, sandy or argillaceous bottom and feeds on crustaceans, insect larvae, rotifers, oligochetes and algae.

Cobitis elongata prefers the sandy bottom of the hills area and feeds on insect larvae, crustaceans, oligochetes, leeches and aquatic vegetation.

Sabanejewia romanica lives in mountain and hill rivers, on sandy bottom; in its food, animal components are prevailing. It consumes insect larvae, crustaceans, oligochetes, algae and vegetal remains.

The snout of these fishes is curved, with an inferior protractile mouth bearing 3 pairs of moustaches. They are provided with a filtration system of biserial symmetric type [7]. Just like in *Noemacheilus barbatulus* [12], in *Misgurnus fossilis* there is a tegumentary valve, both on the roof and on the floor of the bucco-pharyngeal cavity. In the other Cobitidae the floor valve is missing.

HISTOLOGY

The upper lip consists of a thick epithelium with many rows of epidermic cells, a few small mucous cells and numerous profound gustatory buds (19 on one section in *Cobitis elongata*), sustained by the chorion papillae. The superficial layer of epidermic cells is horny, becoming still hornier at the entering of the bucco-pharyngeal cavity. In *Sabanejewia romanica* the chorion is rather thick (Fig. 1 *C*).

In *Misgurnus fossilis* almost the entire depth of the superior epithelium is formed by bag-shaped mucous cells, having a big nucleus at the basis (Fig. 1 *A*). Among the ordinary epidermic cells, rare gustatory buds may be noticed.

The chorion is thick, while underneath, in the lax connective tissue, large chromatophores, blood vessels and nervous endings are to be found. The presence of some particular cells in the epithelium of the upper-lip mucous membrane in this species has also been pointed out by Oppel [15], these cells being denominated "pedunculate goblet cells". The inferior epithelium of the upper lip is thinner, consisting of epidermic cells among which, here and there, the chorion sends papillae to sustain the gustatory buds (one may notice up to 21 buds on one section). Mucous cells are rare and globulous. The last layer of epidermic cells is horny ; the horns being more pronounced in the median area, a structure reminding that of *Noemacheilus barbatulus* appears.

The lower lip (Fig. 1 *B*) presents in all the investigated species an epithelium formed almost exclusively of epidermic cells, strongly horny at the surface; towards the extremities, gustatory buds are observed. In *S. romanica* and *Misgurnus fossilis* the chorion is thicker. In *Misgurnus fossilis* the lax connective tissue is thin, with many nuclei, blood vessels and chromatophores.

The maxillary valve is obvious, having on its superior part an epithelium with bottle-shaped mucous cells, a crescent nucleus at the basis, 1 — 2 gustatory buds ; on the inferior part, the gustatory buds are rare in *C. taenia*, denser and larger in *C. elongata*, *S. romanica* and *M. fossilis*. The superficial cells of the maxillary valve inferior epithelium are slightly horny in *Cobitis* and *Sabanejewia* species, more horny in *Misgurnus*. The chorion is thick and sinuous ; in the lax connective tissue of the maxillary valve axe, there are nervous endings and blood vessels (more numerous in *Misgurnus*). The presence of more abundant mucous cells in the superior epithelium of the maxillary valve and on the mouth roof facilitates the valve movements during breathing.

The mucous membrane of the bucco-pharyngeal cavity presents a series of folds (higher on the roof), consisting of a thick epithelium with polygonal epidermic cells, having large, oval or irregularly shaped nuclei ; the cells are orderly arranged in the superficial layer. Along with these, there are also pear-shaped mucous cells, gustatory buds, while clubbed cells are missing. The chorion is thick in *S. romanica* and *Misgurnus fossilis* and thin in *C. elongata* and *C. taenia*. The lax connective tissue includes blood vessels (large and tapered in *Misgurnus*), nervous endings and adipose cells (exclusively in *C. elongata*).

Only *Misgurnus fossilis* presents on the floor a well developed mandibular valve, the epithelium of which includes numerous mucous cells and rare gustatory buds (Fig. 1 *D*).

At the olfactory capsules level, 2 — 3 large folds, papilla-like, are observed in the species of the *Cobitis l. s.* genus, while in *Misgurnus*, 14 folds. The highest and most extensive ones are found in *C. elongata* (279 μ, Fig. 2 *D*) and in *S. romanica* (288 μ, Fig. 2 *B*) ; in *Misgurnus fossilis* they measure between 135 and 150 μ (Fig. 2 *A*), while in *C. taenia*, 135 μ (Fig. 2 *C*). On the floor the folds are low in *Misgurnus fossilis* (72—90 μ), and higher in the other 3 species — in *C. elongata* 162—243 μ, in *C. taenia* 63 — 162 μ and in *S. romanica* 117 μ. The epithelium of the papillae has different thicknesses according to the species, the largest

being found in *C. taenia* (99 μ). It consists of epidermic cells, 1 — 3 gustatory buds and small pear-shaped mucous cells. The thickest chorion is found in *Misgurnus fossilis* (up to 18 μ), followed by *S. romanica* (9 μ), *C. elongata* (6.7 μ) and *C. taenia* (4.5 μ). The lax connective tissue presents at the papillae level certain particular aspects according to the species. In *Misgurnus fossilis* the connective tissue has large meshes in which blood vessels and nervous endings are to be found. In *C. taenia*, the epithelium being very thick, the penetration distance of the connective tissue is more restrained. Its meshes are smaller and include numerous nuclei, blood vessels and nervous endings. *C. elongata* and *S. romanica* present in the papilla axis a denser connective tissue with an abundant content in hyaluronic acid.

In the sections, towards the eyes, the papilla epithelium maintains its structure, yet an increase of the pear-shaped mucous cells number is noticed. Under the chorion is the lax connective tissue with nervous endings, blood vessels and adipose cells (in *S. romanica* and *C. elongata*). In all the analysed species, bundles of longitudinal muscular fibres appear in the lax connective tissue of the lateral sides of roof and floor.

The epithelium at the base of the gill arches is thick, with numerous pear-shaped mucous cells and gustatory buds (6 — 11 in *Misgurnus fossilis*, 11 — 13 in *C. taenia*, 11 — 21 in *S. romanica* and 16 — 26 in *C. elongata*). The chorion is thin and sinuous in *C. elongata* and *C. taenia*, thicker in *S. romanica*, reaching its maximum in *Misgurnus fossilis*. At the level of the medulla oblongata, a well developed palatal organ is observed — a contractile cushion present in all the analysed Cobitidae species (Fig. 3).

The crypt types may be listed in a short table, with the epithelium and cells corresponding to the analysed species :

A. Narrow crypts, the highest having 27 μ
- Very narrow crypts (4.5 μ at the tip and 9 μ at the aperture), epithelium thick (81 μ), chorion very thin (4.5 μ). Mucous cells slightly tapered, opening at the crypts level. Chorion papillae long (36 μ) and thin . . . *Cobitis taenia*
- Crypts somewhat wider (9 μ at the tip and 18 μ at the aperture), epithelium thinner (63 μ), chorion 4.5 μ. Mucous cells (denser towards extremities) opening at the crypts level. Chorion papillae shorter (18 μ) . . . . . *Sabanejewia romanica*

B. Crypts open, deep or rather deep, high up to 45 μ
- Crypts with 22.5 μ aperture, thin tip (4.5 μ), epithelium 72 μ, chorion somewhat thicker (9 μ). Mucous cells (up to 18 μ) opening at the crypts level or near these. Chorion papillae higher than broad (27/18 μ) . . . . . *Cobitis elongata*

C. Largely opened crypts, 36 — 54 μ high
- Crypts with aperture up to 54 μ, tip 9 μ. Low thickness of epithelium (36 μ), chorion thick (9 — 18 μ). Mucous cells rather large as against epithelium thickness, opening at the crypts level, as well as on the entire epithelium surface. Chorion papillae broad (18/36 μ) . . . . . *Misgurnus fossilis*

Under the epithelium, a muscular layer consisting of dense, striated muscular fibres, laxly arranged in *C. taenia* (Fig. 3 *A*). Among these, stripes of connective tissue, blood vessels and nervous endings. In *Misgurnus fossilis* the striated muscle bundles are very large ; among them, abundant blood vessels and nervous endings. In Cobitidae the presence of adipose cells among the muscle fibres (mentioned in Cyprinidae by Al-Hussaini) is not observed. On the median floor, epithelium with crypts, at the level of which very numerous bag-shaped, globulous mucous cells are opening. The number of elongate gustatory buds is high. The muscle layer under the epithelium is rather thick, with fibres arranged in multiple directions including blood vessels and pharyngeal teeth (Fig. 3 *C*). The number of these latter varies between 10 and 12 on each side, their structure being similar in all the studied species.

Related to the dorsal wall of the bucco-pharyngeal cavity, opposite to the last pharyngeal teeth, some spur-shaped horny formations appear, extending to the lateral sides (Fig. 4 *A*). Their structure resembles that of the bitter bone in carps, having heavy exfoliations in the superficial layers (Fig. 4 *D*). In *Misgurnus fossilis* (Fig. 4 *C*) the horny formations are very broad (up to 585 μ) and high, with a very thick chorion at the basis (up to 45 μ). At this level, the epithelium of the mucous membrane is thickened, presenting crypt-separated folds. In the crypt area, globulous mucous cells open ; these become more intensely stained with azan. In the remaining epithelium, the mucous cells are elongate, bag-shaped and more palely coloured. Gustatory buds (1 — 2 for each fold) are sustained by the long papillae of the chorion. Under the epithelium, the muscle layer, formed by large bundles of fibres, more laxly arranged than previously.

From among the other studied species, *C. elongata* presents the largest horny formation (Fig. 4 *B*), more closely attached to the dorsal wall of the bucco-pharyngeal cavity, parallel with a few buds of pharyngeal teeth. It measures 432 μ in length and 90 μ in breadth (at the basis) and no obviously thickened chorion is present. Epithelium with 2 types of mucous cells : elongate on the roof and globulous at the level of the floor crypts.

In *S. romanica* and *C. taenia* (Fig. 4 *A*) the horny formation takes up the lateral sides of the bucco-pharyngeal cavity (180 μ in breadth/153 μ in length in the former species, and 126 μ/90μ in the latter one). In this area the epithelium consists only of bag-shaped mucous cells in *C. taenia*; bag-shaped and globulous in *S. romanica*. The two cell types might reflect but different stages in mucus elaboration — the globulous ones, in full swing of the elaboration process, the bag-shaped ones, at its end.

In *C. taenia* the muscle fibres are arranged in large, rare bundles in the upper part, smaller and denser at the basis. In *S.romanica* the muscles are arranged in large and dense bundles. These horny formations probably influence the deglutition control and contribute to the effective food trituration in the species in which they are more developed.

Where horny formations end, the posterior area of the bucco-pharyngeal cavity may still be observed for some time; it is lined with an epithelium having a lower number of gustatory buds and a very high number of mucous cells. On the roof, the muscles maintain for some time the arrangement of the palatal organ (with muscle fibres in multiple directions), whilst on the floor towards the periphery begins the arrangement of the circular muscle fibres.

CONCLUSIONS

The comparative study of the bucco-pharyngeal cavity in the 4 Cobitidae species reveals the following:

1. In all the analysed species, the inner face of the upper lip and the lower lip present a horny formation, yet weaker than in *Noemacheilus barbatulus*, which partially extends to the lower part of the maxillary valve.

2. *Misgurnus fossilis* presents a particular constitution of its upper lip, which has an epithelium almost entirely consisting of elongate, bag-shaped mucous cells, with a small nucleus, triangular at the basis.

3. In all the species, the roof of the bucco-pharyngeal cavity presents a maxillary valve; in *Misgurnus fossilis* there is also a mandibular valve on the floor.

4. The bucco-pharyngeal cavity presents, on the roof, papilla-shaped protuberances, the largest being in *S. romanica* and *C. elongata*. In the same species, the epithelium presents the largest gustatory buds, and in their axis, the connective tissue, abundant in hyaluronic acid. The two species live in sandy areas, the papillae playing, probably, a role in the filtration of the water penetrating in the bucco-pharyngeal cavity.

5. In the epithelium of the mucous membrane, the highest number of gustatory buds is observed in *C. elongata*, followed by *S. romanica*. A lower number of buds is noticed in *Misgurnus fossilis*.

6. All the studied Cobitidae have a palatal organ on the roof, playing an important role in favouring both deglutition and water elimination.

7. We point out, for the first time in literature, the presence of two horny formations, the structure of which is similar to that of the bitter bone in carp. These formations are situated in the posterior part of the bucco-pharyngeal cavity, extending from the dorsal wall to the lateral sides. The most developed horny formations are encountered in *M. fossilis* and *C. elongata*, in the food composition of which also enter insect larvae strongly chitinized, ostracods and molluscs. Hence, in these species, mastication takes place at the posterior pharynx level.

REFERENCES

1. Al-Hussaini A. H., 1949, Quart. J. Micr. Sci., **90**, *2*, 109 — 139.
2. Bolk L., Göppert E., Kalius E., Lubosch W., 1937, *Handbuch der vergleichenden Anatomie der Wirbeltiere*, **3**, 1018.
3. Bușniță Th., 1925, Bul. Soc. ști. Cluj, **2**, 133 — 156.
4. Călugăreanu D., 1907, Arch. ges. Physiol., **118**, 42 — 51.
5. — 1908, Rev. Șt. med., 597 — 629.
6. Curry E., 1939, J. Morph., **65**, 53 — 78.
7. Gasiorkiewicz A., Urbanowicz K., 1958, Bull. Soc. sci. lett. Łódz, **9**, *4*, 1 — 10.
8. Lupu H., 1908 — 1909, Arch. Zool. exp. gén., **9**, 417 — 427.
9. — 1914, Ann. Sci. Univ. Jassy, **8**, 52 — 116.
10. — 1928, Ann. Sci. Univ. Jassy, **15**, 272 — 285.
11. McVay J. A., Kaan H. W., 1940, Biol. Bull., **78**, 53 — 67.
12. Meșter L., 1971, St. Cerc. Biol. — Zool., **23**, *5*, 439 — 444.
13. Oppel A., 1896, *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere.* Der Magen, Vienna, **1**, 543.
14. — 1897, *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere.* Schlund und Darm, Vienna, **2**, 560.
15. — 1900, *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere.* Mundhöhle, Bauchspeicheldrüse und Leber, Vienna, **3**, 1050.

Received November 24, 1972

Faculty of Biology
Bucharest, Splaiul Independenței 91—95

ABBREVIATIONS

Bi = lower lip ; Bs = upper lip ; C = chorion ; cbf = bucco-pharyngeal cavity ; Cp = polyhedral cells ; cep = epidermic cells ; CMc = mucous cells ; cr = chromatophores ; cri = crypt ; DF = pharyngeal teeth ; ep = epithelium ; FC = horny formation ; Mg = gustatory bud ; N = nerve ; n = nucleus ; PF = pharyngeal bone ; P = chorion papilla ; pc = horny portion ; pci = citoplasmatic bridges ; SM = striated muscle layer ; Tc = connective tissue ; Tcd = dense connective tissue ; Vm = maxillary valve ; VS = blood vessel ; zb = basal zone ; zm = medial zone ; zs = superficial zone.

The abbreviations are the same for all figures.

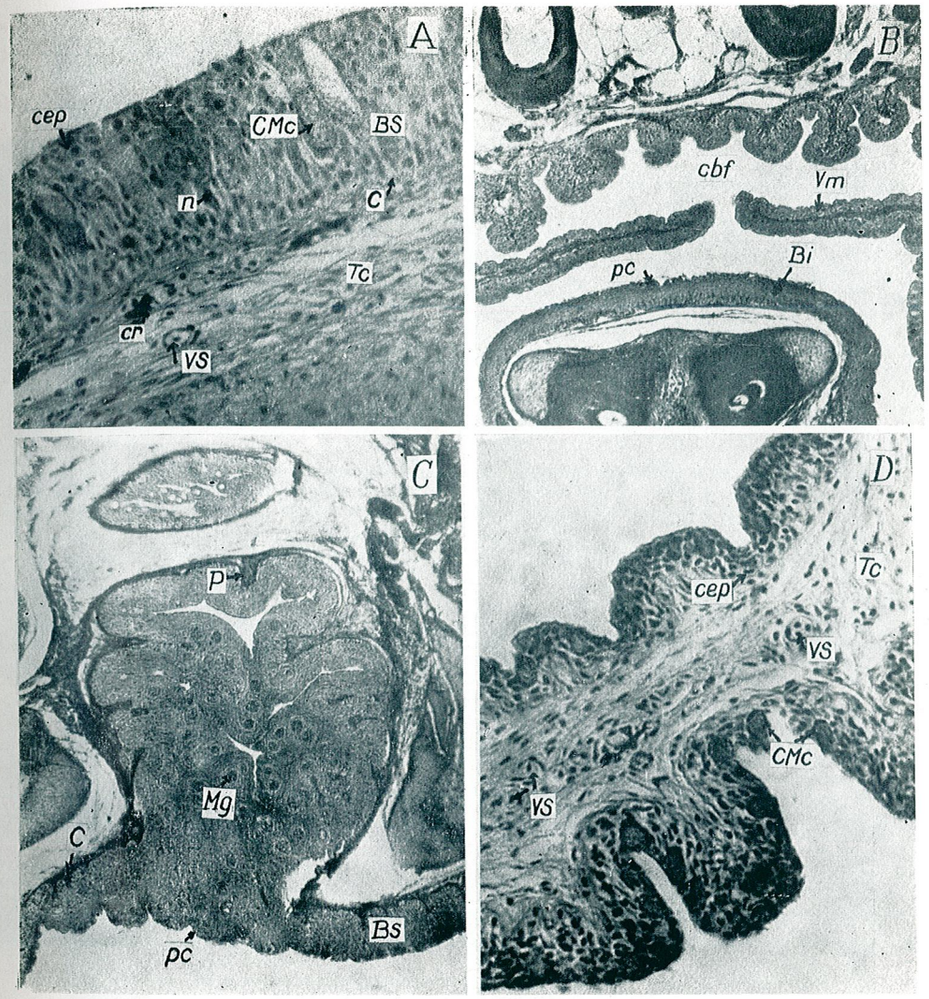


Fig. 1. — Anterior part of head — cross section.
A. Misgurnus fossilis, ♂ = 10 cm.(6 × 24) — epithelium of upper lip ;
B. Cobitis elongata, ♀ = 13.4 cm. (6 × 6.3) — lower lip and maxillary valve ;
C. Sabanejewia romanica, ♂ = 7.2 cm. (6 × 6.3) — upper lip ;
D. Misgurnus fossilis, ♂ = 10 cm. (6 × 25) — mandibular valve.

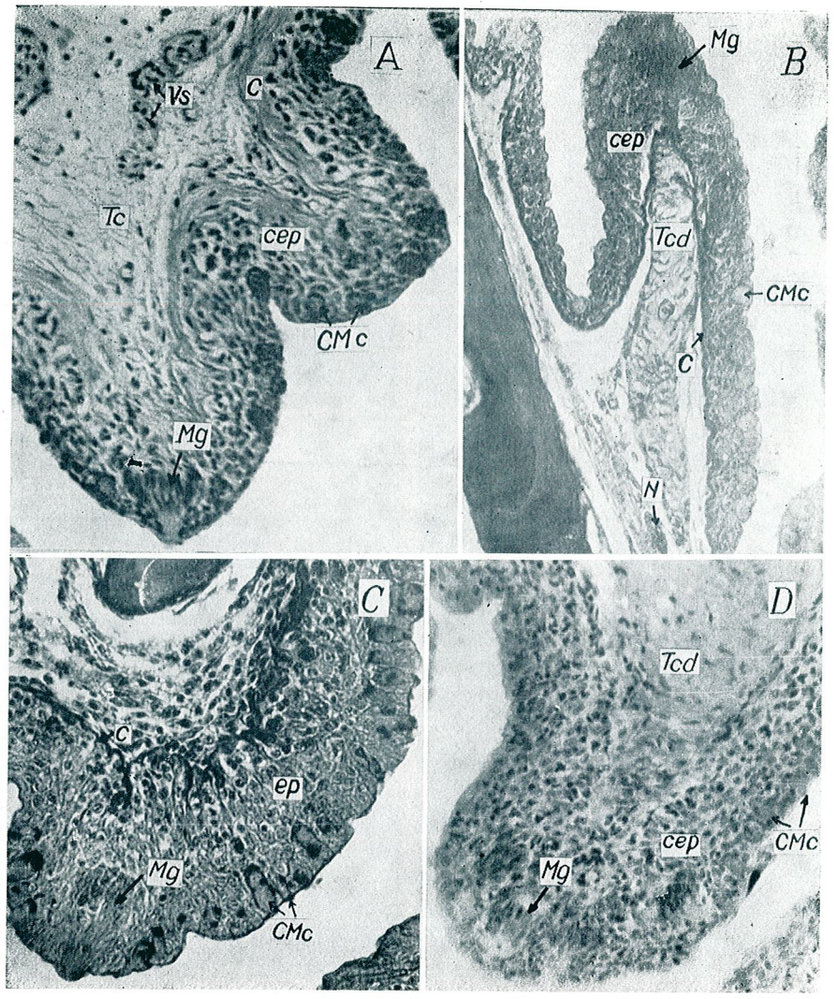


Fig. 2. — Papillae of the bucco-pharyngeal cavity (cross section).
A. Misgurnus fossilis, ♂ = 10 cm. (6 × 25) — roof;
B. Sabanejewia romanica, ♂ = 7.2 cm. (6 × 24) — latero-inferior angle;
C. Cobitis taenia, ♀ = 4 cm. (6 × 24) — roof;
D. Cobitis elongata, ♀ = 13.4 cm. (6 × 24) — roof.

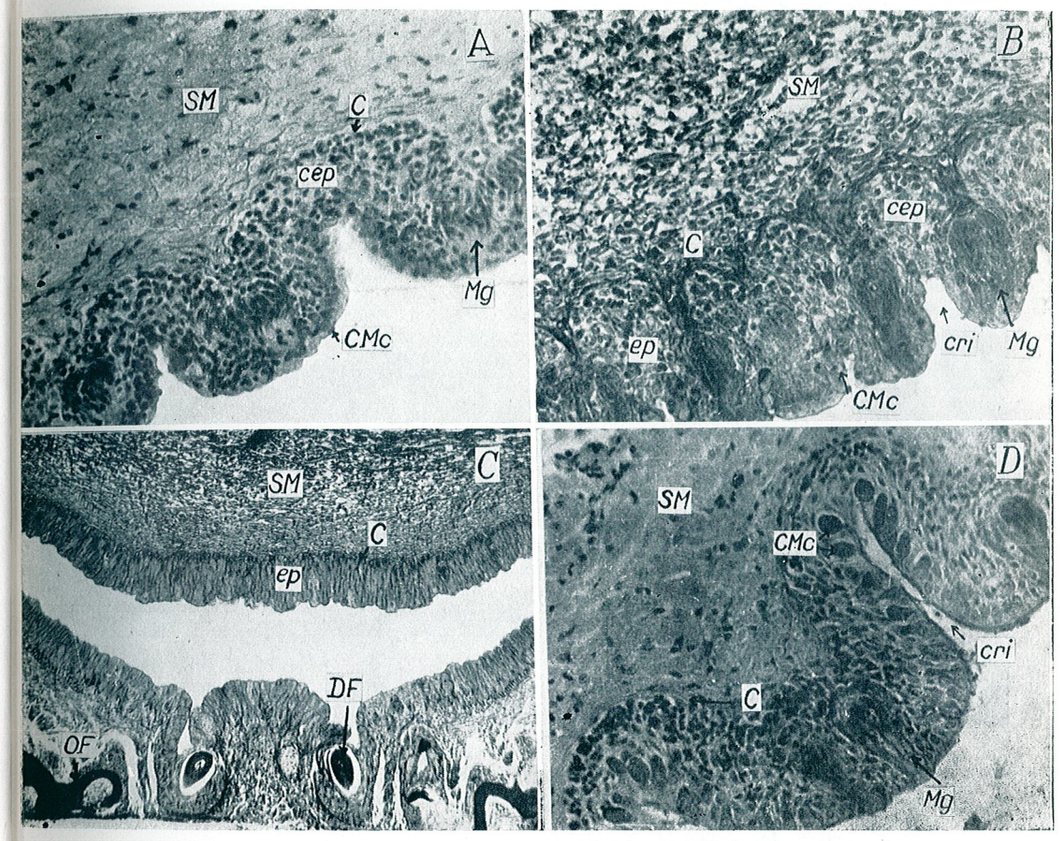


Fig. 3. — Cross sections at the palatal organ level.
A. Cobitis elongata, ♀ = 13.4 cm. (6×25);
B. Cobitis taenia, ♀ = 4 cm. (6 × 6);
C. Cobitis taenia, ♀ = 4 cm. (6 × 24);
D. Sabanejewia romanica, ♀ = 9 cm. (6 × 24).

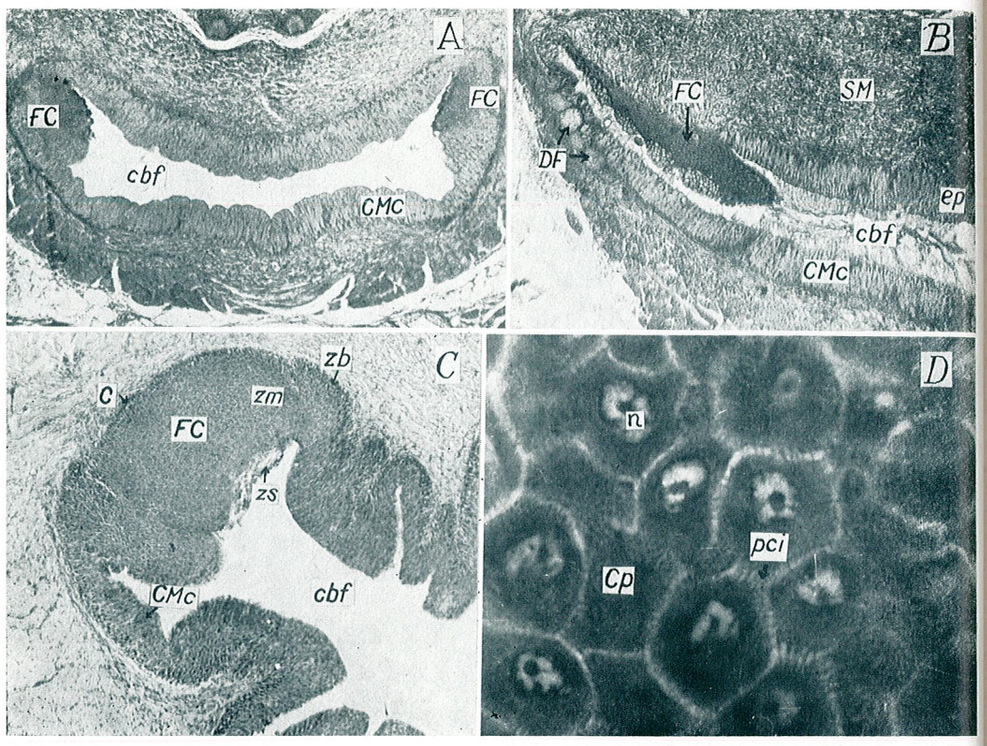


Fig. 4. — Horny formations in cross sections.
A. Cobitis taenia, ♀ = 4 cm. (6 × 6);
B. Cobitis elongata, ♀ = 13.4 cm. (6 × 6.3);
C. Misgurnus fossilis, ♂ = 11.9 cm. (6 × 6.3);
D. Misgurnus fossilis, ♂ = 11.9 cm. (6 × 90).

METABOLISM OF CAROTENOID PIGMENTS DURING THE EMBRYONIC DEVELOPMENT OF *LEPTINOTARSA DECEMLINEATA* SAY

BY

MATILDA JITARIU, G. ACATRINEI and I. PETCU

The authors deal with the metabolic variations of carotenoid pigments during embryogenesis in *Leptinotarsa decemlineata* Say. In newly laid eggs the authors have found α-carotene, three hydroxylated β-carotene pigments in esterified form, as well as mono-hydroxy-mono-keto-carotene, lutein and probably monadoxanthin.

The appearance of echinenone and canthaxanthin has been genetically programmed, these forms existing only when the embryonic tissues are already in the 2nd—3rd stages in the development of the body. At these stages, the total amount of carotenoid pigments significantly exceeds the amount of those pigments in newly laid eggs.

The authors discuss the hypothesis of this synthesis.

It is apparent that invertebrates constitute a rich material for the study of animal carotenoids. Besides Crustacea, about which numerous papers have been written, insects have also become a subject of investigation [4] — [7] [11] [14] — [16] [19] — [21]. From among these papers, we shall quote those of Manunta et al. [14] [15], Merlini and Cardillo [16], Leuenberger and Thommen [11] who have isolated carotenoids from adults of Colorado beetles, giving evidence of the fact that these insects possess the capacity of turning β-carotene into echinenone and canthaxanthin, pigments which do not exist in leaves of *Solanum tuberosum* — the plant on which *Leptinotarsa* feeds.

Yet, since the vital cycle of *Doriphorus* comprises, besides the adult period, stages of embryonic, larval and nymphal development, we set about studying the carotenoidic metabolism during these stages in the animal's life.

The present paper records the results yielded by the analysis of carotenoidic metabolism starting from the period of embryonic development.

REV. ROUM. BIOL. – ZOOLOGIE, TOME 18, No 2, p. 145–152, BUCAREST, 1973

MATERIAL AND METHOD

Eggs were amassed from the field and selected according to the colour they took at various stages of their embryonic development. The selection was followed by a histological analysis meant to exactly define the embryonic stage. A large number of analyses were performed with a view to effecting statistical calculations. To this effect the eggs were kept in absolute ethanol at 4 C° and in the dark.

Exhaustive extractions of pigment were carried out in acetone from dried material, always at the same time, and thereafter weighed. The extractions were saponified by aqueous KOH 60% [8] and kept overnight at the room temperature in nitrogen atmosphere and in dark.

After removing the alcalus, the extractions were transferred in lightpetroleum. Raw extractions were then dried on anhydric SO_4Na_2 and the reading of the optical density was done at 450 mμ by using a Beckman DB spectrophotometer, and the formula $E_{1\ cm.}^{1\%} = 2500$ enabled us to make calculations according to Bonaly's method [1] of total carotenoids in μg/g. of wet tissues.

Isolation and purification of pigments were made on an alumina column activated for two hours at 120 C°. The elution agents used were various concentrations of acetone in lightpetroleum.

The quantitative estimation of each pigment was done by the above indicated method with due account of the optical density of the respective pigment at its absorption maximum. Statistical calculations were made according to Student's method.

The characterization of the pigment was done by taking into consideration its behaviour towards the partition between methanol 95° and lightpetroleum [17], the chromatography in a MgO thin layer against a genuine test, the reduction by BH_4Na for ketonic groups [10] and the recording of visible spectra in different solvents for the shape of the curve as well as in IR to determine a certain molecular structure.

RESULTS

The histological examination of newly laid eggs having a yellow colour revealed the structure of an egg rich in vitellus, which does not however exhibit cellular differentiation. The total carotenoids at this stage are of 180.28 μg/g. wet tissue represented by traces of α-carotene, then by three pigments of almost equal absorption maxima (Table 1) and also

Table 1

Absorption maxima in visible light of pigments 1a, 1a1, and 2a, in different solvents

The pigment	Solvents		
	Lightpetroleum	Benzene	Ethanol
1a	477—448~425	492—464~442	480 — 454
1a1	478—449~423	493—466~442	481 — 453
2a	478—447~425	493—465~438	481 — 453

almost equal to that of β-carotene, though they are not β-carotene. The first among them prevails quantitatively in the entire period of embryonic development, even if at times the amount drops by usage.

The first pigment (1a) is not retained in the purifying column, while the second one (1a1) is diluted on this column in 6% acetone-lightpetroleum; the third pigment (2a) is dissolved on the main column in 6% acetone-lightpetroleum and in 10% acetone-lightpetroleum on the purifying column.

The partition coefficient of the three pigments reveals an epiphasic character. IR spectra recording (Unicam SP 200) shows for pigments

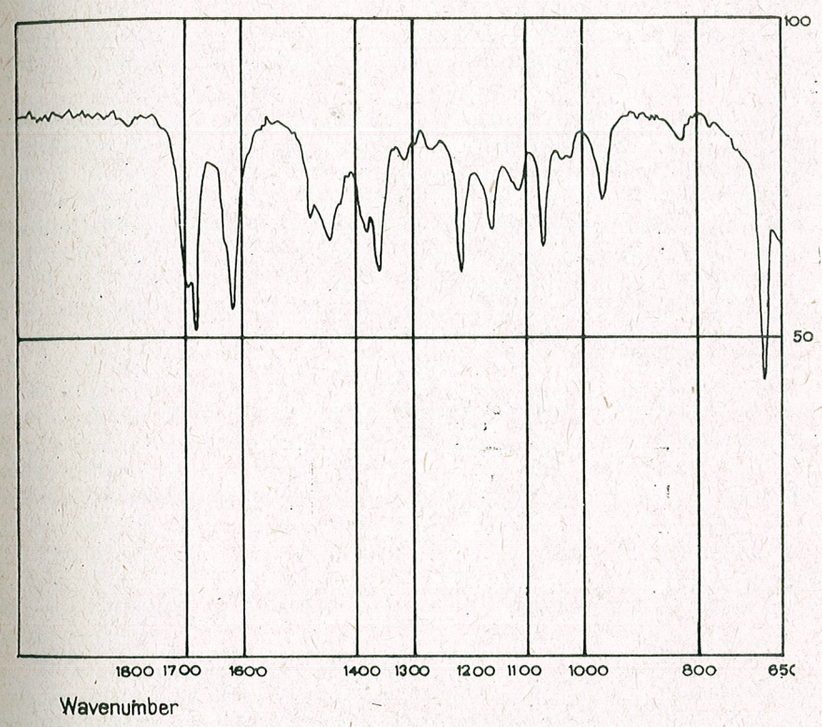


Fig. 1. — Infra-red recording of pigment 1a.

1a and 1a1 an absorption band of almost 965 cm^{-1} given by the out-of-plane vibration of C—H in the configuration *trans* of the —CH = CH— group [12]. At the same time, the band which appears at about 1380 $cm.^{-1}$ is ascribed to the deforming vibration of the methyl group on a *cis* double bond in aliphatic systems [8] [12]. The "aryl" groups are present owing to the C—H out-of-plan vibration given by the 830 cm^{-1} band.

The absorption band at 1680 cm^{-1} of carbonyl denotes the "ester" function of the conjugation with the polyenic chain. Besides the ester group, there exists in the molecule an ether group whose absorption band is of 1080 cm^{-1} [12] (Fig. 1).

As for the third pigment, (2a), it is lacking an ether group.

Hence, after saponification by KOH 60%, the molecules of these three carotenoids are still holding the esteric function. But the presence of this group gives us ground to assume that the three pigments are not β-carotene, but hydroxylated forms of this latter, which esterify themselves.

Besides the pigments mentioned so far, the newly laid eggs contain a pigment (2b) likely to have the form of a mono-hydroxy-mono-keto-carotene. The visible spectrum shows its asymmetric form. The absorption maximum is of 453 mμ with a shoulder of 471 mμ. By turning into a pigment of a finer structure, it reveals an absorption maximum of 476 — 447 mμ and an inflection of 426 mμ following treatment by BH_4Na. Thin layer chromatography of MgO against genuine echinenone yields a Rf of 0.868 and an echinenone of 0.85. Therefore we may assume a structure of mono-hydroxy-mono-keto-carotene.

Finally, in the newly laid eggs there also exists lutein, a finding already pointed to in previous papers [6] [11] [14] — [16]. We also determined it according to the absorption maximum in visible spectrum in light-petroleum, benzene and ethanol, according to the same Rf as that of the genuine test, as well as according to the negative reaction against epoxyde.

The last pigment is quite similar to lutein, but the absorption maximum in lightpetroleum, benzene and ethanol is akin to that of monadoxanthin [13] (Table 2). It is almost completely hypophasic before and after saponification, the partition coefficient being equal to 21.5 : 78.5 and nearer to that of monadoxanthin, which is of 27 : 73, than to that of lutein, which is of 39 : 61. Thin layer chromatography yields a Rf of 0.70 as against lutein which is of 0.91. The reaction towards epoxyde is negative.

Table 2

Comparison between absorption maxima in visible light of lutein and monadoxanthin

Lightpetroleum	Lutein	474—445∼425
	Monadoxanthin ?	473—443∼423
Benzene	Lutein	489—459∼435
	Monadoxanthin ?	488—457∼437
Ethanol	Lutein	476—447∼429
	Monadoxanthin ?	477—447∼426

IR spectrum shows a *cis* configuration (vibration band of about 1380 $cm.^{-1}$) and a weak ester (vibration band of about 1738 $cm.^{-1}$). In the molecule there also exists an OH group (vibration bands of 3250 $cm.^{-1}$ and of 1043 $cm.^{-1}$). All these pigments are present over the further stages of embryogenesis.

In the red egg which represents the 2nd — 3rd stages in the development of the body, besides the forms encountered in the newly laid egg, one also finds echinenone and canthaxanthin. These were tested by thin layer chromatography against genuine tests, as well as by BH_4Na treatment. Thereafter they were changed into isocryptoxanthin and isozeaxanthin, respectively.

In the egg containing embryos ready for hatching (the brown egg) a pigment with an absorption maximum in visible spectrum of 448 mμ and an inflection of 474 mμ appears. This pigment has not yet been minutely analysed. We only point it out.

DISCUSSIONS

In our attempt to assess the significance of the metabolic carotenoid process we came across difficulties brought about by the inequalities presented by the investigated stage.

Speciality literature points to the heterogeneous character of the eggs and thereby to the variety of the incubation period [5]. Despite the strict selection of the eggs according to colour, it was not possible, in this case, to work with a more homogeneous material in comparison to the case when one works with mature individuals. This may at times supply an explanation for the high values of the variability coefficient in the statistical estimation.

In newly laid eggs we noticed at first the presence of already hydroxylated carotenoids, but not as hydrocarbons (i.e. the presence of 1a, 1a1 and 2a pigments as well as lutein and monadoxanthin). The presence of oxygenated forms is proportionally reduced (i. e. the mono-hydroxy-mono-keto-carotene pigment). In these eggs there is no echinenone or canthaxanthin. Only after gastrulation, as the embryonic tissues are completely finalized and assembled (the red egg stage), is the presence of echinenone and canthaxanthin noticeable.

The much later appearance of certain carotenoid forms shows that the metabolism of these substances in the animal is genetically programmed; consequently, a diastase capable of creating certain forms of carotenoids emerge.

It would be appropriate to recall that the appearance of oxygenated forms is connected with a certain stage in the embryonic development when the tissues are likely to need more oxygen because the oxidoreducing processes are much more intense.

Therefore, along with multiplication, cellular differentiation and embryonic tissue formation, the carotenoids in newly laid eggs undergo a metabolic process which epitomizes not only the emergence of oxygenated forms, of echinenone and canthaxanthin, but also a significant increase in the quantity of hydroxylated pigments (1a, 1a1, lutein) (Fig. 2) translated by a significant rise of the totality of carotenoid pigments (Fig. 3).

In the last stage of embryogenesis, almost at the moment of hatching, we remark a significant decline of the total quantity of carotenoid pigments, brought about by the intense utilization first of all of the oxy-

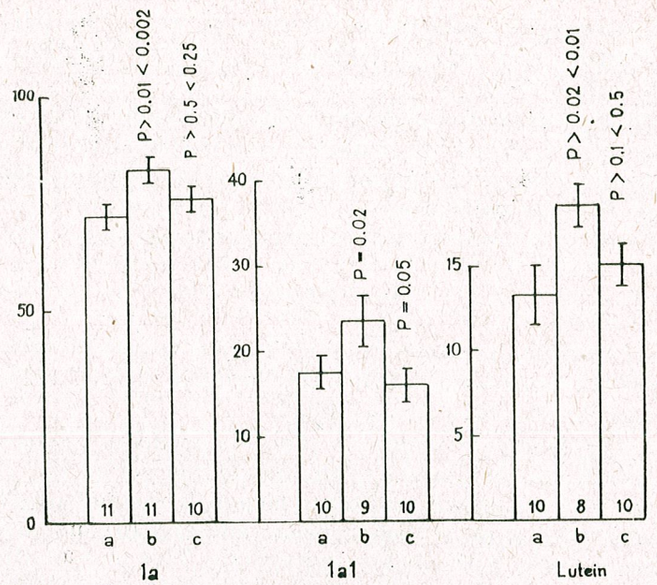


Fig. 2. — The metabolic variation of pigments : 1a, 1a1, and lutein, during embryogenesis.
a = newly laid eggs
b = well constituted embryonary tissues
c = the embryo close to hatching.

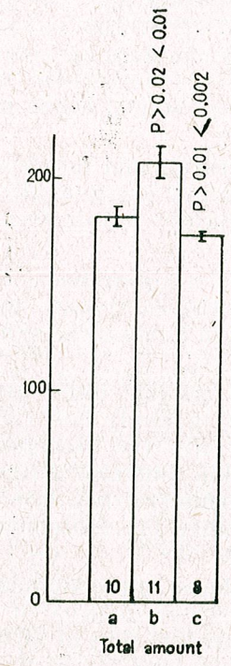


Fig. 3. — The total carotenoid pigments variation during embryogenesis.
a = newly laid eggs
b = well constituted embryonary tissues
c = the embryo close to hatching.

genated pigments — echinenone and canthaxanthin (Fig. 4), mono-hydroxy-mono-keto-carotene—and then by the less intense utilization of the pigment 1a1 (Fig. 2). It is thus obvious that in the formation of the embryo the carotenoid substances also take part.

Since everybody admits that the carotenoid substances are excellent acceptors and donors of electrons [18] and since it is already proved experimentally [2] [3] [8] that the first oxidation of carotenoids is per-

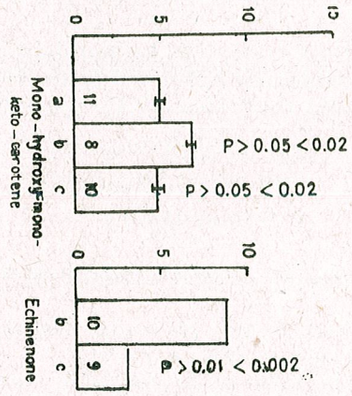


Fig. 4. — The metabolic variation of echinenone, canthaxanthin and mono-hydroxy-mono-keto-carotene during embryogenesis.
a = newly laid eggs
b = well constituted embryonary tissues
c = the embryo close to hatching.

formed by fixing the molecular oxygen, we may assert, on the ground of our results, that the participation of carotenoids in the process of embryogenesis manifests itself as a source of active, atomic oxygen in the metabolic processes, fabulously numerous and intense. This is a hypothesis which requires experimental confirmation.

The problem concerning the process of carotenoid synthesis at the first stage of embryogenesis should also be explained. Since in the adult there is no synthetical process [9] — a logical fact—, at the time of physiologic starvation (embryogenesis, nymphosis), during which an extremely intense metabolism is developing, the animal organism adapts itself to the environmental conditions and creates possibilities to cope with them.

In the vital cycle of this insect, there are stages when a certain type of metabolism prevails, as given by a certain enzymatic picture.

The enzymes which preside over the process of carotenoid synthesis are not yet known. Taking into account that in the body of an insect there exists farnesyl as a precursor of certain hormones, we pose the problem whether the quantity of carotenoid needed at a certain moment during the embryonic evolution is not synthesized from this farnesyl, too.

Acknowledgment

We are most grateful to Mrs. S. Liaaen-Jensen for her having kindly provided us with echinenone extracted from *Aphanizomenon flosaquae*.

We are also grateful to Professor V. Macovei for his helpful interpretation of IR spectra.

REFERENCES

1. Bonaly R., 1967, *Biosynthèse des caroténoïdes cycliques de levures du genre Rhodotorula.* (Thèse), Nancy.
2. Bodea C., Nicoară, E., Gross J., 1955, St. cerc. st. (Cluj), *4*, 51.
3. Bodea C., Gross J., 1957, St. Cerc. chim., **8**, *1 — 2*.
4. Bückmann D., 1968, *Die Biochemie der morphologischen Farbanpassung bei Tagschmetterlingspuppen.* Akad. Verlagsges. Geest-Portig K. G., Leipzig.
5. Busnel R., 1938, *Études physiologiques sur le Leptinotarsa.* (Thèse), Paris.
6. Cardani C., Merlini L., Mondelli R., 1962, Gaz. chim. Ital., **92**, 41.
7. Feltwell J., Valadon L., 1970, Nature (Lond.), **225**, *5236*, 969.
8. Goodwin T. W., 1965, *Chemistry and Biochemistry of Plant Pigments*. Acad. Press, London.
9. — 1952, *The Comparative Biochemistry of Carotenoids*, Acad. Press, New York.
10. Krinsky N., Goldsmith T., 1960, Arch. Biochem. Biophys., **91**, 271.
11. Leuenberger F., Thommen H., 1970, J. Insect Physiol., **16**, 1855.
12. Liaaen-Jensen S., Jensen A., 1965, *Recent Progress in Carotenoid Chemistry*. Pergamon Press, London.
13. Mallams A., 1967, Chem. Comm., 301.
14 Manunta C., Solinas M., 1952, Rend. Accad. Lincei, Classe Fis. Mat. Nat., **12**, *8*, 759.
15. Manunta C., Moritu G., 1948, Rend. Accad. Lincei, Classe Fis. Mat. Nat., *4*, 115.
16. Merlini L., Cardillo G., 1963, Gaz. Chim. Ital., **93**, 949.
17. Petracek F., Zechmeister L., 1956, Analyt. Chem., **28**, 1484.
18. Pullman B., Pullman A., 1963, *Quantum Biochemistry*. Interscience Publishers, New York, 439.
19 Reichmuth W., 1967, Zeitschr. Angew. Zoologie, **54**, *2*, 285.
20. — 1967, Zeitschr. Naturforsch., **22 b**, 1232.
21. Willig A., 1969, J. Insect Physiol., **15**, 1907.

Received December 12, 1972

"Al. I. Cuza" University
Jassy, Calea 23 August nr. 11
and
Center of Biological Researches

EFFECT OF TEMPERATURE ON THE ISOENZYMIC PATTERN OF POND LOACH (*MISGURNUS FOSSILIS* L.)

II. MALATE DEHYDROGENASE AND SUCCINATE DEHYDROGENASE

BY

RADU MEȘTER, DRAGOȘ SCRIPCARIU and STELIAN NICULESCU

The influence of temperature upon the isoenzymic pattern of malate dehydrogenase and succinate dehydrogenase from muscle, liver, serum and hemolysate of warm- and cold-adapted fishes was studied. The electrophoretic pattern of enzymes shows that the homogenates of all investigated tissues of warm- and cold-adapted fishes present the same enzymic pattern, by determining the enzyme activity *in vitro* at 20°C. Low temperature (incubation of the gels at 0°C) induces a decrease of the total enzymatic activity as well as the number of the isoenzymes in loach tissues, regardless of the adaptation temperature. This appears differently according to the enzyme and the investigated tissue.

Malate dehydrogenase and succinate dehydrogenase are two important enzymes of the Krebs cycle. Numerous quantitative and electrophoretic investigations in higher vertebrates established their presence under multiple molecular forms and a series of kinetic parameters [10] [14].

These dehydrogenases were so far less studied in poikilotherms. The adaptation of fishes to low temperatures influences the activity of these enzymes. Kruger [9] has reported an 80% increase of the activity of succinate dehydrogenase from the liver of eels acclimatized at 11°C, as against those adapted at 26°C. An increase of succinate dehydrogenase activity was likewise observed in the muscle of the crucian carp adapted to cold [12]. The adaptation of ide to the temperature of 10°C was accompanied by an increase of malate dehydrogenase activity, while the succinate oxidation was not affected by the adaptation temperature [5]. The stimulation of some enzymes of anaerobic and aerobic glycolysis by

REV. ROUM. BIOL. — ZOOLOGIE, TOME, 18, Nº 2, p. 153–161, BUCAREST, 1973

the adaptation of fishes to low temperatures was also suggested by other authors [2] [6] [7]. Other researches performed on poikilotherms do not record any significant modifications of the malate dehydrogenase and succinate dehydrogenase activity depending on the adaptation temperature of fishes [1] [8].

Our previous data concerning the activity and molecular forms of certain dehydrogenases from the tissues of pond loaches adapted to 0°C and 20°C, evidenced the importance of the study of enzymes at the adaptation temperature of fishes. It was found that low temperature induces a diminution of enzymatic reaction rate by the lowering of the total enzymatic activity and of the functionality of certain isoenzymes, irrespective of the adaptation temperature of fishes [11].

In the present work we purposed to analyse by electrophoresis on polyacrylamide gel, the activity and enzymatic molecular forms of malate dehydrogenase and succinate dehydrogenase from the liver, muscle, serum and hemolysate of pond loaches adapted to 0°C and 20°C, respectively.

MATERIAL AND METHODS

Experiments were performed on loaches (*Misgurnus fossilis* L.) obtained from the Greci pond, Olt district. Adaptation was achieved in the laboratory, at 0°C and 20°C, for 30 days.

The preparation of tissue homogenates. The skeletal muscle obtained from the lateral sides of the body was homogenized in a Potter homogenizer, in distilled water, one part tissue and 3 parts water, at cold. The liver was likewise homogenized in distilled water, one part tissue and 8 parts water w/v. For a better extraction of enzymes, the homogenates were frozen and thawed 2 — 3 times. Total proteic extract, obtained after centrifugation at 7000 g. for 20 min. were used for subsequent electrophoretic investigations. Blood was collected on heparin in 0.75 % NaCl solution, from the caudal vein of fishes by tail cutting. The blood was washed three times with 0.75 NaCl solution and centrifuged, each time at 6000 g, for 20 min. The diluted serum and the erythrocytic hemolysate suspended in distilled water were used for electrophoresis.

Electrophoresis was performed in polyacrylamide gel, at a 7.5 % concentration, according to the method of Davis [4], disk-electrophoresis system. Electrophoresis of protein was run at 3 mA/tube, for two hours.

In view to determine the enzymatic activity of the electrophoretic fractions, the gels were washed with distilled water and then with 0.1 M tris-HCl buffer, pH 7.4 under cold conditions. Subsequently, the gels were incubated in the buffer solutions adequate to each enzyme. Enzymes from both cold- and warm-adapted fishes were evidenced at 0°C and 20°C (corresponding to the adaptation temperature of the animals).

Malate dehydrogenase was determined according to Davidson and Cartner [3], while succinate dehydrogenase according to Nachlas et al. [13].

RESULTS

Malate dehydrogenase. Electrophoretic data on polyacrylamide gel have evidenced the presence of this enzyme in all tissues. Total enzymatic activity (based on the staining intensity of the isoenzymatic fractions and their number) differs from one tissue to the other, depending probably on the contribution of the enzyme to the control of oxidative metabolic processes.

The electrophoretic pattern of malate dehydrogenase from muscular homogenates of warm-adapted fishes present 5 fractions with intense enzymatic activity, by the incubation of gels at 20°C. At low temperature all the 5 isoenzymatic fractions appear, but with a lower activity (Fig. 1). The malate dehydrogenase from cold-adapted fishes is characterized by the presence of 4 enzymatic fractions by the incubation of gels both at 20° C and at 0°C. In this case, the staining intensity of the bands is increased at a higher temperature (Fig. 1). The fact is noticed that one of the enzymatic bands of malate dehydrogenase disappears as a result of the adaptation of fishes to low temperature.

From the liver of both cold- and warm-adapted fishes, 4 fractions with malate dehydrogenase activity were electrophoretically separated by incubation at 20°C. Low incubating temperature evidenced electrophoretically two isoenzymatic fractions with a weak staining activity from both groups of fishes (Fig. 1).

The electrophoretic pattern of malate dehydrogenase from serum of both cold- and warm-adapted fishes is characterized by the presence of three enzymatic fractions. No particular modifications of enzyme activity as a result of the adaptation of fishes to cold or by determination of enzyme at low temperature is recorded. In this latter case, a diminution of the width of the band with high electrophoretic migration is induced, as well as some reduction of the activity of the other two isoenzymes (Fig. 2).

From the hemolysate of both warm- and cold-adapted fishes, two isoenzymatic fractions with malate dehydrogenase activity were separated. The determination of the enzyme at 0°C induces a reduction of the activity of the two isoenzymes in both groups of fishes (Fig. 2).

Succinate dehydrogenase. The electrophoretic pattern of succinate dehydrogenase from muscle homogenates appears under the form of 5 isoenzymatic fractions, in both groups of fishes, when the enzyme determination is made at 20°C. By the incubation of gels at 0°C, only three enzymatic fractions with weak or very weak tinctorial activity appear, irrespective of the adaptation temperature of fishes (Fig. 3).

Succinate dehydrogenase from the liver of both groups of fishes appears under the form of 4 isoenzymes with weak enzymatic activity, by determination of its activity at 20°C. Gel incubation at 0°C induces a reduction of enzymatic activity and of isoenzymatic pattern in cold-adapted fishes as well as in those adapted to 20°C : at a low temperature two isoenzymes with very weak tinctorial activity appear (Fig. 3).

From the serum of cold- and warm-adapted fishes, two isoenzymatic fractions were electrophoretically evidenced on polyacrylamide gel. When the enzyme is determined at 0°C, two isoenzymes with reduced and respectively very reduced enzymatic activity appear in both groups of fishes (Fig. 4).

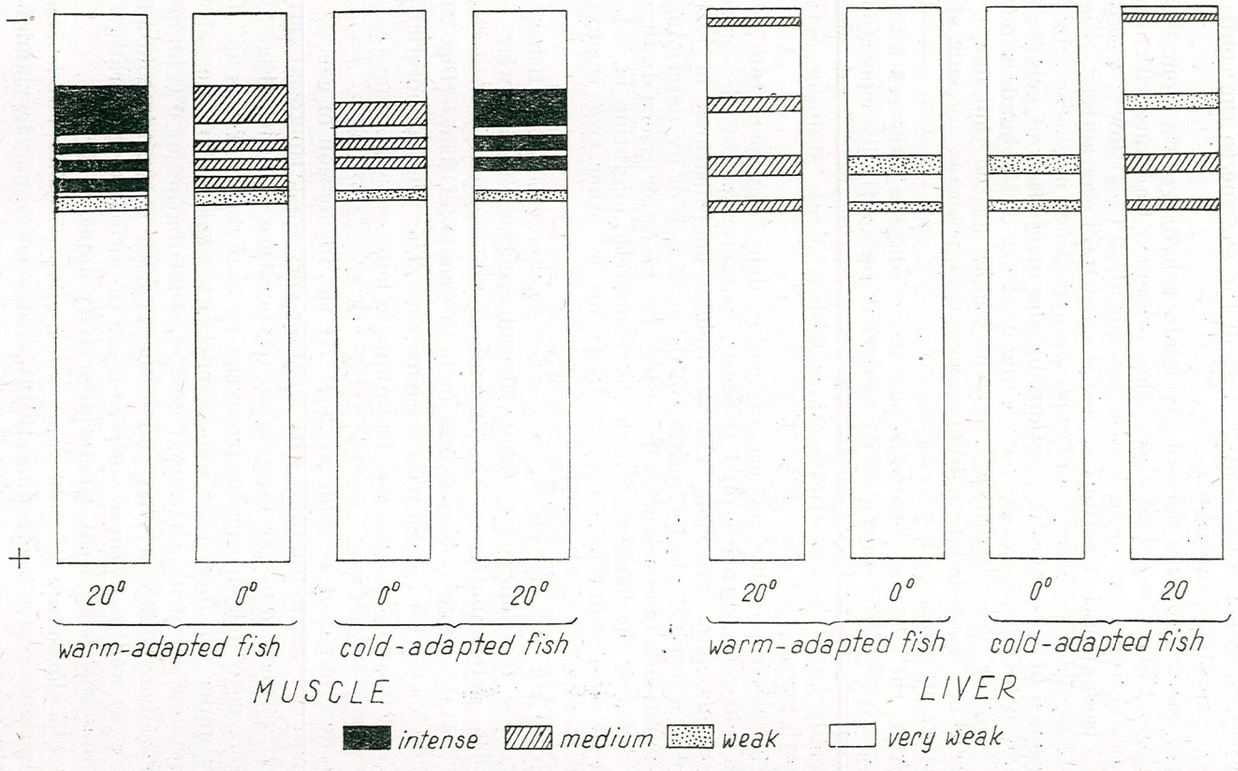


Fig. 1. — Isoenzymatic pattern of malate dehydrogenase from muscle and liver, obtained on polyacrylamide gel. 20° and 0° indicate the temperature at which the gels were incubated when determining the enzymatic activity.

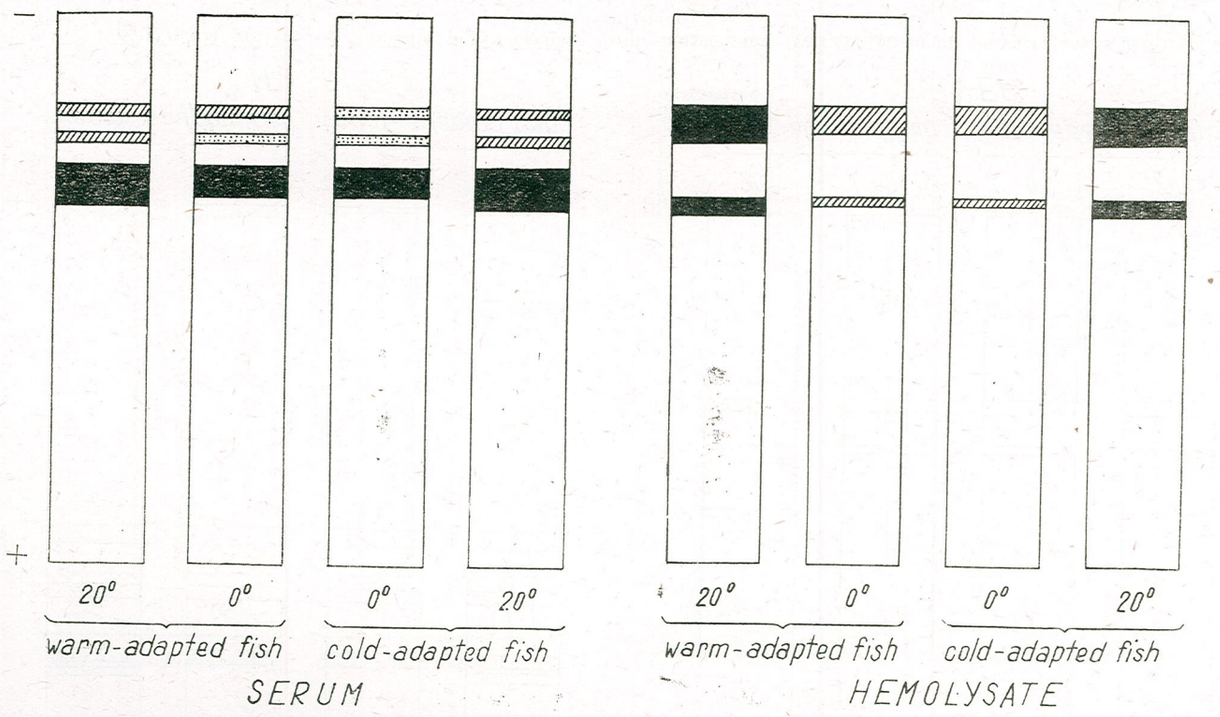


Fig. 2. — Isoenzymatic pattern of malate dehydrogenase from serum and hemolysate, obtained on polyacrylamide gel. Explanation as in figure 1.

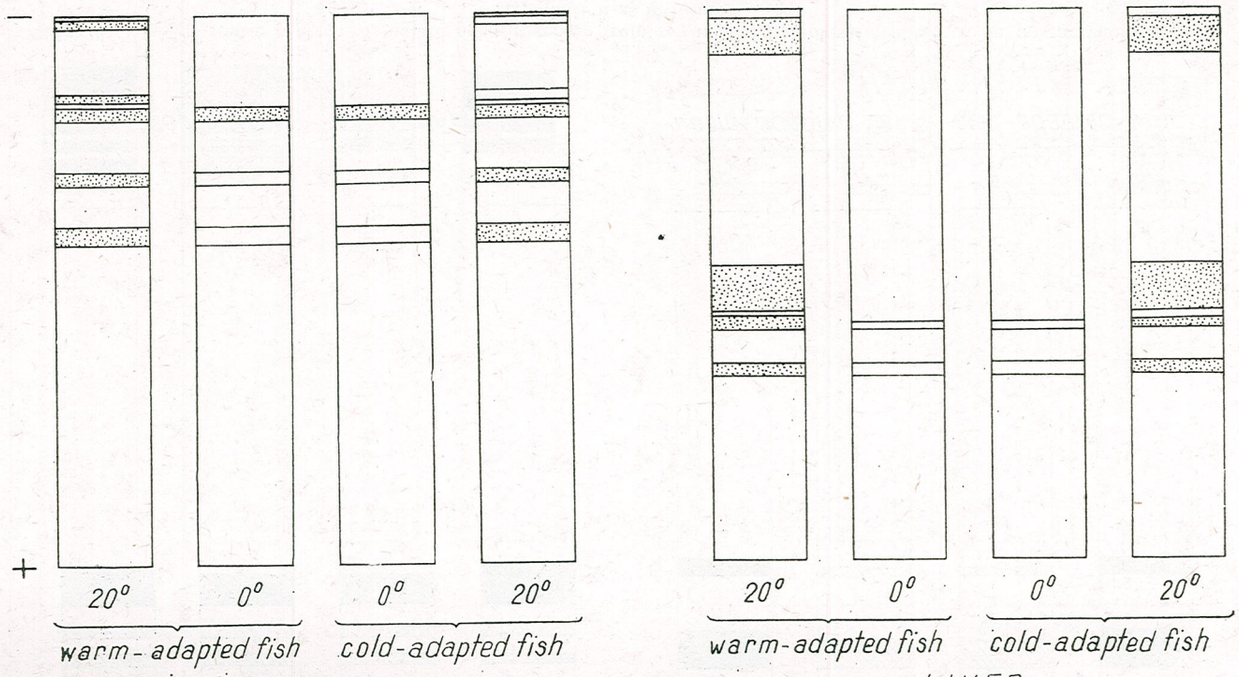


Fig. 3. — Isoenzymatic pattern of succinate dehydrogenase from muscle and liver, obtained on polyacrylamide gel. Explanation as in figure 1.

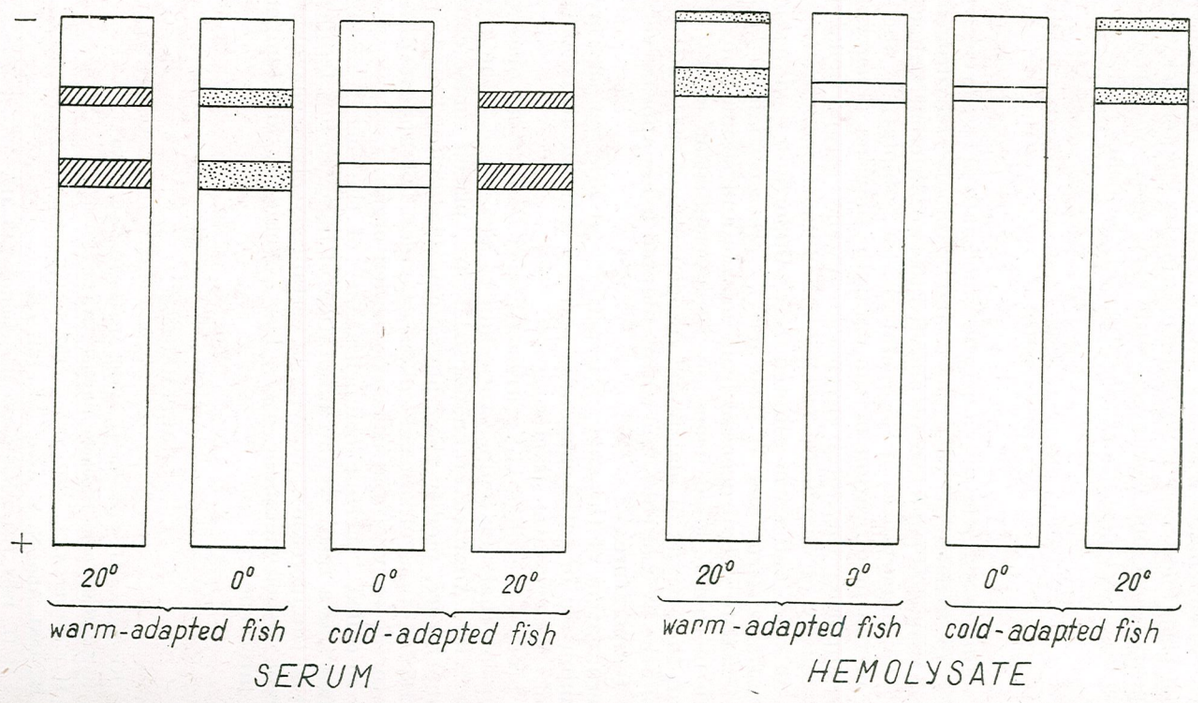


Fig. 4. — Isoenzymatic pattern of succinate dehydrogenase from serum and hemolysate, obtained on polyacrylamide gel. Explanation as in figure 1.

The electrophoretic pattern of succinate dehydrogenase from the hemolysate of cold- and warm-adapted fishes is characterized by the presence of two isoenzymatic fractions with a weak staining activity, when the gels had been incubated at 20°C. Incubation at low temperature leads to the appearance of one isoenzyme with very weak tinctorial activity, irrespective of the adaptation temperature of the fishes (Fig. 4).

DISCUSSION

In accordance with the previously obtained data [11], the electrophoretic results of malate dehydrogenase and succinate dehydrogenase of the studied tissues of pond loaches adapted to two different temperatures underline the importance of temperature (of adaptation and determination) in the modification of the catalytic function of enzymes.

By comparing the total enzymatic activity (proportional to the staining intensity of enzymatic fractions) and the isoenzymatic repartition both in cold- and warm-adapted fishes, important modifications of the enzymatic patterns and of their activity are ascertained. Thus, the activity of malate dehydrogenase from muscle and serum remains relatively high in cold-adapted fishes, by its determination at 0°C. On the other hand, the activity of malate dehydrogenase and succinate dehydrogenase from the liver of cold- and warm-adapted fishes appears very weak at a low temperature.

Determination of the enzymatic activity at low temperature leads to the diminution of total enzymatic activity and the reduction of the number of isoenzymatic fractions.

Our previous results [11], correlated with the present ones, lead to the assumption that low temperature induces a decrease of the glycolytic enzyme activity. Adapted or unadapted fishes, placed under conditions of low temperature, modify their physiological behaviour by a decrease of the catalytic functions of some glycolytic enzymes. This appears differently according to the enzyme and the investigated organ.

The so-called "thermic compensation" of the energy metabolism, which appears as a result of the adaptation of fishes to low temperature, attributed to the increase of activity of certain glycolytic enzymes, seems to be unreal. Other metabolic ways, less affected by low temperature, probably intervene.

REFERENCES

1. Braun K., Kunneman H., Laudien H., 1970, Marine Biology, **7**, 59.
2. Caldwell R. S., 1969, Comp. Biochem. Physiol., **31**, 79.
3. Davidson R., Cartner A. J., 1967, Science, **157**, 1569.
4. Davis J. B., 1964, Ann. N. Y. Acad. Sci., **121**, 404.
5. Jankowsky H. D., 1968, Helgolander wiss. Meeresunters., **18**, 317.
6. Hochachka P. W., 1967, *Molecular Mechanisms of Temperature Adaptation*, ed. Prosser C. L., A.A.A.S., Washington, 177.
7. Hochachka P. W. 1965, Arch. Biochem. Biophys., **111**, 96.

8. Kunneman H., Laudien H., Precht H., 1970, Marine Biology, **7**, 71.
9. Kruger G., 1962, Z. wiss. Zool., **167**, 87.
10. Latner A. L., Skillen A. W., 1968, *Isoenzymes in Biology and Medicine*, Acad. Press, New York.
11. Meșter R., Scripcariu D., Niculescu S., 1972, Rev. roum. Biol., série Zool., **17**, 205.
12. Murphy M. B., 1961, Ph. D. Thesis, Univ. of Illinois (quoted after Caldwell, 1969).
13. Nachlas M., Tson K., De Souza E., Cheng C. S., Seligman A. M., 1957, J. Histochem. Cytochem., **5**, 420.
14. Wilkinson J. H., 1965, *Isoenzymes*, Spon Ltd, London.

Received December 13, 1972

Faculty of Biology
Bucharest, Splaiul Independenței 91—95

ON THE CONSTITUTIVE HETEROCHROMATIN IN SEVERAL RODENT SPECIES

BY

I. VOICULESCU

The occurrence of qualitative differences between centromeric constitutive heterochromatin and that located in other chromosome segments, for rodent species with a high content of heterochromatin, was demonstrated at the cytological (chromosomal) level. The different Giemsa staining affinity of heterochromatin after a special treatment is presumably due to different degrees of repeatability of nucleotide sequences in the heterochromatic zones; the higher the degree of repeatability of nucleotide sequences, the greater the probability of reassociation of DNA strands after denaturation.

The recent publication of new techniques allowing the demonstration of the occurrence and location of constitutive heterochromatin zones on chromosomes [1] [2] and the recognition of the banding pattern characteristic of each chromosome pair [5] [8] [14] gave a further impetus to cytogenetic investigations and allowed a new approach in basic cytogenetics as well as in medical practice. Therefore, it is to be expected that several problems related to chromosome structure and karyotype evolution might receive appropriate solutions.

Already in 1968, Britten and Kohne [3] demonstrated the occurrence in eukaryotic organisms of redundant DNA. It was found that a given type of redundant DNA appeared to be distributed within the genome of eukaryotic organisms either uniformly dispersed, or as clusters of units.

The first investigations on redundant DNA were carried out in *Mus musculus* [11] [12] [16], the content value found for this type of DNA being of about 34%. This value is significantly different from the G—C content of the remaining DNA, which allowed the separation in cesium chloride density gradient of two distinct DNA fractions. As the

REV. ROUM. BIOL. – ZOOLOGIE, TOME 18, No 2, p. 163–169, BUCAREST, 1973

excess DNA migrates as a satellite with respect to the main DNA fraction, it was designated as "satellite DNA". Yasmineh and Yunis [16] demonstrated in the same species that the satellite DNA is located in constitutive heterochromatin. Several other papers [11] [12] indicate as sure the occurrence of heterochromatin, and therefore of satellite DNA, in the centromere region of the chromosomes of *Mus musculus*. To reveal the constitutive heterochromatin in cytologic preparations, the technique of Pardue and Gall [12] as improved by Arrighi and Hsu [1], denoted at the IVth International Conference for Standardization in Human Cytogenetics (Paris, September 1971) as the "C'-staining technique" [13], is suitable.

Extending the investigations based upon this technique to other species, it became possible to demonstrate the constitutive heterochromatin zones characteristic of every species under study, as well as their location on chromosomes. Thus, it was demonstrated in *Microtus agrestis* the occurrence of centromeric constitutive heterochromatin for the autosomes, for the major part of X chromosome and for the entire Y chromosome [2]. Furthermore, by the same technique, it was revealed the large amount of constitutive heterochromatin which covers the short arms of several autosomes in the golden hamster [10]. Similar observations were made by us as to the constitutive heterochromatin in the Romanian hamster [15].

As the constitutive heterochromatin in *Mus musculus* is identifiable with the zones of highly repetitive DNA, the question arises whether it is possible to assume such an identification in the species in which constitutive heterochromatin involves chromosome zones other than the centromeric ones; and whether the sex chromosomes in *Microtus agrestis* (the major part of X and the entire Y) are built up by repetitive DNA. In other words, does repetitive DNA occur in all the heterochromatic zones which show uniform and intensive staining with 'C'-staining technique? If so, is this repetitive DNA identical in nature all over the heterochromatic area in the genome?

Cytogenetic methods so far available, the classical as well as the modern ones, do not allow to approach at this level the aspects supposed to provide a solution to the above questions. Our tentative work succeeded in refining the methodology allowing the exclusive staining of centromeric heterochromatin, even in species known to display constitutive heterochromatin in other regions, too. On this basis, an examination of several aspects related to the above questions became possible, which is the aim of the present report.

MATERIAL AND METHODS

The following mouse and hamster species were used throughout the investigations: *Clethrionomys glareolus*, *Microtus ochrogaster*, *Microtus agrestis*, and *Mesocricetus newtoni*, everyone of these species displaying peculiarities in the constitutive heterochromatin distribution on chromosomes.

Chromosome preparations from bone marrow were performed by flame drying after fixation in 3 :1 methanol-acetic acid mixture. The slides underwent a treatment based upon the technique of Drets and Shaw for the revealing of chromosome bands [8]; after a pretreatment in sodium hydroxide solution, the preparations were incubated in 12 × SSC, pH 7.8, at 64°C and stained in Giemsa solution. Centromeric constitutive heterochromatin became exclusively stained.

To reveal the overall amount of constitutive heterochromatin in the genome, the technique of Arrighi and Hsu [1] was used.

Autoradiographic examinations of the late-replicating zones in the genome of *Mesocricetus newtoni* were performed on bone marrow cells in short term ($4^1/_2$ h.) cultures. The cultures were given 2uc/ml.^{3}H-thymidine and 3 h. after the start of a culture, colchicine was added.

RESULTS

In chromosome preparations from *Clethrionomys glareolus* we observed, using the technique of Arrighi and Hsu [1], a heterochromatic pattern similar to that observed in *Mus musculus*, the first mouse species investigated in this respect [4] [12]. The chromosome complement shown in figure 1 is illustrative for the occurrence of the constitutive heterochromatin in *Clethrionomys glareolus* only in centromeres as more or less marked blocks.

In *Microtus ochrogaster*, the constitutive heterochromatin appears to occur in the centromeres of all the chromosomes, one chromosome pair showing in addition an intercalary heterochromatin zone, well outlined by this technique (Fig. 2).

The species *Microtus agrestis* was chosen for our study because of its unusually big sex chromosomes, among which the proximal third of the short arm and the entire long arm of the X chromosome as well as the Y chromosome are heterochromatic (Fig. 3). The autosomes of *Microtus agrestis* display constitutive heterochromatin only at the centromeres level. The whole picture of the heterochromatin distribution in this species was already revealed by the 'C'-staining technique [2].

The distribution within the genome of the constitutive heterochromatin in *Mesocricetus newtoni* was studied by us in a previous paper [15]. It was demonstrated in this species the occurrence of a large amount of constitutive heterochromatin involving the centromere zones and the short arms of the autosomes from the pairs 5, 8, 9, 12, 13 as well as the long arms of the autosomes from the pair 17. The autosomal pair 11 shows a small block of constitutive heterochromatin in the centromere, the short arms being not heterochromatic, while the autosomes from the pair 15 do not display any distinguishable heterochromatin block in the centromeres, the short arms being however heterochromatic. The centromere zone and the short arms of the X chromosome as well as the entire Y chromosome are heterochromatic (Fig. 4). This heterochromatic pattern obtained by the 'C'-staining method appears to be identical with that obtained by us in autoradiographic examinations in the same hamster species (Fig. 5).

The late-replicating zones occur on the same chromosomes and in just the same areas as those described above as heterochromatic. No doubt that figure 4 shows the entire amount of constitutive heterochromatin in the genome of the species *Mesocricetus newtoni*.

The previously described treatment applied to chromosome preparations from the same four rodent species under study lead to the following results :

The chromosomes in *Clethrionomys glareolus* showed the same configuration, with constitutive heterochromatin blocks located near the centromeres.

In *Microtus ochrogaster* the heterochromatic pattern was also unchanged, with one autosomal pair showing the intercalary blocks of constitutive heterochromatin.

The autosomes in *Microtus agrestis* do not show any differential response to the two techniques, however, the X chromosome displays three degrees of Giemsa staining intensity on its length with the technique modified by us (Figs 6 and 7). Therefore, at the centromere level, the staining is the most intensive, similar to the corresponding zones on the autosomes. The remaining heterochromatic portion of the X chromosome (the proximal third of the short arm and the entire long arm) is obviously more intensively stained than the euchromatic zone (the distal two thirds of the short arm), but less than the centromeric block. Quite frequently, differences in the staining intensity at the centromere level as compared to the remaining chromosome which is heterochromatic are apparent for the Y chromosome, too (Fig. 7).

Chromosome preparations from *Mesocricetus newtoni* were treated by the same technique of differential staining intensity of the big zones of constitutive heterochromatin. By this technique, metaphase figures were obtained with centromeric heterochromatin * exclusively stained (Fig. 8). Heterochromatin blocks occur in the centromeres zone on the same autosomes from the pairs 5, 8, 9, 12, 13, 14 and 17, as well as at the centromere level of X chromosome. The small heterochromatic block in the centromeres of the autosomes of the pair 11 is still apparent. The absence of such a heterochromatic block from the centromere of the autosomes from the pair 15 is to be observed.

With another series of chromosome preparations, we obtained the same degrees of Giemsa staining intensity as those described for the X chromosome in *Microtus agrestis*. In figure 9, the autosomal pairs with heterochromatic short arms (pairs 5, 8, 9, 12, 13, 14 and 15) or long arms (pair 17) display a more marked Giemsa staining affinity as compared to euchromatic regions but however obviously less marked than the zones located close to centromeres. The same pattern is apparent for the X chromosome as well. The Y chromosome, which is entirely heterochromatic, does not show any heterochromatic block intensively stained at the centromere level; the most intensive Giemsa staining is confirmed to the distal two thirds of the long arm (Fig. 9).

* It should be noted that a distinction is to be kept in mind between the centromere and the so-called "centromeric" heterochromatin; the latter denotes the heterochromatic block located close to the centromere in contrast to the heterochromatin located in other chromosome segments, which therefore might be designated as "non-centromeric".

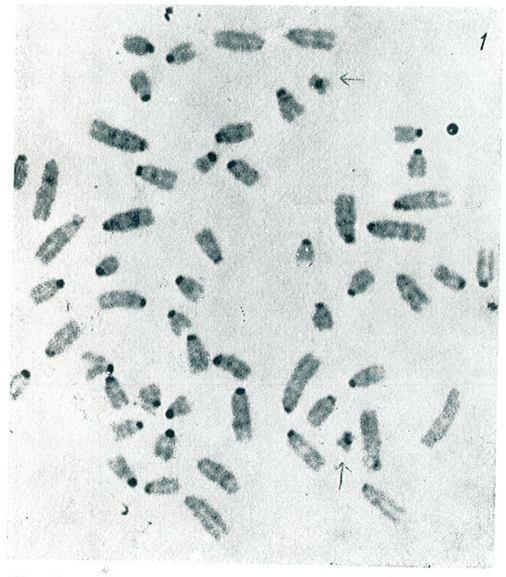


Fig. 1. — Centromeric constitutive heterochromatin in *Clethrionomys glareolus*, as detected by 'C'-staining treatment.

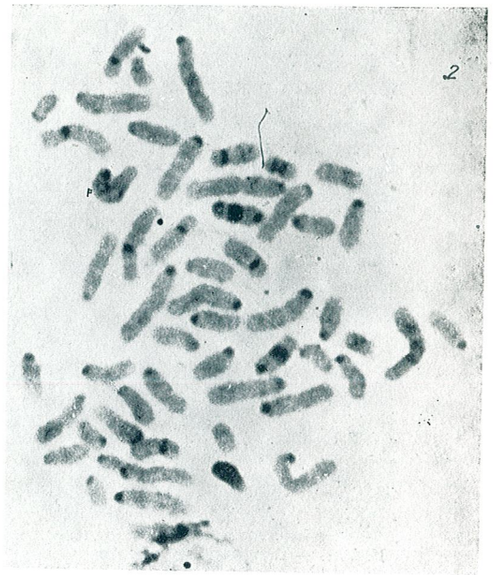


Fig. 2. — The location of constitutive heterochromatin on the chromosomes of *Microtus ochrogaster*; one pair of autosomes shows a block of intercalary heterochromatin.

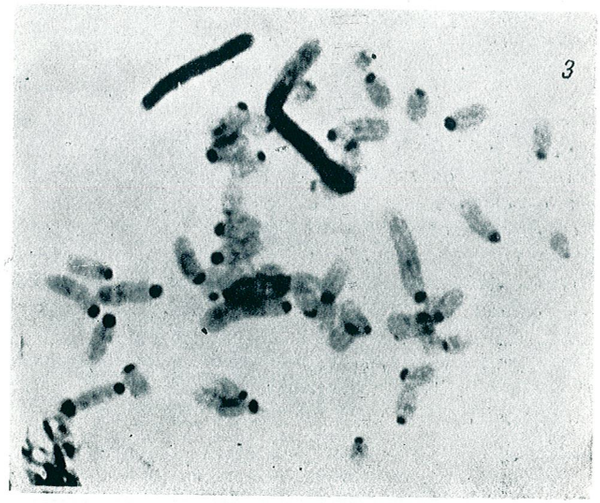


Fig. 3. — Constitutive heterochromatin in *Microtus agrestis*, as detected by 'C'-staining treatment.

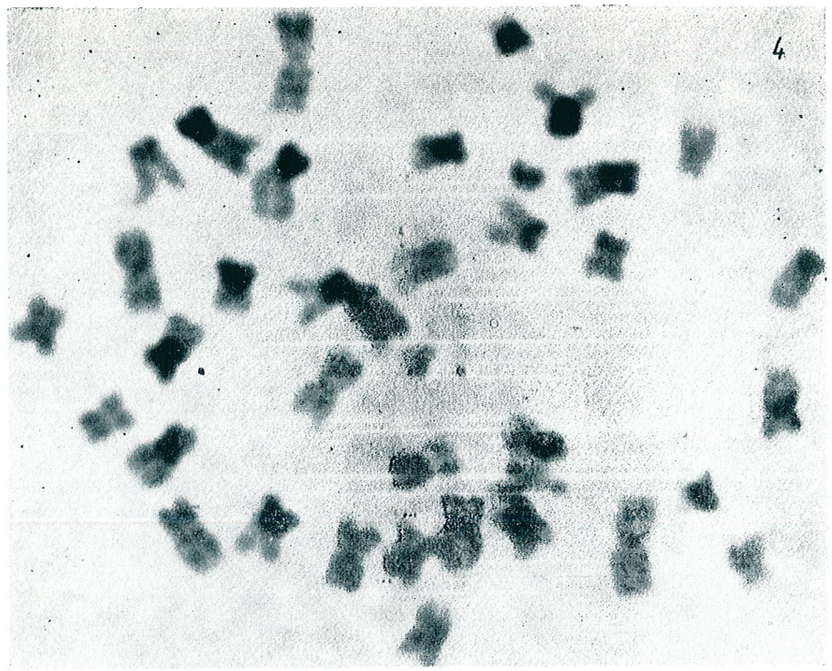


Fig. 4. — Constitutive heterochromatin in *Mesocricetus newtoni*, as detected by 'C'-staining treatment.

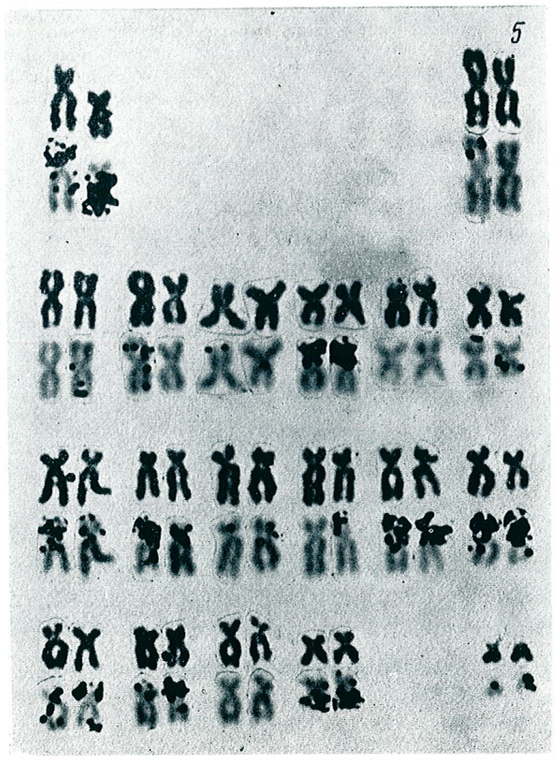
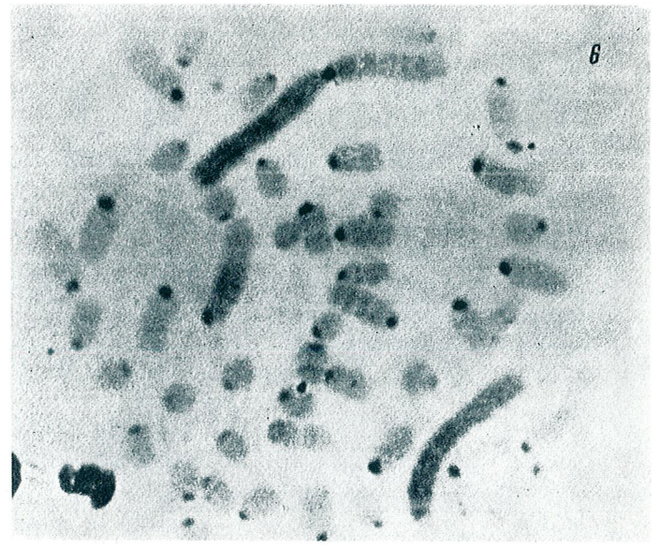
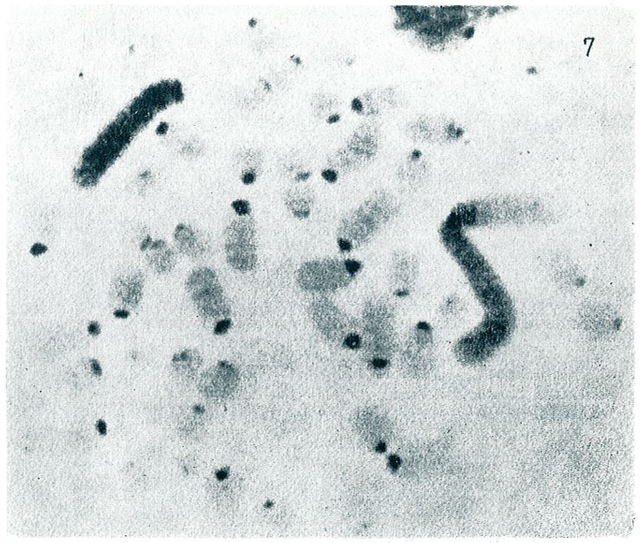


Fig. 5. — The late-replicating zones in the chromosomes of *Mesocricetus newtoni*, as revealed by autoradiography.





Figs 6 and 7. — Heterochromatic zones displaying different Giemsa staining intensities on the sex chromosomes of *Microtus agrestis* (note the centromere zone intensively stained as compared to the remaining heterochromatic portion).

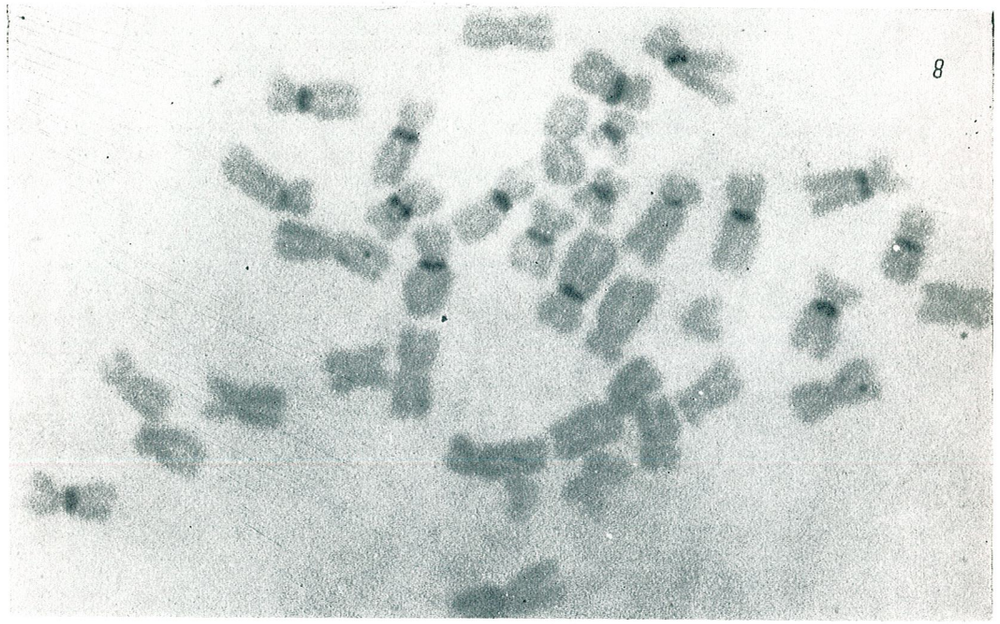


Fig. 8. — The exclusive demonstration of "centromeric" constitutive heterochromatin blocks on the chromosomes of *Mesocricetus newtoni*.

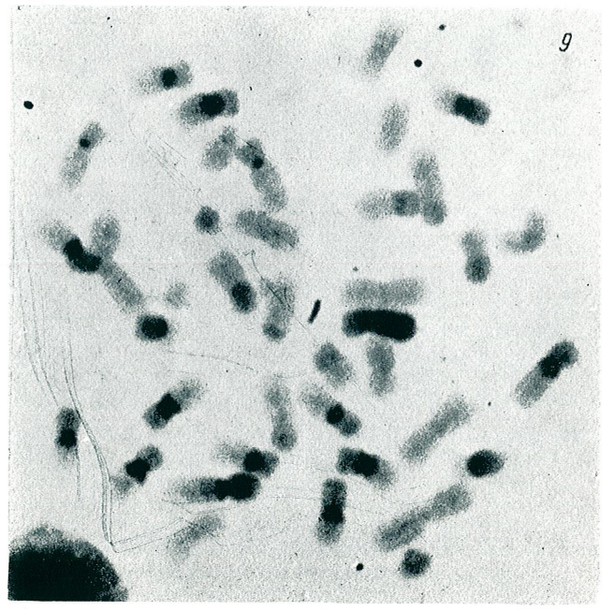


Fig. 9. — Heterochromatic zones with different Giemsa staining intensities on the chromosomes of *Mesocricetus newtoni*.

DISCUSSION

Our results provide a suitable support to several considerations related to the problems to which this paper intended to give an answer. A first point we would like to make is that the so-called facultative heterochromatin in the X chromosome of the mammals does not enter our discussion, all the argumentation being confined to the constitutive one.

The studies of Yunis and Yasmineh [17] led the authors to the conclusion of an equivalence between repetitive DNA and constitutive heterochromatin. Arrighi et al. [2] claimed that several fractions of repetitive DNA might not be expressed as heterochromatin, namely in the case of short segments of repetitive DNA which result in heterochromatin zones microscopically undetectable. However, this observation does not disagree with the assumption suggested by the data of Yunis and Yasmineh and consequently the heterochromatin revealed by the 'C'-staining technique could be considered as consisting in repetitive DNA. The repeated nucleotide sequences submitted to a 'C'-staining treatment undergo, following denaturation, a faster annealing than the unrepeated ones, and therefore the repetitive DNA portions, i. e. heterochromatin, become detectable with Giemsa staining.

Our results indicate at the cytologic level that, even within heterochromatic areas, differences occur in the Giemsa staining affinity. In all the cases described, the heterochromatic portions located at the centromeres level are more intensively stained than those located in other parts of the chromosomes, which provides a further support to the assumption of differences occurring within the heterochromatin in the genome of a given species. As to the nature of these differences, no sure conclusion can be drawn.

In *Mus musculus*, it was demonstrated that the constitutive heterochromatin located close to the centromeres consists in DNA with a high degree of repeatability of the nucleotide sequences [11], [12], [16]. Based upon this observation, one might infer from our results that in "non-centromeric" heterochromatin, nucleotide sequences would display a lower degree of repeatability. The assumption could be made that chromosome segments might contain repeated sequences with different degrees of homogeneity which, because of imperfect reassociations, result in different degrees of Giemsa staining. Yunis et al. [18] tentatively explained the faster reassociation of satellite DNA sequences as compared to the DNA bulk in fixed chromosome preparations, taking into account that under the conditions of the 'C'-staining treatment, the DNA strands undergo hydrolysis and subsequently may slide in different zones of the DNA duplex. Assuming a certain degree of displacement of the additional strands as well as the fact that DNA in metaphase chromosomes is coiled and looped especially in heterochromatin, it seems reasonable to think that a strand dissociated from a segment with sequences many times repeated could reassociate with a complementary strand from another segment. If so, the different degree of association in big heterochromatic zones should be accounted for in terms of the extent of the repeatability of nucleotide sequences.

Biochemical investigations by Corneo et al. [6] [7] revealed for human DNA two satellite fractions highly repetitive which cover 2.5 % of the genome and a "main homogeneous DNA band" of moderate repeatability, which comprises 15 %. Our results provide evidence, for the first time at the cytology level, for the occurrence of such qualitative differences within constitutive heterochromatin, which were detected until now only by biochemical methods. Taking into account that with the technique described by us only highly or very highly repetitive DNA becomes stained, a good agreement appears with the results obtained by Hennig and Walker [9], which found by cesium chloride density gradient centrifugation, only a small fraction of highly repetitive DNA in *Microtus agrestis*, a species which otherwise has a high content of heterochromatin in the genome. It is conceivable that the authors detected only highly repetitive DNA located in the centromeres of the autosomes and of the X chromosome. In the remaining portion of the constitutive heterochromatin, as revealed by our data too, in *Microtus agrestis* as well as in *Mesocricetus newtoni* the repeatability of nucleotide sequences is probably lower.

CONCLUSIONS

1. Constitutive heterochromatin occurs in the centromeres of the chromosomes in the genome of all the mouse species investigated.

2. In the Romanian hamster (*Mesocricetus newtoni*) most autosomes do not show in the centromeres such an amount of constitutive heterochromatin as to be microscopically detectable by Giemsa staining after 'C'-treatment.

3. In the mouse species and in the hamster which possess a large amount of constitutive heterochromatin in their genome a distinction can be made cytologically between "centromeric" heterochromatin and that located in other chromosome segments by means of a special treatment. The different Giemsa staining affinity within the heterochromatin occurring in a given chromosome is presumably related to the degree of repeatability of nucleotide sequences.

4. Presumably a linear relation exists between the degree of repeatability of nucleotide sequences and the Giemsa staining affinity of heterochromatin, as after denaturation, the probability of the reassociation of DNA strands is higher with the increasing number of repeated sequences.

5. The same staining affinity for constitutive heterochromatin located close to the centromeres was observed also in the cases in which, following the chromosome rearrangements underwent by a species during its evolution, this heterochromatin became intercalary (e.g. an autosomal pair in *Microtus ochrogaster*).

REFERENCES

1. Arrighi F. E., Hsu T. C., 1971, Cytogenetics, **10**, 81 — 86.
2. Arrighi F. E., Hsu T. C., Saunders P., Saunders G. F., 1970, Chromosoma (Berl.), **32**, 224 — 236.

3. Britten R. J., Kohne D. E., 1968, Science, **161**, *1341*, 529 — 540.
4. Chen T. R., Ruddle F. H., 1971, Chromosoma (Berl.), **34**, 51 — 72.
5. Chauduri J. P., Vogel W., Voiculescu I., Wolf U., Humangenetik, **14**, 83 — 84.
6. Corneo J. P., Ginelli E., Polli E., 1968, J. mol. Biol., **33**, 331 — 335.
7. — 1970, J. mol. Biol., **48**, 319 — 327.
8. Drets M. E., Shaw M. W., 1971, Proc. nat. Acad. Sci. (Wash.), **68**, 2073 — 2077.
9. Hennig W., Walker P.M.B., 1970, Nature (Lond.), **225**, 915 — 919.
10. Hsu T. C., Arrighi, F. E., 1971, Chromosoma (Berl.), **34**, 243 — 253.
11. Jones K. W., 1970, Nature (Lond.), **225**, 912 — 915.
12. Pardue M. L., Gall J. G., 1970, Science, **168**, 1356 — 1358.
13. * * * 1972, *IVth Intern. Conf. Standard. in Human Cytogenetics*, Paris 1971. The National Foundation-March of Dimes.
14. Schnedl W., 1971, Nature (Lond.), New Biol., **233**, 93 — 94.
15. Voiculescu I., Vogel W., Wolf U., 1972 Chromosoma (Berl.), **39**, 215 — 224.
16. Yasmineh W. G., Yunis J. J., 1970, Exp. Cell Res., **59**, 69 — 75.
17. Yunis J. J., Yasmineh W. G., 1970, Science, **168**, 236 — 265.
18. Yunis J. J., Roldan Liliana, Yasmineh W. G., Lee J. C., 1971, Nature (Lond.), **231**, 532 — 533.

Received November 30, 1972

The "Traian Săvulescu" Institute of Biology
Department of Genetics
Bucharest 17, Spl. Independenței 296

AVIS AUX AUTEURS

La « Revue Roumaine de Biologie — Série de Zoologie » publie des articles originaux d'un haut niveau scientifique, de tous les domaines de la biologie animale : morphologie, physiologie, génétique, écologie, taxonomie, 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, conseils, etc. 2. Comptes rendus des livres de spécialité parus en Roumanie.

Les auteurs sont priés d'envoyer leurs articles, notes et comptes rendus dactylographiés à double intervalle (31 lignes par page) en deux exemplaires.

Les tableaux et l'explication des figures seront dactylographiés sur pages séparées et les diagrammes exécutés à l'encre de Chine noire, sur du papier calque.

Les tableaux et les illustrations seront numérotés avec des chiffres arabes. La répétition des mêmes données dans le texte, les tableaux et les graphiques sera évitée. Les références bibliographiques citées par ordre alphabétique des auteurs comporteront le nom de l'auteur, l'initiale, l'année, le titre de la revue, abrégé conformément aux usances internationales, le tome, le numéro, la page. Les travaux seront accompagnés d'un court résumé en anglais, de maximum 10 lignes. Les textes des travaux ne doivent pas dépasser 15 pages dactylographiées (y compris les tableaux, la bibliographie et l'explication des figures).

Les auteurs ont droit à 50 tirés à part gratuits.

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

La correspondance relative aux manuscrits, à l'échange de publications, etc., sera adressée au Comité de rédaction, 296, Splaiul Independenței, Bucarest 17.

PRINTED IN ROMANIA