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

ÉDITIONS DE L'ACADÉMIE DE LA RÉPUBLIQUE SOCIALISTE DE ROUMANIE
3 bis, rue Gutenberg, Bucarest, Roumanie

REVUE ROUMAINE DE BIOLOGIE

— SÉRIE DE ZOOLOGIE —

TOME 17 1972 N° 6

SOMMAIRE

REV. ROUM. BIOL.—ZOOLOGIE, TOME 17, N° 6, p. 371—434, BUCAREST, 1972

DREI NEUE ARTEN DER GATTUNG *TRICHOUROPODA* BERLESE 1916 (UROPODIDAE)

VON

Z. FEIDER und MARINA HUȚU

The authors describe three species of genus *Trichouropoda* they enframed in the groups of species established by Hirschmann within the genus according to their degree of relationship. These species are: *Trichouropoda macrochaeta* collected from the hollow dust, found again in the arbour and which enters the „elegans" group; *Trichouropoda moldavica* which belongs to the „ovalis" group; and *Trichouropoda hirschmanni* of group „orbicularis". The last two were collected from the leafy arbour.

The species were determined following the structure of the hypostoma, epistoma tristernum and chelicera.

In Rumänien wurden bis jezt bekannt gegeben folgende Arten der Gattung *Trichouropoda*: *T. ovalis* C. L. Koch 1839, *T. obscura-similis* Hirschmann, Zirngiebl-Nicol 1961, *T. karawaewi* Berlese 1904, *T. spatulifera* Moniez 1892, *T. romanica* Feider, Huțu 1971, *T. diaveolata* Hirschmann, Zirngiebl-Nicol 1961, *T. orbicularis* C. L. Koch 1839.

Hier beschreiben wir drei neue Arten dieser Gattung, die in einer vorheriger Arbeit nur angeführt wurden: *T. macrochaeta* n. sp. Feider, Huțu, die nach den Gruppen die Hirschmann aufgestellt hat [4] der Gruppe, „um elegans" angehört, *T. moldavica* n. sp. Huțu, aus der Gruppe „um ovalis" und *T. hirschmanni* n. sp. Feider, Huțu, aus der Gruppe „um orbicularis". Letztere widmen wir dem hervorragenden Acarinologen Herr Werner Hirschmann.

Die morphologische Beschreibung der Arten wird in die Reihenfolge der systematischen Bedeutung der Organe gemacht.

Trichouropoda macrochaeta n. sp. Feider, Huțu

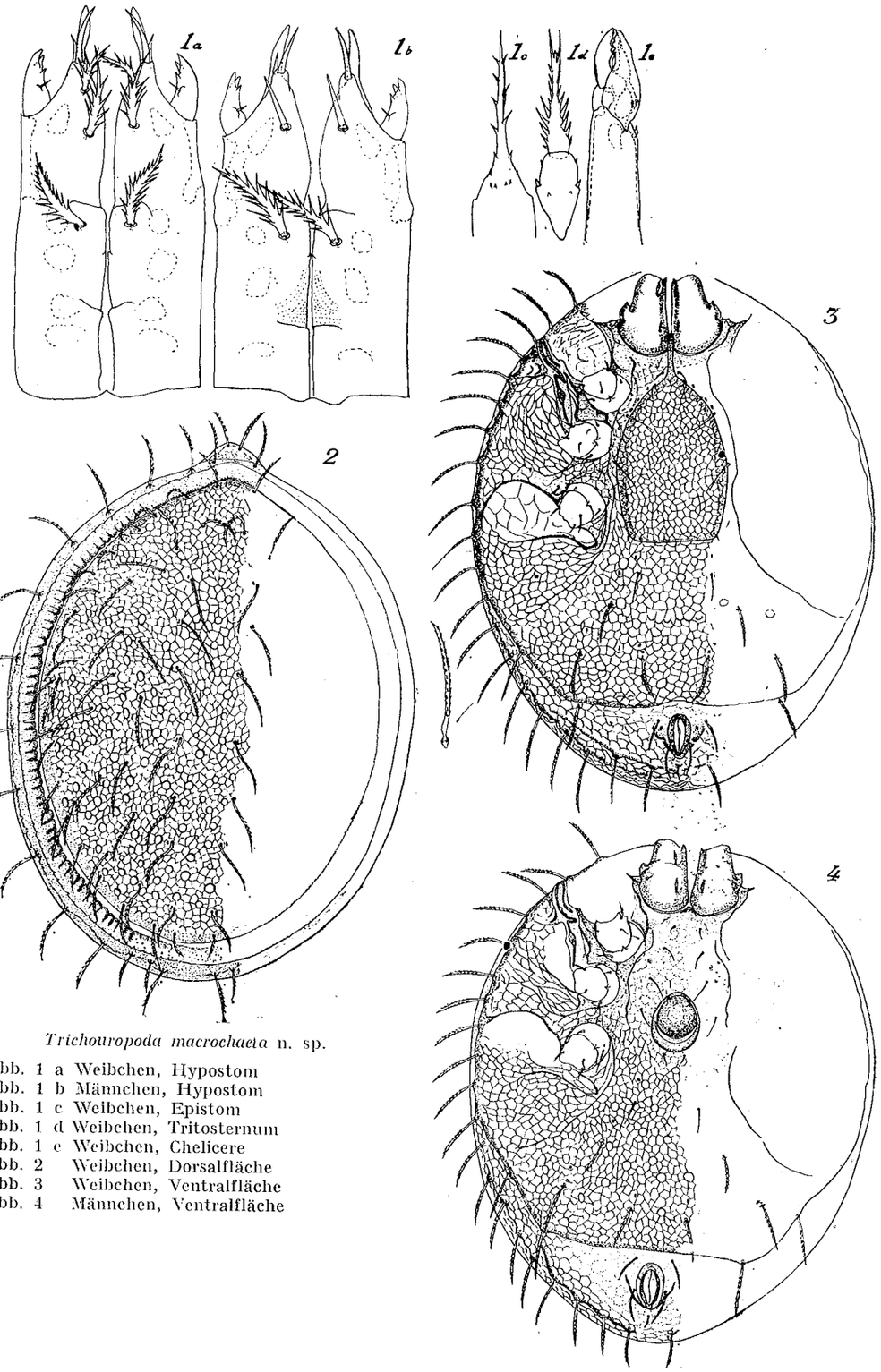
Die Art wurde benannt nach den Haaren der Körperoberfläche, die im Verhältnis zu anderen Arten aus Rumänien, sehr lang sind.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, No 6, p. 373–380, BUCAREST, 1972

Größe der Idiosoma: Weibchen — Länge: 820 µ, Breite: 650 µ; Männchen—Länge: 810 µ, Breite: 610 µ.

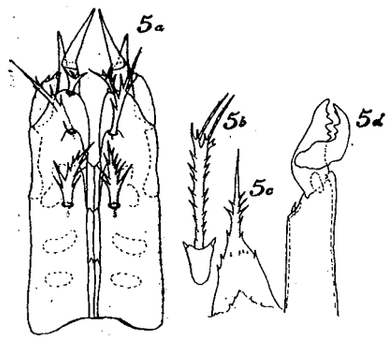
Hypostom: Weibchen (Abb. 1a): Corniculi gedrungen, mit 2 abgerundeten Endzacken, ein Seitenzacken und dazu ein spitziger Zacken auf der Fläche; Laciniae gleichlang aber etwas breiter als c_1; c_1 glatt, zugespitzt, ein wenig nach innen gebogen; c_2 und c_3 an c_1 genähert; c_2 etwa gleichlang wie c_1 mit 4 starken Nebenzacken auf der Außenseite und 3 kleinere auf der Innenseite; c_3 breiter als c_1, mit 4 Paar Nebenzacken; c_4 länger als die anderen Coxalhaare und mit vielen Nebenzacken; 3 Querleisten als Strukturdoppelbögen ausgebildet; von der ersten und dritten Querleiste geht beiderseits je eine kurze Strukturlinie aus. Männchen (Abb. 1b): c_1 und c_4 ähnlich wie beim Weibchen nur c_1 etwas schmäler; c_2 fehlt wie bei allen verwandten Arten; c_3 glatt, gleich c_1. *Epistom* (Abb. 1c): Basalteil verhältnismäßig kurz mit 2 Paar kurzen Seitenzacken und 2 Paar kleinen Flächenzähnen; Distalteil lang, mit wechselständigen Zacken und kurzer Endspitze. *Tritosternum* (Abb. 1d): Grundglied länglich, mit je 1 Paar Vorderrand-, Seiten- und Flächenzacken; Lacinia kurz, Proximalhälfte dicker, besonders an der Basis, mit 4 Paar starken Seitenzacken; Distalhälfte schmäler mit 4 Paar kleiner Zacken; Endabschnitt in 2 kleinen seitlichen, glatten Spitzen und eine mittlere, ganz kurze aber dickere aufgeteilt. *Chelicere* (Abb. 1e): Fixuslade mit 4, Mobilis mit 3 abgerundeten Zähnchen; Schere länglich gestreckt. *Dorsalfläche* (Abb.2): Marginale einheitlich mit Innenrand ab s_6 tief kreneliert; Marginalhaare nicht vermehrt, lang, doppelreihig fein gefranst, Z_5 und I_4 kürzer, ebenfalls gefranst; Dorsalhaare mit gleicher Form wie die der Marginale; Dorsale mit wabenförmigem Strukturlinienmuster und dazwischen unregelmäßig verbreitete abgerundete Strukturgruben. *Ventralflächen*: Weibchen (Abb. 3): Endopodial- und Metapodiallinie gut sichtbar, geschlängelt; Metapodiallinie verwächst nicht mit Carina ventralis, die gut ausgeprägt ist und in eine Querstrukturlinie übergeht, die die Analabgrenzungslinie darstellt; Operculum groß, plätteisenförmig mit hinteren Ecken leicht abgerundet, reicht mit dem hinteren Teil nur wenig über Coxen IV hinaus; Mittelspitze mit langen einspitzigem, kolbenförmigem Fortsatz der weit den Vorderrand des Sternum überragt; v_1 und v_5 kurz, v_5 ein wenig von Operculum bedeckt; von v_2 bis v_4 die Länge der Haare zunehmend, v_4 gleichlang wie x_1; x_2, V_2, V_6, V_7, V_8 lang, doppelreihig fein gefranst; V_3 und V_4 gleich gestaltet, etwas kürzer und dünner; 2 x- Haare; 20 Randhaarpaare; Analöffnung von einem V_4 enthaltenden Strukturlinienring umgeben; die gesamte Ventralfläche mit demselben Strukturmuster wie auf Dorsale. Männchen (Abb. 4): Endopodiallinie kurz nicht mit Metapodiallinie verwachsen, die sonst wie beim Weibchen verläuft; Operculum eiförmig, von einem glatten Chitinband im unteren Abschnitt umgeben; v_1 und v_5 kurz; v_2 etwa zweimal, v_3, v_4 und x_1 dreimal so lang wie v_1; Analöffnung etwas größer als beim Weibchen; Haarform, Haarzahl, Peritrema und Strukturmuster wie beim Weibchen.

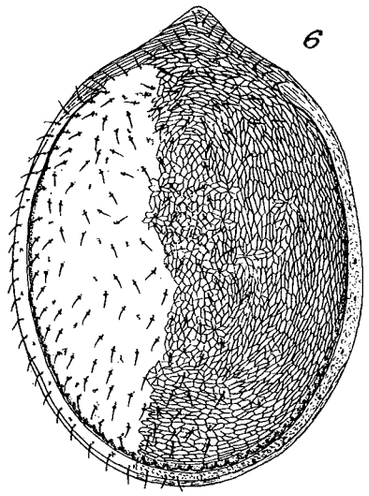
Nach dem Bau der Gnatosomaunterseite und Tritosternum wie auch nach dem Gesamthabitus der Erwachsenen scheint diese Art nahe verwandt mit *T. hispanica* (Hirschmann, Zirngiebl-Nicol, 1961).

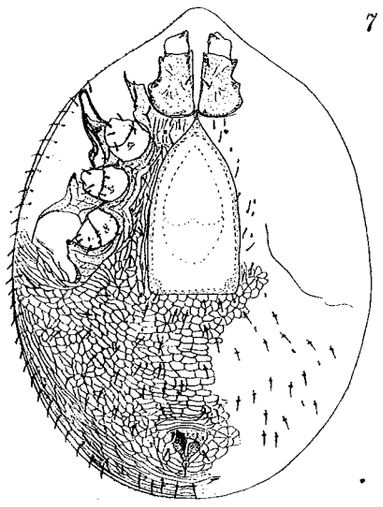


Trichouropoda macrochaeta n. sp.

Abb. 1 a Weibchen, Hypostom
Abb. 1 b Männchen, Hypostom
Abb. 1 c Weibchen, Epistom
Abb. 1 d Weibchen, Tritosternum
Abb. 1 e Weibchen, Chelicere
Abb. 2 Weibchen, Dorsalfläche
Abb. 3 Weibchen, Ventralfläche
Abb. 4 Männchen, Ventralfläche

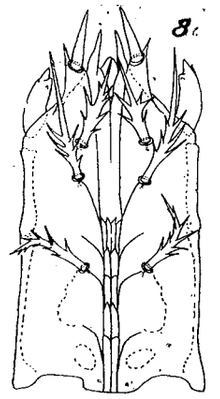


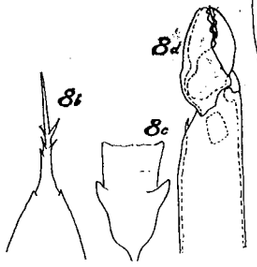


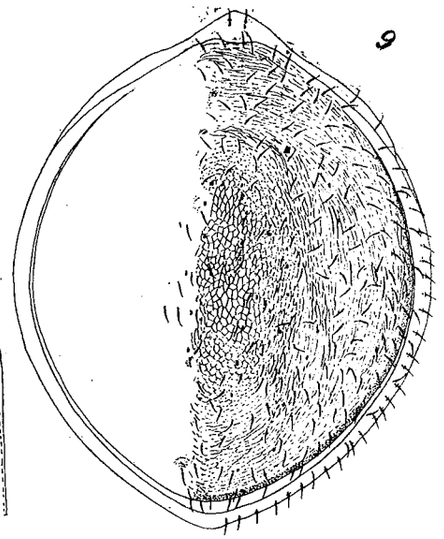


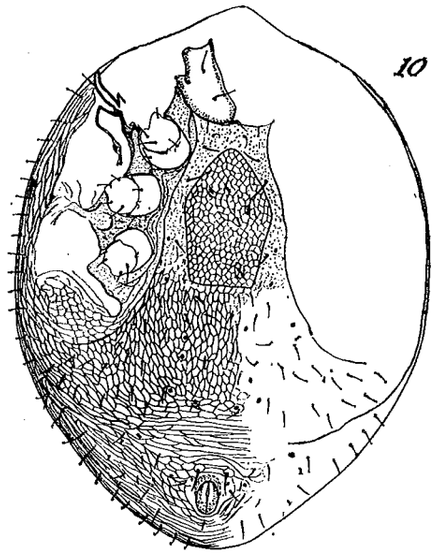
Trichouropoda moldavica n. sp.

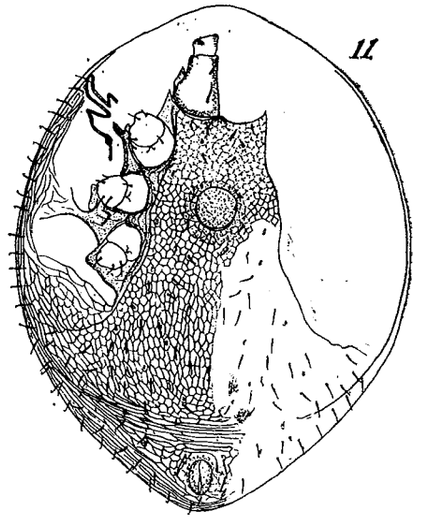
Abb. 5 a Weibchen, Hypostom
Abb. 5 b Weibchen, Epistom
Abb. 5 c Weibchen, Tritosternum
Abb. 5 d Weibchen, Chelicere
Abb. 6 Weibchen, Dorsalfläche
Abb. 7 Weibchen, Ventralfläche











Trichouropoda hirschmanni n. sp.

Abb. 8 a Weibchen, Hypostom
Abb. 8 b Weibchen, Epistom
Abb. 8 c Weibchen, Tritosternum, Basalteil
Abb. 8 d Weibchen, Chelicere
Abb. 9 Weibchen, Dorsalfläche
Abb. 10 Weibchen, Ventralfläche
Abb. 11 Männchen, Ventralfläche

Terra typica : Babadag (Kreis Tulcea – Rumänien). Datum : 3.IV.1956. Biotop : Mulm aus Baumhöhle. Holotyp : Weibchen, Paratyp : 3 Weibchen, Alotyp : 2 Männchen. Bîrnova (Kreis Iași — Rumänien). Datum : 15.IV.1957, Biotop : Verrottete Blätter. 3 Weibchen und 1 Männchen Alle Exemplare bie Verfassern.

Trichouropoda moldavica n. sp. Huțu

Größe der Idiosoma : Weibchen — Länge 655 μ und Breite 480 μ. *Hypostom* : (Abb. 5a) : Corniculi zylindrisch mit 3 kleinen Endzacken ; Lacinien nicht sichtbar ; c_1 dick, mundwärts gerichtet, mit kräftigem seitlichem Innenkantenfortsatz ; c_2 klein, mit Geweihform, die bei den Arten dieser Gruppe kennzeichnend ist ; c_3 verhältnismäßig kurz, 1 1/2 × $\times c_1$, mit 4 Nebenzacken, auf einem Ansatzhöcker sitzend von dem beiderseits je eine Strukturlinie ausgeht ; c_4 breit, mit 4 Zacken auf der Außenseite und 3 auf der Inneren, Größe der Nebenzacken gegen der Spitze allmählich kleiner werdend, Endteil in 2 ungleichen Spitzen gespalten ; 3 Querleisten mit je 1 Paar Zäckchen, von Q_3 aus läuft vorwärts je eine Strukturlinie die vor Q_2 in die Coxalflächen biegt. *Epistom* (Abb. 5b) : Basalteil fast dreieckig, mit 2—3 kurzen Seitenzacken und 4 Flächenzähnen ; Distalteil kurz, mit 6 ungleichen Zacken und einer verhältnismäßig langen, glatten Endspitze. *Tritosternum* (Abb. 5c) : Grundglied klein, mit 2 Vorderrandzacken ; Lacinia gegen die Spitze dicker werdend mit 8 Paar etwa gleichgroßen Zacken ; Endabschnitt in 3 kurze gabelartige Äste und 2 längere dünne fein beborstete Spitzen geteilt. *Chelicere* (Abb. 5d) : Fixuslade massiv mit 4 Zähnen von denen ein mittlerer stärker ist ; Mobilis mit einem mittleren starken und 2 seitlichen kleinen Zähnen ; Rollplatte klein, länglich — rund. *Dorsalfläche* (Abb.6) : Marginale zwischen $z_1' - z_1$ mit Dorsale verwachsen ; Innenrand glatt ; etwa 35 Paar kurze, nadelförmige Marginalhaare und etwa 7 Paar Marginalporen ; Dorsale mit kreneliertem Hinterrand und Strukturlinienmuster das ringsum den Poren eine radiale Anordnung nimmt ; Dorsalhaare stark vermehrt, gleich die der Marginale. *Ventralfläche* (Abb. 7) : Endopodiallinie fehlt ; Metapodiallinie im stumpfen Winkel gegen die Randverwachsungsnaht laufend ; Operculum groß, plätteisenförmig mit rechten Winkeln an geradem Hinterrand, weit über Coxen IV hinausreichend ; die kurze Mittelspitze erreicht den Sternumvorderrand ; alle Ventralhaare etwa gleichlang, nadelförmig ; im Genitalbereich 3 *vx*-Haare ($vx_2 - vx_4$) mit betreffenden Poren ; etwa 20 Paar x-Haare und 32 Randhaarpaare ; Analöffnung T-förmig mit 2 seitlichen Chitinklappen wie bei *T. sardensis* (Hirschmann, Zirngiebl-Nicol 1961) ; Peritrema mit langem gewundenem Vorderast und ganz kurzem Blindschlauch ; gesamte Ventralfläche (ausgenommen das Operculum) mit gleichem Strukturmuster wie Dorsale.

Nach der Form der Gnatosomaunterseite, Tritosternum und Epistom ähnelt die Art am besten mit *T. interstructura* (Hirschmann, Zirngiebl-Nicol 1961) aus der Gruppe „um ovalis".

Terra typica : Roman (Rumänien). Datum : 2.I.1939. Biotop : verrottete Laubwaldblätter. Holotyp : Weibchen beim Verfasser.

Trichouropoda hirschmanni n. sp. Feider, Huțu

Größe der Idiosoma : Weibchen — Länge : 740 μ, Breite : 510 μ ; Männchen — Länge ; 710 μ, Breite : 480 μ.

Hypostom : Weibchen (Abb. 8a) : Corniculi schlank, mit 2 Endzacken und einem winzigen Flächenzacken ; Laciniae kürzer und schmäler als c_1 ; c_1 breit mit Ansatzstelle die die gesamte Vorderkante des schmalen Mundfortsatzes einnimmt ; c_2 breit, Länge : 1 1/2 × c_1, mit starken ungleichlangen Nebenzacken ; c_3 — Länge : 2 × c_1, etwas schmäler als c_2, auf der proximalen Hälfte 3 Paar Nebenzacken von denen die auf der Außenseite kräftiger sind ; c_4 etwa gleichlang c_2 aber schmäler, mit 9—10 ungleichlangen Nebenzacken ; c_3 an c_2 genähert, so daß die Strecken $c_1 - c_2$ und $c_3 - c_4$ etwa gleich sind ; 5 Querleisten und 4 Strukturlinien : eine $c_3 - Q_2$, eine von Q_3 aus, eine $c_4 - Q_4$ und endlich eine von Q_6 auslaufend. Männchen : Konnte nicht eindeutig beobachtet werden ; c_2 scheint kolbenförmig, verkürzt und an c_1 genähert ; c_3 scheint glatt und verkürzt dem Weibchen gegenüber. *Epistom* (Abb. 8b) : Basalteil groß, dachförmig, mit 1 Paar kleinen Seitenzacken ; Distalteil verhältnismäßig kurz mit 5—6 Seitenzacken und glatter Endspitze die fast die Hälfte der Distalteillänge darstellt. *Tritosternum* (Abb. 8c) : nur das Grundglied konnte gut beobachtet werden, Lacinia am Präparat nicht sichtbar ; Grundglied massiv mit 1 Paar mächtigen Seitenzacken und 1 Paar Flächenzacken. *Chelicere* (Abb. 8d) : Entspricht in der Form *T. orbicularis* (C. L. Koch 1839), nur sind die Zähne kleiner und stumpfer ; Rollplatte fast rechteckig. *Dorsalfläche* (Abb. 9) : Marginale glatt, zwischen $z_1' - z_1$ mit Dorsale verwachsen ; Marginal- und Dorsalhaare kurz, nadelförmig, vermehrt ; Strukturlinienmunster polygonal, verwischt, sichtbarer in der Mitte der Dorsale. *Ventralflächen* : Weibchen (Abb. 10) : Endo- und Metapodiallinie, verwachsen, enden im Bogen auf der Bauchfläche ; Carina ventralis gut ausgeprägt, biegt in die Bauchfläche ein etwa in Höhe von V_7 wo mit dem querlaufenden Strukturlinienmuster eine Analabegrenzung entsteht ; Operculum mit artspezifischem pentagonalem Umriß, Vorderrand schmal gerundet in Höhe von v_2 endend, Hinterrand nur wenig die Coxen IV überragend, gesamte Oberfläche des Operculum mit unregelmäßigen fünfeckigen Strukturlinienmuster bedeckt ; v_1 und v_5 kurz, nadelförmig ; v_2 — und v_4 — Länge 1 1/2 × v_1 ; v_3 das längste v-Haar etwa 2 1/2 × v_1 ; alle anderen Ventralhaare kurz, nadelförmig, ausgenommen V_4 das gleichlang v_3 ist ; Randhaare etwas länger als die der Ventrale, auf der hinteren Hälfte des Körpers mit gekrümmten Spitzen ; Analöffnung länglich, mit ovalem Chitinring ; Ventralfläche hinter dem Operculum mit länglich-polygonalem Strukturlinienmuster bedeckt, das verschiedene Richtungen nimmt, die Zone neben und vor dem Operculum mit verwischtem Muster ; Peritrema in charakteristischer Form gewunden, mit kurzem am Ende verbreiteten Blindschlauch. Männchen (Abb. 11) : Endo- und Metapodiallinie wie auch Carina ventralis wie beim Weibchen ; Operculum verhältnismäßig klein, rund ; alle Ventralhaare einschließlich $v_1 - v_5$ kurz, nadelförmig mit Ausnahme vom V_4-Paar das verlängert ist wie beim Weibchen ; Analöffnung und Chitinring dem Weibchen gegenüber etwas größer ; Strukturlinienmuster auf der vorderen Hälfte rundlich, teilweise

verwischt, auf der hinteren Hälfte polygonal-länglich mit senkrechter Richtung bis auf die Analzone wo es querlaufend ist; Peritrema wie beim Weibchen.

Nach dem Bau der Gnatosomaunterseite und die Form der Chelicere gehört die Art zur Gruppe „um orbicularis", obwohl keine von den Arten dieser Gruppe im Gesamthabitus zu *T. hirschmanni* näher steht.

Terra typica: Boca — Samarineşti (Kreis Gorj — Rumänien). Datum: 6.II.1957. Biotop: verrottette Laubwaldblätter. Holotyp: Weibchen, Alotyp: 1 Männchen, bei den Verfassern.

LITERATUR

1. Berlese A., Redia, 1903, **1**, 324–386.
2. Hirschmann W., Acarologie, Gangsystematik der Parasitiformes, 1957, **1**, 10–13.
3. — Acarologie, Gangsystematik der Parasitiformes, 1959 **2**, 21–22.
4. Hirschmann W., Zirngiebl-Nicol I., Acarologie, Gangsystematik der Parastiformes, 1961, **4**, 1–41.
5. — Acarologie, Gangsystematik der Parasitiformes, 1964, **6**, 1–22.
6. — Acarologie, Gangsystematik der Parasitiformes, **8**, 23–25.
7. — Acarologie, Gangsystematik der Parasitiformes, 1969, **12**, 46–47; 100–104.

Eingegangen am 12. April 1972

Biologisches Forschungszentrum, Iaşi

CERIANA WORELLI sp. n. (DIPTERA, SYRPHIDAE)

PAR

VL. BRĂDESCU

In the present work a new species named *Ceriana worelli* sp. n. (Diptera, Syrphidae) is described. The author made a comparative study of the species *Ceriana binominata* (Verr.), with which this new species has resembling characters.

Pendant la révision d'un matériel des Syrphides compris dans les collections du Muséum d'Histoire Naturelle «Gr. Antipa» de Bucarest, nous avons identifié trois exemplaires (1♂ 2 ♀♀) d'une nouvelle espèce appartenant au genre *Ceriana* Rafinesque.

Moyennant la littérature de spécialité existante pour ce genre, nous arrivons à l'espèce méditerranéenne *Ceriana binominata* (Verrall, 1901). Quoiqu'il existe quelques ressemblances assez prononcées avec l'espèce mentionnée ci-dessus, une analyse plus approfondie a précisé une série de traits caractéristiques qui nous ont conduit à la conclusion qu'il s'agit d'une espèce nouvelle, que nous dénommons *Ceriana worelli*. en mémoire du prestigieux entomologiste amateur qui a été Eugen Worell.

Ceriana binominata, évidemment apparentée à la nouvelle espèce, a été collectée pour la première fois par F. Kowarz (juin 1871) sur le territoire de la Roumanie, dans la zone des Portes de Fer (Danube), et décrite par Loew en 1873 sous le nom de *Ceria tridens*. En 1901, Verrall, en vue d'éviter l'homonymie avec une espèce de Californie, l'a dénommée *Ceria binominata*. E. Séguy (1961) a rétabli les droits de priorité en ce qui concerne la dénomination du genre: *Ceriana* Rafinesque 1815.

Dans le voisinage de notre pays, *Ceriana binominata* a été encore signalée en Bulgarie — région de Tîrnovo (P. Drensky, 1934) — et en Yougoslavie — Monts Fruška, près de la localité Novi Sad (S. Glumac, 1958).

Notre espèce provient de la Dépression de Sibiu (ou de Cibin), les exemplaires étant collectés le 24.VI.1934 (2 ♀♀) et le 9.V.1946 (1 ♂)

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, N° 6, p. 381–384, BUCAREST, 1972

aux alentours de la ville de Sibiu (la colline Guşterița, actuellement quartier urbain).

MÂLE

Tête. Front noir, plus ou moins luisant; les poils blanchâtres, courts et rares, plus épais sur les parties latérales, comportent jusqu'à 3/4 de la longueur du style terminal des antennes. Sur le fond noir du front, bien délimitées et tangentes aux bords des yeux, se détachent trois taches jaunes-oranges : l'une, située au centre, étroite et ressemblant à des ailes déployées, moule l'angle interoculaire sur une partie un peu plus grande que la largeur de l'apophyse antennifère ; les deux autres sont à peu près rondes et situées d'un côté et de l'autre du front et envoyent, chacune à part, un prolongement effilé vers la tache centrale sans la touchant, tout de même. La coloration et le dessin de la face sont similaires à ceux de *Ceriana binominata* (y compris le dessin imitant le chiffre 7). Le profil de la face, légèrement incliné vers le calus facial (très faiblement développé), revient doucement vers l'épistome. La face et les gênes avec des poils rares et courts, fort fins, blanchâtres, plus développés sur les parties latérales et sur celle inférieure. La zone occipitale, avec une bande transversale jaune-orange, de longueur à peu près égale à la largeur antérieure du thorax ; devant le triangle ocellaire, situé sur une tache noire de forme rhombique, la couleur jaune s'étend sur toute la largeur du vertex. Sur le triangle ocellaire et sur le vertex, des poils blanchâtres, courts, de longueur croissante vers les parties latérales. L'apophyse antennifère égale au quart du premier article antennaire ; le premier article un peu plus court que les 2^{e} et 3^{e} réunis, le 2^{e} à 4/5 du 3^{e} article. L'apophyse antennifère et les deux tiers basaux du premier article, jaunes rougeâtres ; le tiers apical du premier article, ainsi que le 2^{e} et 3^{e}, bruns. La pubescence des antennes brune noirâtre, celle du style terminal blanche.

Thorax. Mésonotum noir, mat, finement ponctué ; deux paires de taches jaunâtres : l'une sur les callosités humérales, l'autre sur les parties latérales du mésonotum, devant la suture thoracique transversale. Sur la suture thoracique transversale, deux petites taches blanches argentées, tomentées, orientées transversalement. Les pleures présentent deux taches jaunes bien délimitées: l'une oblongue, à orientation verticale ; la deuxième, ovale, est située en dessous. Scutellum noir, mat, finement ponctué ; à la base, une bande transversale jaune, de la largeur du tiers du scutellum. Mésonotum et scutellum avec des poils blanchâtres, fort courts, un peu plus longs sur les bords ; à la partie postérieure du scutellum quelques poils noirs. Les ailes avec r 4 + 5 peu courbée (fig. 1). Cuillerons et balanciers blancs jaunâtres. f 1 et f 2 noirs, faiblement colorés en jaune rougeâtre aux extrémités, surtout vers celle apicale ; f 3 à coloration distribuée en trois secteurs presque équivalents, le noir au milieu ; tibias et tarses jaunes, faiblement colorés de brun à la partie apicale. La pilosité des pattes, courte, blanchâtre en grande partie.

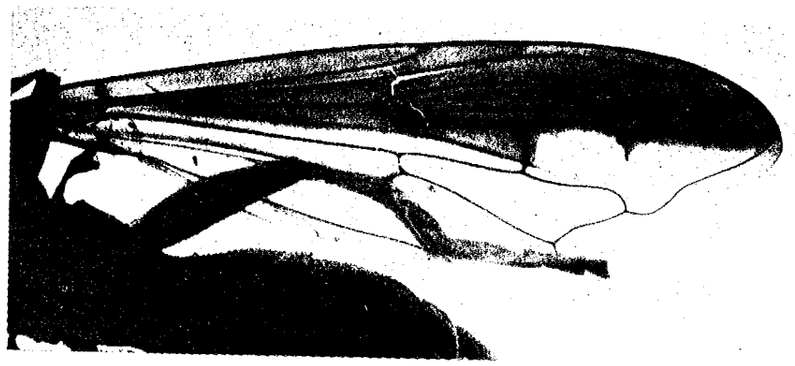


Fig. 1. — *Ceriana worelli* sp. n. ; aile (original).

Fig. 2. — *Ceriana worelli* sp. n. ♀ ; les deux bandes filiformes, fort courbées, blanchâtres argentées, du tergite IV (original).

Abdomen. Plus finement ponctué et un peu luisant par rapport au mésonotum. Le tergite II, dans sa partie la plus étroite, comporte la moitié de la largeur de la partie postérieure du tergite III. Par ce caractère, ainsi que par la courbure évidente de l'abdomen, l'insecte offre l'aspect d'une guêpe. La pilosité blanchâtre est mieux développée sur le tergite IV. Sur la partie apicale de l'abdomen, des poils plus longs, érects, de couleur prédominante brune noirâtre. Les bords latéraux du tergite I avec des poils plus longs et blancs. Hypopygium avec des poils longs blancs jaunâtres. Outre les taches latérales jaunes de la partie antérieure du tergite II et les bandes transversales de même couleur des parties postérieures des autres tergites, le tergite IV présente encore deux bandes filiformes (d'environ 0,3 mm), fortement courbées, couvertes d'un toment blanchâtre argenté. Ces deux bandes commencent dans le sens longitudinal, du côté de la zone centrale et du bord postérieur du premier tiers du tergite, forment ensuite une large courbure, pour finir dans le sens transversal, et s'arrêtant avant d'atteindre les bords latéraux de l'abdomen à une distance égale avec le tiers de la longueur du tergite (fig. 2). Toutes les espèces paléarctiques connues du genre *Ceriana* sont dépourvues d'un tel ornement. Sternites noirs, plus luisants que les tergites; à bandes jaunes, correspondantes à celles des tergites; celle du sternite IV plus étroite. Sternite II à pilosité blanchâtre; sternites III et IV, à pilosité plus courte, blanche jaunâtre, avec des réflexes dorés.

FEMELLE

Fort semblable au mâle. Différences : la tâche jaune centrale du front, interrompue au milieu, présente des extension latérales plus développées ; les deux autres taches latérales jaunes ont une forme presque triangulaire. La bande transversale jaune-orange de l'occiput, plus large. Tergites II et IV un peu plus courts que chez le mâle. Sur les sternites, seulement la bande jaune transversale du sternite V est plus large ; à la partie terminale du sternite V, des poils noirs, un peu plus longs que ceux de couleur claire.

Pour une différenciation plus évidente, nous faisons suivre en parallèle la diagnose des deux espèces :

Ceriana worelli sp. n.	*Ceriana binominata* (Verrall, 1901)
— 2e article antennaire 4/5 de la longueur du 3e.	— 2e article antennaire 1/2 de la longueur du 3e.
— f 3 à coloration jaune égale vers les deux extrémités (par 1/3 de la longueur du f 3).	— f 3 moins coloré de jaune à la partie basale qu'à celle apicale.
— Tergite IV avec deux bandes filiformes (d'environ 0,3 mm) fort courbées, blanchâtres argentées.	— Tergite IV dépourvu de pareilles bandes.
— Longueur : 13,00—13,50 mm	— Longueur : 10 mm

La nouvelle espèce s'intègre dans la clef indiquée par P. Sack, comme suit :

12. 2e et 3e articles antennaires de longueur à peu près égale. Bande faciale médiane, noire, en forme de fuseau. . . . **C. subsessilis** Illig.

— 2e et 3e articles antennaires évidemment inégaux. Bande faciale médiane, noire, tridentée à la partie supérieure. Le dessin jaune de la partie faciale gauche représente la forme du chiffre 7. . . **13**

13. 2e article antennaire à 1/2 de la longueur du 3e.
f 3 moins coloré de jaune à la partie basale qu'à celle apicale. Tergite IV sans dessins blanchâtres argentés. Taille petite (10 mm) . . . . . **C. binominata** Verr.

— 2e article antennaire à 4/5 de la longueur du 3e.
f 3 également coloré de jaune à toutes les deux extrémités (par 1/3 de la longueur totale de f 3). Tergite IV avec deux bandes à toment, formant des dessins blanchâtres argentés. Espèce plus robuste (13,00—13,50 mm) . . . **C. worelli** Brădescu

Les types sont déposés au Muséum d'Histoire Naturelle « Gr. Antipa » de Bucarest. Holotype : 1 ♂, capturé à Sibiu (la colline Guşteriţa), le 9.V.1946. Allotype : 1 ♀, Sibiu (la colline Guşteriţa), le 24.V.1934. Paratype : 1 ♀, Sibiu (la colline Guşteriţa), le 24.V.1934. Leg. E. Worell.

Nous remercions à cette occasion une fois de plus le Dr. M. Băcescu, directeur du Muséum d'Histoire Naturelle « Gr. Antipa » de Bucarest, pour m'avoir offert la possibilité d'étudier le matériel des collections du Muséum. Également, nous accomplissons un devoir, en remerciant le Prof. M. Ieniştea pour le large esprit de collaboration scientifique en ce qui concerne les divers problèmes de spécialité.

BIBLIOGRAPHIE

1. Drensky P., Mitt. bulg. Ent. Ges., 1934, **8**, 109—131.
2. Glumac S., Zbornik za prirodne nauke, 1958, **17**, 37—78.
3. Kowarz F., Verh. der k.-k. zool.-bot. Ges. in Wien, 1873, **23**, 453—464.
4. Loew H., Ber. ent. Zeit., 1873, **17**, 33—52.
5. Sack P., *Syrphidae*, in Lindner — *Die Fliegen der Paläarktischen Region*, Stuttgart, 1932, *31*, 1—451.
6. Séguy E., Mém. Mus. Nat. Hist. Nat., Série A, Zool., 1961, **23**, 1—248.
7. Verrall G. H., *British Flies*, London, 1901, **8**, 1—691.

Reçu le 10 mars 1972

Entreprise géologique de prospections

THE STATUS OF SOME NOMINAL SPECIES OF CULTRINAE AND XENOCYPRIDINAE (PISCES, CYPRINIDAE)

BY

PETRU BĂNĂRESCU

The status of seven nominal species of Cyprinidae is analyzed. *Leptocephalus mongolicus* Basilewsky is the common species known as *Erythroculter mongolicus* and wrongly identified with *Culter mongolicus*. The generic name *Erythroculter*, although younger than *Chanodichthys*, must be retained as nomen conservandum. *Culter mongolicus* Basilewsky, *Leuciscus recurviceps* Richardson and *L. machaeroides* Richardson are unidentifiable. *Culter flavipinnis* is the southern subspecies of *Erythroculter illishaeformis*, *flavipinnis* having priority over *recurvirostris*. *Hemiculter kweichowensis* is probably a subspecies of *H. dispar*. *Leuciscus xanthurus* is the same species as *Xenocypris argentea*; the last name must be retained.

Valuable revisional papers were published in recent years on genera and species of Cultrinae and Xenocypridinae, mainly by Chinese ichthyologists [22—24]; I too contributed with several papers [1—4]. Yet there are still some specific names whose status remained obscure. I try here to clarify a few of them.

1. **Leptocephalus mongolicus** Basilewsky, 1855 (Fig. 1)

Basilewsky [5] gives an adequate illustration on Table IV, figure 2 of this nominal species, which is reproduced here (Fig. 1). This is very suggestive for the rather common East Asian fish recorded by many authors as *Erythroculter mongolicus* but identified, first by Berg [6], then by his followers, not with *Leptocephalus mongolicus* Basilewsky, but

with *Culter mongolicus* of the same author. The fish illustrated by Basilewsky has a wide, oblique mouth, like *E. mongolicus*, about 78 scales in lateral line (*E. mongolicus* has 73—79); one can count only some 15 branched anal rays, but in the description, Basilewsky mentions a total amount of 22 rays (e. g. 19 or 20 branched); *E. mongolicus* has 19—21, as against 22—31, rarely 21 in the other species within the genus *Erythroculter*.

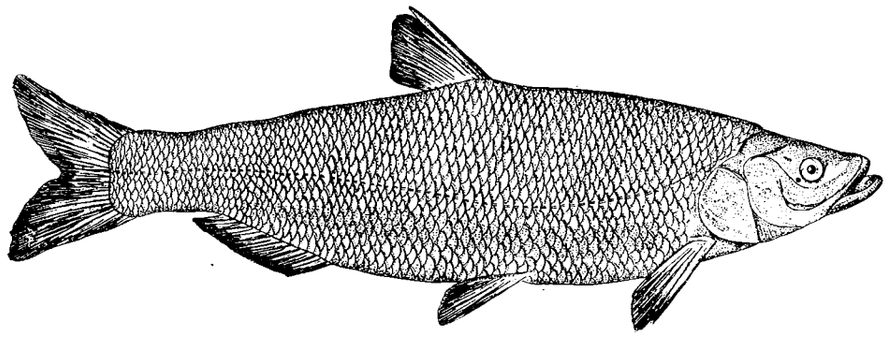


Fig. 1. — *Leptocephalus mongolicus* Basilewsky. Redrawn after Basilewsky.

This nominal species became the type of the genus *Chandichthys* Bleeker, 1859. Both Chu [11] and I [1] showed that the species described by Bleeker [9] as *Chanodichthys mongolicus* and identified by him with Basilewsky's *Leptocephalus m.* actually is *Erythroculter m.* of Berg and subsequent authors. Yet one could still have doubts on the identify of Blekeer's and Basilewsky's species. The examination of the illustration proves it is the same species.

In describing *L. mongolicus*, Basilewsky mentions, on p. 232, "*vesica aerea 3-loba*"; the air bladder divided into three chambers is an important generic character for *Erythroculter*. Yet in describing *Chanodichthys m.*, Bleeker [9] mentions: "*venter plano, post pinnas ventrales non carinato; vesica natatoria bipartita*". He was wrong; probably the specimen was stuffed and the post-ventral keel could not be recognized and the third, smallest chamber of the air-bladder may have been overlooked.

In defining the genus *Chanodichthys*, Berg [8] mentions: some 80 scales, abdomen rounded (e. g. also behind pelvics), air-bladder bipartite. He identified as *Ch. mongolicus* one Amur drainage specimen now apparently lost (I could not find it in the Leningrad Museum in July 1967) with 81 scales and a rounded abdomen. This specimen probably was a hybrid, not identical with Basilewsky's and Bleeker's species. Nikolski [15] didn't find in the Amur any specimen corresponding to Berg's so called *Ch. mongolicus*.

Leptocephalus mongolicus being the generotype of *Chanodichthys* Bleeker, 1855, this genus has priority over *Erythroculter* Berg, 1909, the species *L. mongolicus* and *Culter illishaeformis* (*C. erythropterus* sensu Berg) being congeneric. Yet the only author who realized this synonymy

is Chu [11] who used the named *Chanodichthys* for the species usually ascribed to *Erythroculter*. All other authors who used this either did it in a very broad sense (e. g. Günther [12]) or included in it mainly species of *Megalobrama*, or adopted Berg's above mentioned point of view. The generic name *Erythroculter* is in general use since more than 35 years for species from China, Korea, and Soviet Far East, many of which are economically valuable; its replacement by *Chanodichthys* would led thus to much confusion. I propose to retain *Erythroculter* as *nomen conservandum*.

2. **Culter mongolicus** Basilewsky, 1855

Basilewsky gives a very short and vague description of this species ("*Corpus latius praecedentibus, rectum, squamis mediocribus grisescentibus vestitum; dorso arcuato; abdomine carinato. Pinnae griseae. Linea lateralis arcuata, media*") and no illustration at all. After this description, it is not possible to identify the species; yet all authors, since 1909, identified it with the species usually called *E. mongolicus*, which actually is, as shown above, Basilewsky's *Leptocephalus m.* This regretable confusion is due to Berg [6]. Even if accepting that Basilewky's *Leptocephalus m.* and *Culter m.* are the same species, the first name has page priority (231 over 237) and must be retained.

3. **Leuciscus recurviceps** Richardson, 1846

This nominal species is based on the drawing of a fish by a Chinese artist, brought to the British Museum by Reeves. According to Berg's 1934 paper [7] this fish is unidentifiable; Rendahl [16] identified it with *Culter alburnus*.

Tchang [19] used the name *Culter recurviceps* for *E. dabryi*; Lin [13] considered *recurviceps* a valid species of *Culter*, subgenus *Erythroculter*, characterized by 66—68 scales and 23—24 branched anal rays, while *dabryi* (listed by him without having examined any specimen) is accepted as a species of *Culter* s. str., with L. lat. 65—73, A 2/25—29. H. W. Wu [21] records as *C. recurviceps* two specimens with A 2/25—26, L. lat. 65—69; these values, as well as those mentioned by Lin for *recurviceps*, are typical for *E. dabryi* (= *oxycephalus*) characterized by A 2/25—28, rarely 23, L. lat. 64—71.

Nichols [14] too considers *recurviceps* a possible synonyme of *dabryi*, while L. Yih & C. K. Wu (in H. W. Wu & oth., 22) formally synonymize *recurviceps* with *dabryi*, although they use the last-quoted name, while the first one, if valid, has priority.

Whitehead recently published [20] a very good illustration of the drawing by a Chinese artist after which *L. recurviceps* was described. One recognizes a Cultrine fish with almost vertical mouth, 29 branched anal rays, some 64—66 scales in lateral line and a denivelation at the nape. After the rays and scales count, this fish may be either *Culter alburnus* (A 2/25—29; L. lat. 63—72), *Erythroculter dabryi* (A 2/(23) 25—29; L. lat.

65—71) or *E. illishaeformis flavipinnis* (= *E. i. recurvirostris*) (A 2/24—26; L. lat. 68—75). The shape of the almost vertical mouth suggests rather *E. illishaeformis*. It seems thus more probable that this fish is the same as *E. illishaeformis flavipinnis* (Tirant, 1883). The name *recurviceps* has priority not only over *recurvirostris*, but even over *illishaeformis* (Bleeker, 1871) and over *dabryi* (Bleeker, 1871) as well. But the identity of *L. recurviceps* is far from being sure, there is no holotype of it and my opinion is that it must be rejected as a *nomen dubium*.

4. **Leuciscus machaeroides** Richardson, 1846

This nominal species was almost generally overlooked by students on the fish fauna of East Asia. Rendahl [16] mentions briefly that this and other specific names proposed by Richardson after painting must be "am besten völlig der Vergessenheit überlassen"; neither Nichols [14] nor B. L. Yih&C. K. Wu (in H. W. Wu&oth., 22) mention this name.

An adequate reproduction of the painting, brought by Reeves to the British Museum, after which *L. machaeroides* was described, was recently published by Whitehead [20]. One recognizes an elongate Cultrine fish, with short anal fin (11 branched rays), pointed head and snout and strongly recurved lateral line, with about 52 scales. This illustration is very suggestive for *Hemiculter leucisculus* (Basilewsky, 1855); but there is no spine in the dorsal fin, whose last simple ray, is, according to the illustration, slender and flexible. The dorsal spine is very clearly indicated in other painting from the same set (e. g. *Abramis terminalis*, *Leuciscus recurviceps*, *L. xanthurus*), one cannot thus say it was overlooked by the artist. The presence of a slender, not spinified, last simple dorsal ray demonstrate the fish is the same as *Hemiculterella sauvagei* Warpachowski, 1887. The name *machaeroides* has, evidently priority over *sauvagei*. Yet the identification of the firstnamed being not sure, while the second is based on still existing hoholtype, *H. sauvagei* must be retained.

5. **Culter flavipinnis** Tirant, 1883

Unlike the preceeding nominal species, this one is based on a holotype, still existing in the Lyon Museum, which was reexamined by Chevey [10]. This author concluded *C. flavipinnis* is the same was *Erythroculter pseudobrevicauda* Nichols&Pope, 1927; Tirant's name has priority and must be retained, but the species belongs to *Erythroculter*. In my revision of this genus [1] I overlooked the name *flavipinnis*. It was demonstrated in that paper that *E. pseudobrevicauda* is a synonym of *C. recurvirostris* Sauvage 1884, this one being a subspecies of *Erythroculter illishaeformis*. Chevey [10] showed *flavipinnis* is the same fish. According to the priority law, the right name of the southern (North Vietnam and Hainan) subspecies of the North and Central Chinese *Erythroculter illishaeformis* is thus: *Er. illishaeformis flavipinnis* (Tirant, 1883)

6. **Hemiculter kweichowensis** Tang, 1942

This species too was described after fish specimens and according to modern criteria. Yet it was totally overlooked in the reviews and revisions of the genus *Hemiculter* [3] [22] [23].

According to its description [18], this fish has the keel confined to the ventral part of the abdomen, as in *H. dispar*; it differs from the subspecies of the last named in having 60 scales in the lateral line (as against 46—58) and 12 branched anal rays (as against 13—15 in *H. d. hunanensis* and *H. d. hainanensis*, about 17 in *H. d. dispar*). Tang (18) mentions also a larger eye than in *H. dispar*: eye diameter 28.9% of head, 7.4% of standard length and 98% of interorbital width, (at a st. length of 110 mm). I found even higher values of the eye diameter in *H. d. hunanensis* from the Minkiang drainage in Fukien.

This nominal species was described after a single specimen from Kweiyang, Kweichow province, on a southern tributary to the Yangtze. It seems to be a fourth subspecies of *Hemiculter dispar*.

7. **Leuciscus xanthurus** Richardson, 1846

This fish was described after a Chinese painting from Reeves collection; this painting was reproduced by Whitehead [20]. It is evidently a *Xenocypris*, with about 56 scales in lateral line. The only species having this low number of scales is *X. argentea* Günther, 1868. I consider both species as identical, but the last name *argentea*, although younger, must be retained, as far as it received general acceptance and is based on a holotype in the British Museum.

One name proposed by Richardson after a fish-painting can be identified without doubt and received general acceptance: *Abramis terminalis*, ascribed at present to *Megalobrama*.

Acknowledgements. M^me^ le Prof. N.-L. Bauchot, Paris, and Drs M. Boeseman, Leiden, P.H. Greenwood, London and P.J. Whitehead, London, sent xeroscopies of not available papers; P.J. Whitehead sent informations about Reeves collection of Chinese fish drawings.

REFERENCES

1. BĂNĂRESCU P. Rev. roum. Biol., Zool., 1967, *12*, 4, 215—227.
2. — Rev. roum. Biol., Zool., 1967, *12*, 5, 297—308.
3. — Trav. Muséum Hist. Nat." Gr. Antipa", 1968, *8*, 523—529.
4. — Rev. roum. Biol., Zool., 1971, *16*, 1, 9—19.
5. BASILEWSKY S. Nouv. Mém. Sov. Nat. Moscou, 1855, *10*, 215—263.
6. BERG S. L. *Ryby basseina Amura*. Zap. Akad. Nauk., *24*, 9, VII + 270 p., St. Petersbourg, 1909.
7. — C. R. Acad. Sci. U.R.S.S. (N.S.), 1934, *2*, 264—266.
8. — Ryby presnych vod S.S.S.R., *2*. Izd. Akad. Nauk, Moskva—Leningrad, 1948.

9 Bleeker P. Verhandel. Akad. Wetensch. Amsterdam, Add. Natuurk., 1871, *12*, 1–91, 13 pls.
10. Chevey P. Bull. Mus. nat. Hist. Nat., Paris, 1937, (2), *9*, 4, 247–251.
11. Chu Y. T. Biol. Bull. St. John's Univ., Shanghai, 1935, *2*, 1–225.
12. Günther A. *Catalogue of Fishes in the British Museum*, 7. London, 1868.
13. Lin S. Y. Lingnan Sci. J., Canton, 1934, *13*, 4, 615–632.
14. Nichols J. T. *The Fresh-water Fishes of China*. New York, 1943.
15. Nikolski G. V. *Ryby basseina Amura*. Moscow, 1956.
16. Rendahl H. Ark. f. Zool., 1928, *20 A*, 1, 1–190.
17. — Ark. f. Zool., 1932, *24 A*, 16, 1–134.
18. Tang D. S. Lingnan Sci. J., Canton, 1942, *20*, 2–4, 147–166.
19. Tchang Ti-L. *Contribution à l'étude morphologique, biologique et taxinomique des Cyprinidés du Bassin du Yangtze*. Thèses, Paris 1930.
20. Whitehead P. J. Bull. Brit. Mus. (Nat. Hist), Histor. Series, 1969, *3*, 7, 193–234, 29 plts.
21. Wu H. W. Sinensia, 1939, *10*, 1–6, 92–142.
22. Wu H. W.&oth. *The Cyprinide Fishes of China*, 1. Technical Printing House, Shanghai, 1964 (in Chinese).
23. Wu Tz. Tz., Yih, B. L. Acta Hydrobiol. Sinica, 1959, *2*, 157–169 (in Chinese).
24. Yin B. L., Chu Tz. J. Acta Hydrobiol. Sinica, 1959, *2*, 170–199 (in Chinese).

Received April 13, 1972

The "Traian Săvulescu" Institute of Biology

Department of Animal Systematics and Evolution.

COMPARATIVE STUDIES ON THE WEBERIAN APPARATUS IN THE SUBFAMILY DANIONINAE AND CULTRINAE (CYPRINIDAE-PISCES)

BY

CONSTANTINA SORESCU

The author makes a comparison of the Weberian ossicles and the first four vertebrae of *Danioninae* and *Cultrinae* in order to establish their phyletic relationship. Within the last-named subfamily, one can distinguish primitive species, whose Weberian apparatus and the first four vertebrae bear some similarity with that of *Danioninae* (their presumed ancestors), specialized and evolved species. The author comes to the conclusion that the *Danioninae* are primitive, from the *descending Cultrinae*.

The comparative study of the Weberian apparatus in some Cyprinidae (subfam. Leuciscinae, Xenocypridinae, Abramidinae) with a view to establishing some phylogenetic relationships, as well as the history of these researches, was the object of a previous paper [17].

The assertions of the above-mentioned work, on the phylogenetic value of the Weberian apparatus, are supported also by subsequent researches on the Weberian apparatus in the subfam. Danioninae and Cultrinae. The representatives of these subfamilies people the waters of China, Japan, India, Singapore and Europe and, in our opinion, their comparative study could lead us to some accurate conclusions regarding their phylogeny. Asiatic species were obtained from the collection of the "Tr. Săvulescu" Institute of Biology, by courtesy of Dr. P. Bănărescu, to whom thanks are due.

METHOD OF WORK

Skeletal samples were obtained by transparent preparation and maceration of the soft tissue in KOH 4%, staining of the Weberian ossicles with alizarine and mounting in gelatin glycerin (according to R. Tilak's method). The preparation of the spinal column was carried out by routine skeletization.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, N° 6, p. 391–398, BUCAREST, 1972

The studied species were: *Osteichthyes-Pisces*; Subfam. *Danioninae*: *Squaliobarbus curriculus, Zacco temmincki, Zacco platypus, Zacco pachycephalus, Ochetobius elongatus, Aspidoparia morar*; Subfam. *Cultrinae*: *Rasborinus lineatus altior, Chalcalburnus chalcoides mento, Alburnus alburnus, Hemiculter bleeckeri, Erythroculter illishaeformis, Culter alburnus, Pelecus cultratus, Pseudoxygaster gora, Parabramis pekinensis, Pseudolaubuca sinensis, Paralaubuca typus, Ischikauia steenackeri, Salmostoma baccaila.*

The comparative study of the 20 species, so different as concerns body shape, size, way of life and environment enabled me to establish some characteristics of the Weberian apparatus and of the great vertebra, specific of each subfamily (Table 1), and to use them as phyletic indices. Out of the skeletal specimens studied, only the scaphium, tripus and great vertebra evince variations among subfamilies.

In some species, the Weberian apparatus and the great vertebra evince an intermedary form between the two subfamilies under discussion; these species were considered links which prove the descendence of one family from the other.

Subfam. **DANIONINAE**

The same as for the subfam. Leuciscinae [17], the Weberian apparatus and the great vetebra of Danioninae are primitive. Weberian ossicles have a rough massive aspect. The scaphium has a thick wall; its ascending process, well developed, has an auricular shape, with rounded edges; the articular process of the scaphium, as in Leuciscinae, is formed only of a single ramus. We consider this a primitivity feature, as the appearance of the second ramus in the other subfamilies increases the articulation area of the ossicle in the body of the first vertebra, which results in a better fixation of the whole apparatus and, implicitly, in a more accurate functioning.

The intercalarium keeps the characteristic shape of the whole fam. Cyprinidae. It is long, with a horizontal position and articulated to the great vertebra by an articular process. The two processes of the intercalaria (articular and ascending) are equal.

The tripus is the strongest Weberian ossicle. In Danioninae it is long, with a strongly curved external side throughout its length. The transformatory process prolonges the curve of the external side of the ossicle, resulting in a solid line (Fig. 1). In our opinion, this character also demonstrates the primitive aspect of Danioninae, since in the other subfamilies the aspect of the tripus change, viz. the external curve becomes slighter and the curving angle of the transformatory process is emphasized. This change seems to permit a better functioning of the apparatus as a whole. The cranial extremity of the tripus is long (Fig. 1). The articular process of the tripus in Danioninae, the same as in Leuciscinae [17] is well developed, long and wide, placed towards the centre of the osssicle. The articulation surface of the adductor muscle of the tripus is concave and very large (Fig. 1).

The first four vertebrae which accompany Weberian ossicles vary in correlation with the modification of the latter. We shall use the abbreviation V_1, V_2, V_3, V_4 for vertebrae and the term *the great vertebra* when referring to all four.

V_1 in Danioninae has no variations in shape. It has the shape of a plane-concave disk, with slightly developed dorsal ribs, the same as in all primitive Cyprinidae with unfused vertebrae.

V_2, amphicelous, is not fused to V_3. This character, in addition to the others, underlines the primitive character of Danioninae. Dorsal ribs are well developed.

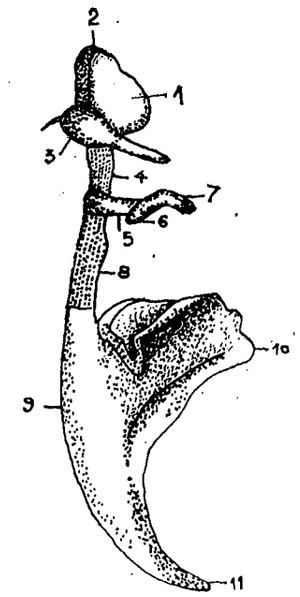


Fig. 1. — Weberian apparatus in *Zacco pachycephalus*. *1*, scaphium; *2*, ascending process of scaphium; *3*, articular process of scaphium; *4*, ligament of scaphium; *5*, intercalarium; *6*, ascending process of intercalarium; *7*, articulary process of intercalarium; *8*, ligament of tripus; *9*, tripus; *10*, articulary process of tripus; *11*, transformatory process of tripus.

V_3, amphicelous, not fused to V_2, has no ribs. It has the shape of a small neel with a cavity in the middle. In Danioninae this cavity is superficial and has a large opening, as this is the articulation site of the articular process of the tripus, which is wide and long (Plate I, *A*).

V_4, amphicelous, has also pleural ribs, in addition to the well developed dorsal ribs. The same as in all primitive Cyprinidae, the articulation end of the dorsal ribs to the vertebra is very wide and prolonged antero-laterally with a sharp excrescence (Plate I, *A*) and *ossa suspensoria*, resulting from the medio-ventral fusion of pleural ribs, is posteriorly oriented (Plate I, *B*).

Thus, in the subfam. *Danioninae*, the primitivity of Weberian apparatus and of the great vertebra is demonstrated by the following characters: the articular process of the scaphium has a single ramification; the external side of the tripus is strongly curved throughout its length; the cranial extremity and the articular process of the tripus are long; V_2 and V_3 are not fused; *ossa suspensoria* is posteriorly oriented.

Subfam. CULTRINAE

The study of the Weberian apparatus and of the great vertebra in East and South-Asiatic Cultrinae, the same as in European ones, permitted us to distinguish within this subfamily some primitive species, some evolved ones and others adapted to the pelagic life.

Out of studied Cultrinae the following species were considered as primitive : *Rasborinus lineatus*, *Chalcalburnus chalcoides*, *Alburnus*

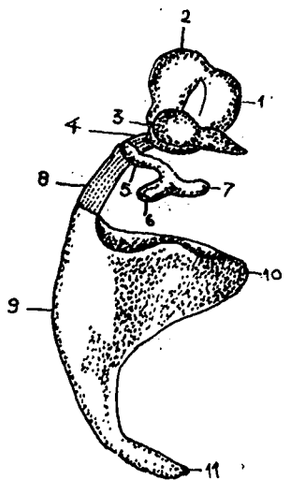


Fig. 2. — Weberian apparatus in *Rasborinus lineatus altior*. Same legend as in fig. 1.

alburnus, *Hemiculter*, *Erythroculter illishaeformis*, *Culter alburnus*. *Rasborinus lineatus* is placed at the limit between Danioninae and Cultrinae, by the characters of the Weberian apparatus and of the great vertebra. In specialized, pelagic Cultrinae (*Pseudoxygaster gora* and *Pelecus cultratus*) the Weberian apparatus resembles in many respects that of evolved Cultrinae (*Parabramis pekinensis*, *Pseudolaubuca sinensis*, *Paralaubuca typus*, *Ischikauia steenackeri*, *Salmostoma baccaila*).

In *Rasborinus lineatus*, the Weberian apparatus is greatly similar to that of Danioninae, having a single character of Cultrinae (the shape of the transformatory process of the tripus). As in Danioninae, the articular process of the scaphium has a single ramus, the external side of the tripus is curved throughout its lentgh and the articular process of the tripus is wide and long (Fig. 2). Only the transformatory process of the tripus is the same as for the other Cultrinae : short, thin and sharply curved, forming an obtuse angle with the rest of the ossicle (Fig. 2). The great vertebra of *Rasborinus lineatus* also has *Danionina* features : V_2 not fused to V_3, cavity for the articular process of the tripus at the level of V_3 superficial and *ossa suspensoria* oriented posteriorly. Due to this similarity of the Weberian apparatus and of the great vertebra in *Rasborinus* and *Danioninae*, the same as of the similarity of their cranium [18], it may be considered that *Rasborinus lineatus* is a primitive *Cultrina*, which proves the descendance of Cultrinae from Danioninae.

In primitive Cultrinae (of the studied species), there start appearing some superiority features of the Weberian apparatus and of the great vertebra. Thus the second ramus of the articular process of the scaphium is also formed but it remains still slightly developed. The external side of the tripus becomes rectilinear, as this ossicle is curved only at the level of the transformatory process, which is short, thin and sharply curved, forming an obtuse angle with the tripus (Fig. 3). V_1, because of the fusion of V_2 with V_3, becomes a biplane disk, and the articulation ca-

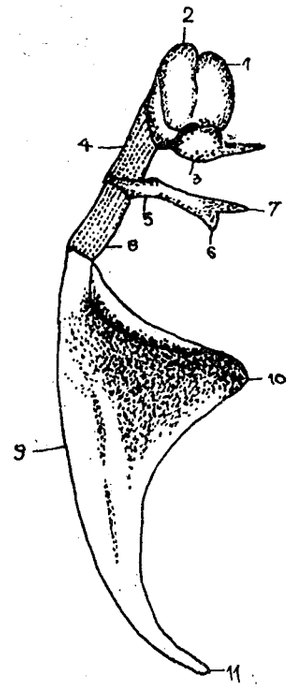


Fig. 3. — Weberian apparatus in *Erythroculter illishaeformis*. Same legend as in fig. 1.

vity of the tripus in V_3 becomes deeper (Plate I, C). Out of primitivity characters encountered in Danioninae, the shape and size of the articular process of the tripus (Fig. 3) is also maintained in primitive Cultrinae (long, wide, placed to the centre of the ossicle), the same as the conformation of V_4 (presence of anterior excrescens, *ossa suspensoria* posteriorly oriented). Evolved Cultrinae, the same as specialized ones, present the Weberian apparatus and the great vertebra modified in the same direction. The new characters point out their superiority. It is worth noticing the appearance and development of the second ramus of the articular process of the scaphium. The external side of the tripus is no longer curved throughout its length, but only at the ends. It is rectilinear on the median line. The articular process of the tripus, though wide, is shortened, which enables a better articulation in cavity V_3 which becomes deeper. The transformatory process of the tripus has a typical Cultrina

conformation : short, thin, sharply curved, forming an obtuse angle with the rest of the ossicle (Figs 4 and 5). V_1 becomes a biplane disk, as V_2 and V_3 got fused, V_4 is also modified. The anterior excrescence of dorsal ribs is reduced (Plate II, *D*) and *ossa suspensoria* is vertically oriented (Plate II, *E* and *F*).

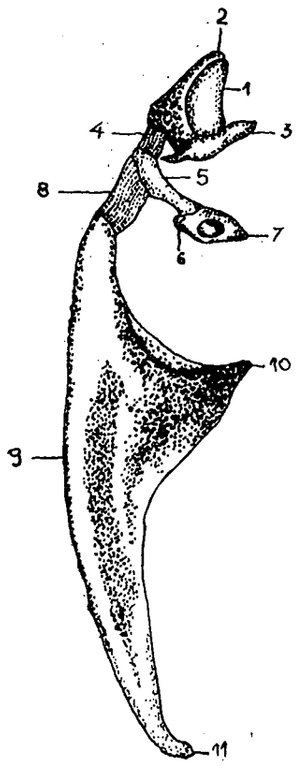


Fig. 4. — Weberian apparatus in *Pelecus cultratus*. Same legend as in fig. 1.

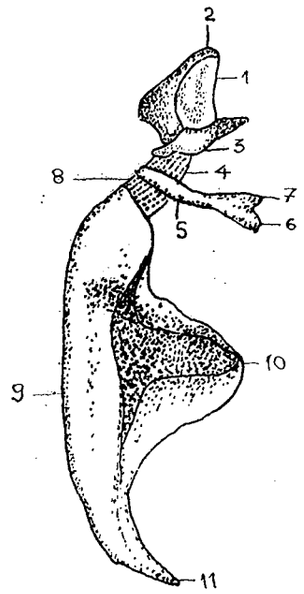


Fig. 5. — Weberian apparatus in *Ischikauia steenackeri*. Same legend as in fig. 1.

Table 1 presents synoptically the characters of the Weberian apparatus and of the great vertebra in Danioninae and in the three groups of Cultrinae.

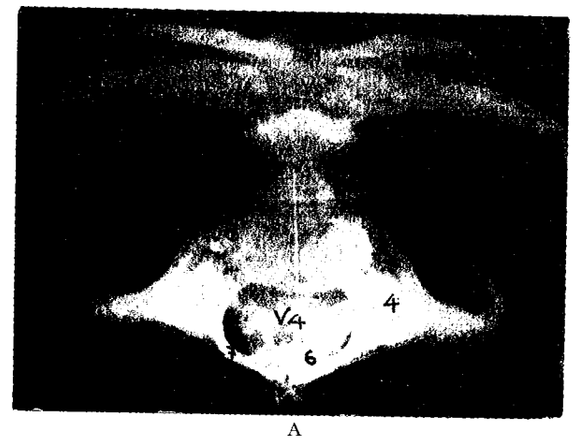
CONCLUSIONS

1. The comparative study of Weberian apparatus and of the great vertebra in 20 species of Danioninae and Cultrinae of East and South Asia and of Europe, enables us to establish that the variations of these skeletal pieces, which remain constant within the subfamily, irrespective of the way of life and environment, may be used as phylogenetic indices.

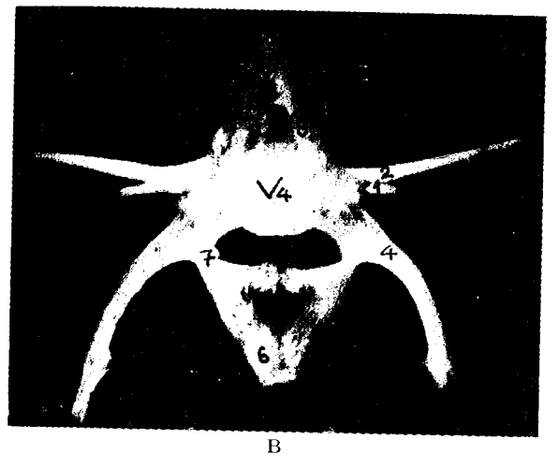
Plate I. — **A**, Great vertebra of *Squaliobarbus curriculus* (ventrally seen). *1*, dorsal rib of V_1 ; *2*, dorsal rib of V_2 ; *3*, cavity for articular process of the tripus ; *4*, dorsal rib of V_4 ; *5*, anterior excrescence of dorsal rib ; *6*, *ossa suspensoria* ; *7*, hemal arc ; *8*, neural arc ; *9*, neural spina of V_4.

B, Vertebra IV in *Squaliobarbus curriculus* (posterior aspect).

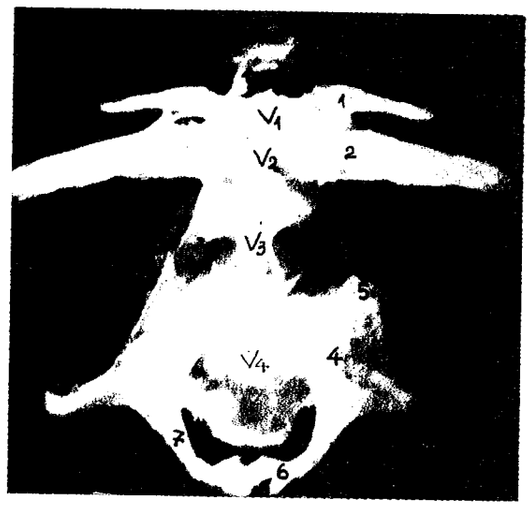
C. Great vertebra of *Chalcalburnus chalcoides mento* (ventral view).



A

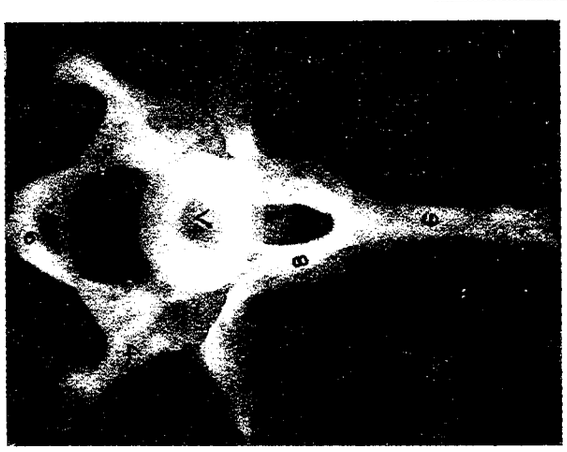


B



C

D



E

F

Plate II. — **D**, Great vertebra in *Peleeus cultratus* (ventral view). **E**, Vertebra IV in *Peleeus cultratus* (posterior aspect). **F**, Vertebra IV in *Pseudolaubuca sinensis* (posterior aspect).

Table 1

Variations of Weberian ossicles and of the great vertebra

Weberian ossicles and the great vertebra		Danioninae	Cultrinae			
			Rasborinus lineatus	Primitive	Evolved	Specialized
Scaphium	Ascending process	Auricular	Auricular	Triangular	Triangular	Triangular
	Aricular process	With 1 ramus	With 1 ramus	With 2 unequal ramus	With 2 equal ramus	With 2 equal ramus
Intercalarium	Shape	Long, wide, with well developed and equal ascending and articulary processes				
Tripus	External side	Strongly curved throughout its length		Medially right, curved at the level of the transformatory process		Medially right, curved at the level of the cranial extremity and of the transformatory process
	Cranial extremity	long				
	Articular process	Well developed, wide and long, placed to the middle of the bone, strongly concave surface for adductor muscle				Well developed, wide yet shorter
	Transformatory process	Long, thick, strongly curved	Short, thin, sharply curved, forming an obtuse angle with rest of the tripus			
Vertebra I		Plane-concave disk with a pair of slightly developed dorsal ribs			Biplane disk, with a pair of well developed dorsal ribs	
Vertebra II		Amphicelous, not fused to V_3, with a pair of strong dorsal ribs			Amphicelous fused with V_3, with a pair of strong dorsal ribs	
Vertebra III		Amphicelous ; long, not fused to V_2 ; superficial cavity of articular process of the tripus			Amphicelous ; fused with V_2, its body remains long in correlation with the long cranial extremity of the tripus ; deepened cavity for the articular process of the tripus	
Vertebra IIII		Amphicelous, dorsal ribs with very wide, articulation end and sharp anterior excrescence ; *ossa suspensoria* triangular, posteriorly oriented				Amphicelous, dorsal ribs with very wide articulation end, reduced anterior excrescence ; vertically oriented *ossa suspensoria*

2. Primitivity characters of Weberian apparatus and of the great vertebra, observed in subfam. Danioninae determine us to consider this subfamily as primitive.

3. The melding of primitivity characters of Danioninae and Cultrinae in the Weberian apparatus and in the great vertebra of *Rasborinus lineatus* justifies us to consider it as a link proving the descendance of Cultrinae from Danioninae.

4. The existence of some Danioninae characters in the Weberina apparatus and in the great vertebra of primitive Cultrinae is a new proof of the descendance of Cultrinae from Danioninae.

5. The differentiation of Cultrinae into 3 groups (primitive, specialized and evolved) demonstrates the existence of a parallel evolution also within the subfamily.

6. The resemblance of the Weberian apparatus and of the great vertebra in evolved Cultrinae and in specialized ones (by adaptation to the pelagic life) determines us to consider that though the two groups of Cultrinae had a parallel evolution, they derive from a common ancestor, probably a primitive Cultrina.

REFERENCES

1. Alexander R. Mc. N., Proc. Zool. Soc. London, 1962, **139**, *3*, 451–473.
2. Băcescu-Meşter Lotus, Trav. Mus. Hist. Nat., 1965, **5**, 247–259.
3. Bănărescu P., *Pisces, Osteichythes* in Fauna R.P.R., Edit. Acad. R.P.R., Bucureşti, 1964, **13**.
4. Beccari N., *Anatomia comparata dei vertebrati*, Firenze, 1955.
5. Beer De G. R., *The development of the Vertebrate skull*, Clarendon Press, Oxford, 1937.
6. Chranilov N. S., Jahrb. Anat., 1927, **49**, 501.
7. Goodrich E. S., *Studies on the structure and development of Vertebrates*, London, 1930.
8. Gregory W. K., Trans. Amer. Phil. Soc., 1933, **22**, 75.
9. Nussbaum J., Anat. Anz., 1908, **32**, 513.
10. Ramaswami L. S., Proc. Nat. Inst. Sci. India, 1952, **18**, *6*, 495–536.
11. — Proc. Nat. Inst. Sci. India, 1953, **19**, *3*, 323–345.
12. — Acta Zool., 1955, **36**, 127–156.
13. — Proc. Zool. Soc. Calcutta, 1957, 294–302.
14. Regan C. T., Ann. Mag. Nat. Hist., 1911, **8**, 13–32.
15. Sagemehl M., Morph. Jahrb., 1891, **17**, 489–595.
16. Sorescu Constantina, St. cerc. biol. Seria Zoologie, 1971, **23**, *4*, 319–329.
17. — Rev. Roum. Biol. Zool., 1970, **15**, *6*, 403–408.
18. Tilak R., Anat. Anz., 1967, **120**, *4*, 350–352.
19. — Zool.-Anz., 1967, **178**, *1–2*, 61–74.

Received March 21, 1972

Faculty of Natural Sciences
Craiova

LES CRITÈRES DE L'ESPÈCE
LE CRITÈRE CYTOGÉNÉTIQUE

PAR

EUGEN V. NICULESCU

The author shows that the cytogenetical criterion cannot be used in taxonomy, since it presents a great intraspecific individual and racial variation and since a normal interspecific variation is observed in the same time with a great interspecific constant; often, an intergeneric variation and an intrageneric one are also found, i. e. just the contrary of a good specific and generic criterion. The author suggest the "new" species erected only basing upon the chromosomal formula cannot display specific validity.

A la suite des recherches effectuées au commencement du XX^{e} siècle par les différents cytologistes, s'est accréditée dans la science l'idée que le nombre des chromosomes est constant à l'intérieur de l'espèce et variable d'une espèce à l'autre. Ainsi on croyait qu'on avait découvert un critère idéal pour l'identification et la délimitation des espèces. Cette idée a persisté longtemps inaltérée.

En 1948, C. Motaş [13] écrivait les lignes suivantes : « *Ces chromosomes dont la forme, dimensions et nombre sont constants au sein de la même espèce, selon les conceptions actuelles génétiques sont les porteurs des facteurs héréditaires ou gènes* ». Même en 1958 on pouvait lire que « *tous les individus d'une même espèce vivante possèdent le même nombre de chromosomes* » [1]. Mais depuis 1958 et jusqu'aujourd'hui se sont accumulés un grand nombre de faits qui nous montrent que cette loi a de nombreuses exceptions et qu'elle ne peut plus être acceptée. On a constaté des variations individuelles et raciales, ainsi qu'une constance interspécifique fréquente — donc le contraire d'un critère idéal. Quoique la liste des exceptions à la loi générale s'accroisse toujours, une nouvelle science s'est toutefois créée — *la cytotaxonomie* — et l'on continue à considérer le critère cytogénétique comme un bon critère taxonomique, les

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, N° 6, p. 399–406, BUCAREST, 1972

travaux sur la cytologie des Rhopalocères ayant comme but « d'*améliorer leur systématique à l'aide d'un nouveau critère* » (H. de Lesse).

Nous allons examiner maintenant les travaux cytologiques les plus récents (H. de Lesse, K. Maeki et C. I. Remington) pour voir quelle est la valeur du critère cytologique et si la systématique des Rhopalocères « s'est améliorée » à l'aide de ce nouveau critère. Pour cela nous examinons la variation chromosomique chez les Rhopalocères.

1. VARIATION INDIVIDUELLE

Un bon caractère d'espèce ne comporte pas des variations individuelles, le caractère spécifique étant constant chez tous les individus de l'espèce. Or, la formule chromosomique présente des variations individuelles chez un grand nombre d'espèces. Ainsi chez *Heliconius doris* L. n = = 24, 25, 26, 27, *Auca nycteropus* Reed n = 7—8, *Steroma andensis* Feld. n = 12—13, *Phyciodes moesta* Salv. n = 32, 33, 34, *Catasticta flisa* Her. Sch. n = 25, 26, 28, *C. reducta* Butl. n = 29, 31, *Mechanitis isthmia veritabilis* Butl. n = 17, 18, *Oeneis chryxus* Doubl. n = 29—30, *Dismorphia psamathe* L. n = 23—24. Chez certains spécimens de *Lysandra amandus* Schn. de Maroc n = 22, chez d'autres n = 23 (en France n = 24); *L. argester* Bergstr. n = 147, 148. Très variées sont les formules chromosomiques aussi chez les *Agrodiaetus* : *A. demavendi* Pfeiffer n = 67—74 et 76, *A. alcestis* Zerny n = 19—22, *A. interjectus* de Lesse n = 29—32, *A. admetus* Esp. n = 77, 78, 79—80, *A. cyanea* Stgr. n = 16, 17, 18, 19, 20, *A. carmon* Her. Sch. n = 81, 82, *A. poseidon* L. n = 18—27, *A. transcaspica* Stgr. n = 19—53. Les espèces du genre *Agrodiaetus* « *sont affectées très fréquemment d'une variation exceptionnelle de leurs formules chromosomiques chez une même espèce* » [7]. Cette variation restreint incontestablement la valeur du caractère cytologique et par conséquent son application en systématique, ce que reconnaît même H. de Lesse [3]. Mais en même temps nous constatons chez *Agrodiaetus damon* Den. et Schiff. un nombre très constant (n = 45) dans toute l'aire de distribution géographique. Donc la variation individuelle est parfois absente, mais souvent elle est très grande et dans ce cas elle diminue la valeur du caractère cytologique. Cette variation « *inconstante* » ne confère donc pas au critère cytologique une valeur taxonomique.

2. VARIATION GÉOGRAPHIQUE

Les populations d'une race et les races d'une espèce ont très souvent des formules chromosomiques différentes. L'amplitude de cette variation est très large et a été constatée chez un grand nombre d'espèces. Ainsi chez *Lysandra coridon* Poda d'Espange et d'Italie (la région centrale des Apennins) n = 87, en France, Angleterre, Suisse n = 88, dans les massifs alpins situés au nord de Venise n = 89, en Yougoslavie n = 90, en Roumanie n = 91 et en Bulgarie n = 92 [10]. Chez *L. bellargus* Rott. n = 45, 46, 47, 48 chez *L. argester* Bergstr. n = 147—151 dans les différentes régions d'Espagne centrale et les Alpes occidentales, en Italie

n = 147—148. Chez *Agrodiaetus cyanea* Stgr. n = 16—22 suivant les races, *Mechanitis menapis menapis* Hew. n = 24, *M. m. mantineus* Hew. n = 20, *Heliconius sapho congener* Weym. n = 33, *H. sapho* ssp. n = 56, *Pareuptychia hesione* Sulz. n = 15, 21, 24, 42—44, suivant les races [9].

3. VARIATION INTERSPÉCIFIQUE

La variation interspécifique de la formule chromosomique revêt des aspects différents. Parfois nous constatons une variation que nous pouvons nommer « *normale* » ou « *régulière* », les espèces se distinguant par une, deux ou plusieurs unités. Ainsi par exemple *Morpho amathonte* Deyr. a n = 27, *M. leontinus* Feld. n = 28; *Doxocopa elis* Feld. n = 31, *D. linda* Feld. n = 32; *Mestra apicalis* Stgr. n = 31, *M. semiflua* Feld. n = 33, etc. [9].

Mais chez beaucoup d'autres espèces les formules chromosomiques diffèrent par un grand nombre d'unités et dans ce cas la variation interspécifique apparaît comme « *irrégulière* », « *discontinue* » en « *sauts* ». Ainsi chez *Dismorphia theucarila* Doubl. n = 18, chez *D. critomedia* Hbn. et *D. nemesis* Latr. n = 31, tandis que chez *D. hipositicta* Feld. n = 48. Deux espèces d'*Euptychioides* sont séparées par 25 unités : *E. vesta* Butl. n = = 25, *E. albofasciata* Hew. n = 50 [9]. La variation irrégulière se voit très bien dans les formules chromosomiques du genre *Charaxes* (H. de Lesse, 1966) : *Ch. laodice* Drury n = 13; *Ch. zelica* Butl. n = 13; *Ch. eupale* Drury n = 17; *Ch. etheocles* Cr. n = 22; *Ch. candiope* God., *Ch. lucretius* Cr., *Ch. numenes* Hew., *Ch. smaragdalis* Butl., *Ch. tiridates* Cr., *Ch. bipunctatus* Rotsch., *Ch. etesippe* God. n = 25; *Ch. nichetes* Grose-Smith., *Ch. anticlea* Drury, *Ch. zingha* Stoll, *Ch. pleione* God., *Ch. subornatus* Schultze, *Ch. kahldeni* Hom. et Hew. n = 26; *Ch. brutus* Cr. n = = 30; *Ch. cynthia* Butl. n = 33; *Ch. protoclea* Feisth. n = 44; *Ch. fulvescens* Auriv. n = 57.

Du tableau ci-dessus on constate que la variation irrégulière est accentuée aussi par le fait qu'un grand nombre d'espèces ont la même formule chromosomique. Par conséquent, au lieu d'une *variation interspécifique*, comme c'est le cas d'un bon critère d'espèce, nous avons, dans ce dernier cas, au contraire, une *constance interspécifique*.

Les espèces possédant un même nombre de chromosomes sont très nombreuses. Voici encore quelques exemples. Chez cinq espèces de *Pedaliodes* n = 29, chez cinq espèces d'*Aldelpha* n = 30, mais la 6ème a n = 27 et la 7ème n = 42, 45. Sept espèces de *Perisama* ont n = 29, mais deux autres espèces ont n = 30. Les espèces des genres *Vanessa*, *Junonia*, *Anartia*, *Hypanartia*, *Metamorpha*, *Siproeta* (*Vanessini*) ont n = 31, toutefois *Anartia amatha* L. a n = 30—31 et 32. Les espèces des genres *Yramea*, *Euptoieta*, *Phyciodes*, *Closyne* (*Argynninae*) ont n = 31, toutefois *Phyciodes mœsta* Salv. a n = 32, 33, 34 et *Ph. margaretha* Hew. a n = = 34—35. Les genres *Dione*, *Dryadula*, *Agraulis*, *Heliconius* (*Eueides*), *Dryas* (*Heliconiinae*) ont n = 31. Le même nombre de chromosomes (n = = 31) se retrouve aussi chez les espèces des genres *Urbanus*, *Astraptes*, *Epargyreus*, *Oechydrus*, *Antigonus*, *Anisochoria*, *Ebrietas*, *Theagenes*, *Erynnis* (*Pyrginae*), toutefois *Achlyodes*, de la même sous-famille, a n =

= 15. *Pyrgus* et *Heliopetes* n = 30. Les genres *Butleria*, *Cynea*, *Hylephila*, *Polites* (*Hesperiinae*) ont n = 29 (H. de Lesse [9]). La même situation se trouve aussi en d'autres familles. Cinq espèces de *Leptophobia* ont n = 26, quatre espèces de *Tatochila* ont n = 28 ; un grand nombre d'espèces de *Colias* ont n = 31, mais chez un spécimen de *C. lesbia* F. n = 32. Le même nombre (n = 31) se trouve aussi chez *Eurema* (13 espèces étudiées par H. de Lesse), mais chez un spécimen d'*Eurema albula* Cr. n = 28 et chez un autre n = 29. On pourrait donner beaucoup d'autres exemples du grand nombre d'espèces examinées par H. de Lesse. Ils nous montrent combien « *irrégulière* » est la variation interspécifique : d'une part *le même nombre de chromosomes chez un grand nombre d'espèces congénériques, d'autre part des formules chromosomiques séparées par une centaine d'unités.* Outre les exemples déjà mentionnés, signalons-en encore deux. Chez *Leptidea sinapis* L. n = 26, *L. morsei* Fenton n = 53 et 54, *L. amurensis* Mén. n = 61 et *L. duponcheli* Stgr., très voisine de *L. sinapis* L., n = 104. Mais l'exemple le plus frappant nous est offert par *Actinote* où deux de ses espèces sont séparées par 136 unités : *A. alcione* Hew. a n = 14, *A. erinome* Feld. n = 150 !

De l'exposé ci-dessus il résulte que le critère cytogénétique ne peut être considéré comme un bon critère d'espèce pour les deux motifs suivants :

1. Il y a une grande *variation intraspécifique* tant individuelle que raciale.

2. La variation interspécifique est parfois régulière et « *normale* », mais souvent elle est irrégulière et en « *sauts* ». Cette variation est d'autant plus inconstante et irrégulière que souvent elle se transforme en une *constance interspécifique*, le même nombre de chromosomes se trouvant chez un grand nombre d'espèces appartenant au même genre ainsi qu'à plusieurs genres de la même sous-famille.

Nous voici arrivés au problème du genre, donc à la *signification systématique supraspécifique du caractère cytologique.*

De l'exposé ci-dessus il résulte déjà que la formule chromosomique ne peut constituer un critère de genre, en d'autres termes les genres ne peuvent être séparés à l'aide de la formule chromosomique. Si tous les genres de la sous-famille des *Pyrginae* ont le même nombre de chromosomes, il va sans dire que le critère cytogénétique ne peut être utilisé dans la sous-famille mentionnée, vu cette *constance intergénérique.* Mais d'autre part nous constatons une *variation intragénérique* qui, également, rend impossible de trouver un caractère de genre. Si toutes les espèces d'un genre avaient un nombre constant, par exemple 30, alors ce nombre pourrait constituer un caractère du genre considéré. Mais si 15 espèces ont n = 30 les 10 autres espèces du genre ont n = 28, 29, 31 , etc. alors le nombre 30 n'est plus un bon caractère générique car celui-ci, on le sait, doit être commun à toutes les espèces composant le genre. Bien plus, un bon caractère de genre doit être propre à un seul genre d'une tribu ou sous-famille. Mais si nous trouvons le nombre 31 chez 9 genres de la même sous-famille (*Pyrginae*) ce n'est plus un bon caractère à l'échelon générique. Au contraire, si les 9 genres avaient n = 28, 29, 30, etc. alors chaque genre a une formule constante qui devient une caractéristique du genre, pouvant être utilisée en taxonomie. Par conséquent le critère cytogénétique présente souvent une *constance intergénérique* et une *variation intragénérique*, c'est-à-dire le contraire d'un bon critère générique.

H. de Lesse [7], en essayant de trouver une signification supraspécifique des formules chromosomiques chez les Rhopalocères, admet que le *nombre modal* [1] de plusieurs genres et même familles est 30. Mais nous ne pouvons guère caractériser les genres par leur formule chromosomique vu la grande variation intragénérique. Ainsi les *Erynnis* néotropicaux ont n = 31, mais parmi les espèces paléarctiques deux *Erynnis* ont seulement n = 30. De même les *Pyrgus* néotropicaux ont n = 30, mais *P. alveus* L. a n = 24, *P. bellieri* Obth. n = 27, *P. carthami* Hbn. n = 29, *P. malvae* L. n = 31. H. de Lesse (1966) considère que n = 24 est un nombre ayant un caractère générique pour le genre *Lycaena*. Ce point de vue ne peut pas être accepté puisque *L. rubidus* Edw. a n = 38 et *L. heteronea* Bsdv. a n = 68 (d'après K. Maeki et C. Remington [11]).

Dans son travail de 1967, H. de Lesse [9] affirme « *qu'un nombre modal de famille, sous-famille, tribu, genre ou sous-genre se dégage la plupart du temps, lorsqu'un nombre suffisant d'espèces a pu être étudié (par exemple 27 et 28 dans le genre* **Tatochila**, *21 pour le sous-genre* **Heliconius**) ». Mais quand la variation intragénérique est trop grande on ne peut pas établir un nombre modal *représentant le caractère du genre.*

Ainsi chez le genre *Lysandra* ont été trouvées des formules chromosomiques différentes, l'amplitude de la variation étant très large : 24, 26, 31, 32, 38, 45, 82, 84, 87, 88, 89, 90, 91, 92, 131—134, —147—151, 190—191 ; de même chez les *Agrodiaetus* ont été trouvés des nombres variant de 10 à 125 [8]. H. de Lesse, en voulant illustrer par des exemples la notion de nombre modal, n'a indiqué que deux genres : *Tatochila* et *Heliconius*. Il n'a pu donner plusieurs exemples puisque la variation est trop grande au sein d'une famille pour trouver un nombre modal. Ainsi par exemple chez les *Hesperiidae*, à côté de genres ayant n = 31, se trouvent d'autres avec n = 15 (*Achylodes*) ou n = 27 (*Alera*). Chez les *Dismorphiinae* il y a des espèces possédant n = 18, 23, 24, 31, 48, 61 et 104 ; quel peut être le nombre modal chez cette sous-famille ? Chez les *Satyridae* également , il est difficile d'établir un nombre modal puisque n = 7, 8, 9, 10, 13, 15, 17, 20, 21, 24, 25, 27, 28, 29, 30, 38, 42, 43, 50 et 60 chromosomes suivant les espèces. Chez les *Nymphalidae* les nombres de chromosomes sont compris entre 13 et 150 ! Par conséquent tant à l'échelon générique qu'à l'échelon familial l'établissement du nombre modal est assez difficile, vu la grande variation intragénérique et intrafamiliale. H. de Lesse [9] a essayé de démontrer que les *Brassolinae* sont des *Satyridae* en utilisant la formule chromosomique de ceux-ci. Nous avons montré dans un autre travail [14] que son argumentation à propos de la position systématique des *Brassolinae* à l'aide de la formule chromosomique n'est pas fondée. « *Nous ne discuterons pas ici la question de savoir si les* **Brassolinae** *sont ou non de* **Satyridae**, *nous voudrions seulement souligner le fait que la formule chromosomique ne peut pas nous suggérer cette position systématique des* **Brassolinae.** *Primo, nous remarquons que cette formule chromosomique a été établie chez deux genres seulement* **Opsiphanes** *et* **Caligo**). *Secundo, nous constatons qu'il y a aussi*

[1] Par nombre modal on entend le nombre de chromosomes le plus fréquent dans un ensemble supraspécifique.

une variation chromosomique au niveau familial (*tous les* **Satyridae** *n'ont pas n = 29*). » Nous avons montré ci-dessus la grande variation au sein de cette famille. « *Si nous voulons établir la position systématique des* **Brassolinae**, *nous devrions en appeler, non pas au critère cytogénétique mais à la nervulation, à l'exosquelette, à l'armure génitale et à la morphologie des chelnilles.* » N'est pas juste non plus l'affirmation de H. de Lesse (1961) selon laquelle il y a « *une nette corrélation entre certaines coupures* (il s'agit du genre *Argynnis* s. l.) *et les résultats cytologiques* ». En ce qui concerne les *Argynnini* sensu Niculescu ainsi que les *Satyrinae* nous ne voyons aucune corrélation.

Ainsi par exemple chez les *Boloria* trois espèces ont n = 30, chez deux autres n = 31 ; chez les *Clossiana* deux espèces ont n = 30, mais chez quatre autres espèces n = 31. *Brenthis ino* Rott. a n = 12, 13, 14, *B. daphne* Den. et Schiff. n = 13, *B. hecate* Den. et Schiff. n = 34. Les « genres » *Fabriciana*, *Mescacidalia* et *Pandoriana* ont n = 29, de même qu'*Argynnis* — mais *A. anadyomene* Feld. a n = 36. Si les genres *Fabriciana*, *Mesoacidalia* et *Pandoriana* avaient un nombre modal différent, cela pourrait constituer un argument pour la validité de ces genres et alors le parallélisme serait évident — si bien entendu les différences morphologiques entre ces genres étaient vraiment de valeur générique. Mais ces genres sont basés « *sur de légères différences des genitalia* » (H. de Lesse) et, d'autre part, le nombre des chromosomes est le même (n = 29). Est-ce qu'on peut affirmer que la formule chromosomique confirme ces coupures génériques? Pas du tout! Les genitalia, et à plus forte raison les données cytologiques, ne confirment pas la validité des genres *Mesocidalia*, *Fabriciana* et *Pandoriana*. C'est pourquoi l'affirmation de H. de Lesse à propos du parallélisme nous surprend. L'argument de H. de Lesse ne soutient pas sa thèse mais la nôtre. Nous avons démontré plusieurs fois l'invalidité de ces trois genres. L'armure génitale ne fournit pas des indices pour une séparation des trois genres et l'examen cytologique non plus.

En sus H. de Lesse [7] affirme que « *dans la sous-famille des Satyrinae, trois grandes révisions génériques basées sur la morphologie des genitalia ont trouvé, dans l'étude cytologique, des confirmations semblables* ». La première révision est celle du genre *Satyrus* (faite par H. de Lesse) où « *on constate tout de même que des nombres différents caractérisent certains des genres créés* ». Par conséquent les bons genres ont des nombres de chromosomes différents !. H. de Lesse justifie son genre *Arethusana* (n = 28) par rapport au genre *Satyrus* (n = 27). Mais en examinant les formules chromosomiques des autres *Satyridae*, nous constatons, toujours d'après H. de Lesse, que les genres *Hipparchia*, et *Pseudotergumia* n'ont pas des nombres différents mais tous les deux ont n = 29 et les *Chazara* et *Pseudochazara* n = 28 (comme *Arethusana*) — mais trois espèces de ce dernier genre montrent n = 27 comme certaines espèces de *Satyrus* !. Donc l'affirmation de H. de Lesse mentionnée ci-dessus, est infirmée par les données cytologiques présentées par lui-même. Quant aux séparations spécifiques effectuées par H. de Lesse et basées sur les formules chromosomiques, nous les considérons comme non valables. En 1959 H. de Lesse décrit la sous-espèce *hamadanensis* de Lesse appartenant à l'espèce *Agrodiaetus dama* Stgr. ; dans la même année il publie

un autre article [4] où il décrète *hamadanensis* une bonne espèce en raison de sa formule chromosomique. Vraiment, *A. dama* a n = 41 et *A. hamadanensis* a n = 22. Est-ce que cette différence dans le caryotype est suffisante pour accorder à la forme *hamadanensis* le statut spécifique? Nous répondons non à cette question en nous basant toujours sur les travaux de H. de Lesse. Il présente un grand nombre d'exemples de sous-espèces dont la formule chromosomique est nettement différente de l'une à l'autre. Outre les exemples ci-dessus nous mentionnons encore *Pareupthychia hesione* Sulz. d'Argentine avec n = 15, mais en Equateur n = 21, 43, 44 ; *Agrodiactus transcaspica elbursica* Forst. n = 16, *A. t. caspica* Stgr. n = 53. Une formule chromosomique différente n'est donc pas toujours un caractère spécifique. La validité spécifique de *hamadanensis* se trouve sous le signe interrogatif comme d'ailleurs toutes les « espèces » qui diffèrent seulement par le caryotype, mais les genitalia sont identiques (*A. sennanensis*, *A. tankeri*, *A. baytopi*, etc. [4], [5] [6]). Pour nous mieux convaincre de la valeur de critère cytogénétique et combien « valides » sont ces espèces, nous examinerons un peu l'« espèce » *Lysandra coelestissima* Vrty. En 1960 H. de Lesse sépare *coelestissima* de *L. coridon* Poda et la considère bona species à la suite de l'examen cytologique (n = 87, tandis que *L. coridon* a n = 88). En 1960 n'étaient pas connues les formules chromosomiques des populations de *coridon* de divers pays (89, 90, 91, 92) et on pensait que n = 87 était le nombre d'une espèce distincte. Quand on eut constaté que les populations et les races pouvaient avoir des formules chromosomiques différentes, H. de Lesse revint sur sa décision de 1960 et redescendit *coelestissima* au rang de sous-espèce de *coridon* [10]. Un autre cas discutable est celui *L. albicans* H. S. A propos de la validité spécifique d'*albicans*, H. de Lesse dit : « *La formule* n = *82 de* **L. Albicans** *semble donc bien avoir la valeur d'un caractère spécifique* ; *elle permet ainsi de séparer* albicans **d'hispana** (n = 84—85) ». Dans un autre travail (1952) le même auteur affirme : « *La validité spécifique de* **L. albicans** *se trouve confirmée par l'étude cytologique. Toutefois j'ai compié sur deux individus de* **L. albicans** *n = 85 et non n = 84 comme dans la majorité des cas* ». Nous pensons que ces lignes ne demandent plus de commentaires. Quelle est la valeur d'un chromosome de plus ou de moins? Il peut être *une variation individuelle, un caractère racial, spécifique ou même générique*. La sytématique des Rhopalocères ne s'est point « améliorée » avec les genres *Mesoacidalia*, *Fabriciana*, *Arethusana*, *Chazara*, *Pseudochazara*, etc. et avec leurs formules chromosomiques non plus. Ces genres, ainsi que les espèces *coelestissima*[2], *coerulescens*, *asturiensis*, *arragonensis*, *hamadanensis*, *tankeri*, etc. ne font qu'encombrer la taxonomie sans aucun profit pour la science.

Pour conclure nous soulignons encore une fois que le critère cytogénétique n'est pas un bon critère pour aucun taxon. Parfois il peut fournir des éléments d'identification à l'échelon spécifique ou générique, mais en tous cas le statut taxonomique des taxa examinées doit être confirmé par l'habitus et surtout par les genitalia. Nous considérons comme une erreur taxonomique les séparations spécifiques basées sur le caractère cytologique en l'absence des structures différentes dans les genitalia.

[2] *Coelestissima* est encore considérée bona species par L. Higgins [2], W. Manley [12], etc. quoique H. de Lesse s'y oppose !

BIBLIOGRAPHIE

1. Delsol M., Cahiers d'Etudes Biologiques, 1958, *5*, 3—20.
2. Higgins L. G. et Riley N. D., *Guide des papillons d'Europe*, traduit et adapté par P. C. Rougeot. Delachaux et Niestlé, Neuchâtel, 1971.
3. Lesse H. de, Lambillionea, 1959, **59**, 1—2, 5—10.
4. — Bull. Soc. Linnéenne Lyon, 1959, **28**, *10*, 312—315.
5. — Bull. Soc. Ent. Mulhouse, 1959, juin, 45—48.
6. — Bull. Soc. Ent. Mulhouse, 1960, octobre, 75—78.
7. — Bull. Soc. Ent. France, 1961, **66**, 71—82.
8. — Rev. Fr. Ent., 1961, **28**, *2*, 93—100.
9. — Ann. Soc. Ent. Fr. (N. S.) 1967, **3**, *1*, 67—136.
10. — Ann. Soc. Ent. Fr. (N.S.) 1969, **5**, *1*, 469—522.
11. Maeki K. et Remington C. L., Journ. Lep. Soc., 1959 (1960), **13**, 193—203; **14**, 127—147.
12. Manley W. B. L. and Allcard H. G., *A Field Guide to the Butterflies and Burnets of Spain*, E. W. Classey, Hampton, 1970.
13. Motaș C., Natura, 1948, **37**, *1*, 7—13.
14. Niculescu E. V., Rev. Verviétoise Hist. Nat., 1971, **28**, *7—9*, 1—8.

Reçu le 3 mars 1972

ELECTRON-MICROSCOPIC OBSERVATIONS ON SPERMATOGENESIS IN *EURYDEMA VENTRALIS* KOL (HETEROPTERA-PENTATOMIDAE) WITH SPECIAL REFERENCE TO MITOCHONDRIA AND ANNULATE LAMELLAE

BY

VIORICA TRANDABURU

Mitochondria and annulate lamellae have been studied in all spermatogenesis stages, on *Eurydema ventralis* Kol. Annulate lamellae have been observed only in spermatogonial cells and exclusively in the cytoplasm of those cells.
Mitochondria polarized in spermatogonia, diffusely spread in spermatocytes, merge in spermatids forming the nebenkern which has a very complicated structure. Subsequently, the nebenkern simplifies its structure, so that it looks as consisting of two mitochondrial bodies. In advanced spermatids and in spermatozoa, the mitochondrial bodies present another structure.

Electron microscopical studies about the mitochondrial transformations determining the nebenkern have been described in a series of insect species by Tahmisian et al. [31], Gatenby and Tahmisian [31], Brelland et al. [6], André [1] [2], Baccetti and Bairati [3], a.s.o.

Minute investigations by means of the electron microscope concerning the further evolution of the nebenkern have been carried out in Lepidoptera (*Macroglossidae* and *Pieridae*) by André [1] [2] and by Pratt [23] in Hemiptera (*Murgantia histrionica*).

Annulate lamellae have been rendered evident in some animals both in oocytes [15] [16] [24] and in spermatids and spermatocytes [26] [30]. They have been pointed out in spermatogonia of Orthoptera by Sakai and Shigenaga [28] and in Homoptera, by Folliot [9].

In the present paper we intend to describe on *Eurydema ventralis* Kol. the annulate lamellae as well as the mitochondria evolution.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, No 6, p. 407—412, BUCAREST, 1972

MATERIAL AND METHOD

Adult specimens of *Eurydema ventralis* Kol. collected from Işalniţa – Craiova were dissected in fixation solution under the binocular and the testes were removed. Fragments $< < 1 mm^3$ were fixed for 2 hours in a 2.5 % glutaraldehyde solution in 0,15 M phosphate buffer at pH 7.4. The material was washed in phosphate buffer and then post-fixed in the same buffer to which 1 %$Os O_4$ was added [17]. The preparations were dehydrated in acetone and embedded in Vestopal W. The ultrathin sections obtained with an ultratome LKB were stained in an aqueous solution of uranyl acetate and contrasted with lead citrate [25]. The grids were examined in a Jem- 7 electron microscope (80 Kv).

RESULTS

Spermatogonia present a large spheric nucleus with the heterochromatin periferrally located in a very thin layer or in small heaps, while the euchromatin is scattered in the nucleus [Fig. 1). In the cytoplasm of these cells, elements of the smooth endoplasmic reticulum, isolated ribosomes or polysomes are irregularly spread. Mitochondria and dictyosomes are situated at a pole of the nucleus in an area where centriolus was also observed under light microscope. Also in the cytoplasm of spermatogonia, flat cisterns perforated by pores, arranged in piles and forming annulate lamellae, are found (Fig. 1). Sometimes these are connected to the nuclear membrane, which demonstrates that they originate from the nuclear membrane. A characteristic fact is that in subsequent stages of spermatogenesis, no more annulate lamellae had been observed in germinative cells.

Spermatocytes present a large nucleus, with heterochromatin arranged in heaps scattered all over the nucleus, while the euchromatin is finaly dispersed. In the cytoplasm, many mitochondria diffusely spread, a weakly developed granular endoplasmic reticulum, one centriolus, ribosomes and dictyosomes, are found.

Young spermatids have a spherical nucleus with larger heaps of heterochromatin than in spermatocytes. In the cytoplasm of these cells, there are : ergastoplasm, ribosomes isolated or polysome-like, dictyosomes and some inclusions which may represent autophagosomes, not encountered in spermatocytes.

Mitochondria and dictyosomes begin to gradually cluster at one pole of the nucleus, where this process acquires intensity (Fig. 2). Through the transformations they underwent, mitochondria form the nebenkern. Thus, mitochondria which had reached at the posterior pole of the nucleus, began to enlarge, branch out and twist, in a very complicated manner. The ramification is more pronounced to the periphery of this group of mitochondria (Fig. 3). In a further stage, the peripheral mitochondria merge first. These begin to branch out, catching among their ramifications the inward not yet merged mitochondria (Fig. 4). Then, the merging of peripheral mitochondria becomes more pronounced (Fig. 5). The merging of these mitochondria results in very lobated forms. The outer mitochondria branch out to the center of the nebenkern, forming a sort of core (Fig. 6). At this stage we found only two external mitochondrial formations and a

still bipartite core. The central part of nebenkern keeps in touch with cytoplasma in some points (Figs 6 and 7). Mitochondria fusion continues and thus structural simplification of nebenkern is achieved; this latter appears to consist of only two large mitochondrial formations, in the matrix of which tubules are noticed (Fig. 7).

In advanced spermatids also with spherical nucleus, the nebenkern is equally divided in two mitochondrial bodies, flanking the axial filament. The two mitochondrial bodies result either by division of the nebenkern in two formations, or by separations of the two mitochondrial formations of the nebenkern and their flanking the axial filament. At the beginning, the mitochondrial bodies are still spherical, then they taper along the axial filament.

In both advanced spermatids and spermatozoa the two elongate mitochondrial bodies present the same submicroscopic structure, different from the one in the previous stage. In each mitochondrial body, a matrix with fibrillo-granular structure is apparent. From the inner membrane of the mitochondrial body, circular cristae shaped lamellary expansions start; these do not significantly penetrate within the matrix (Fig. 8).

DISCUSSIONS

In spermatogonia, mitochondria localization at one pole of the nucleus, as observed by us by means of light microscope in *Eurydema ventralis*, has been also described in other Heteroptera species by Chichering [7], Payne [18], Steopoe [29], Poisson [21], Trandaburu [33] [34]. In the species *Viviparus contectoides* (Gasteropoda), *Murgantia histrionica* (Heteroptera) and *Atractomorpha bedeli* (Orthoptera), electron microscopical researches carried out by Kaye [14], Sakai and Shigenaga [27] revealed in cytoplasma, small mitochondria with few cristae, clustered near the nucleus. In *Gelastocoris oculatus*, Payne [18] [19] noticed, both by light and electron microscopy, the presence in the spermatogonia of a "nuclear cap" represented by aggregate mitochondria with long axes perpendicular to the nuclear membrane.

In *Eurydema ventralis* spermatogonia, the cytoplasma is characterized by the presence of annulate lamellae. Sometimes, annulate lamellae have been noticed as connected to the nuclear membrane, which demonstrates their origin in the perinuclear cistern. These were no longer found in ulterior stages of spermatogenesis. Our results are in good agreement with those of Sakai and Shigenaga [28] in *Atractomorpha bedeli* and with those of Folliot [9] in *Philaenus spumarius*. Folliot [9] described in spermatogonia, annulate lamellae both in cytoplasma and nucleus.

Annulate lamellae have also been found in germinal cells of different animals. Thus in rat spermatids [30], in *Cambarus virilis* (Crustacea—Decapoda) spermatocytes [26], in the oocytes of some animals [15] [16] [24] [30].

Most authors deem that these organites derive from the nuclear membrane : yet Sakai and Shigenaga [28] have demonstrated in *Atractomorpha bedeli* that these annulate lamellae originated in "tubular body" in cytoplasma.

In spermatocytes, the diffuse spreading of mitochondria all over the cytoplasma also found by means of the electron microscope in *Eurydema ventralis*, has been mentioned by Collins and Richter [8] in *Buenea* sp., by Pratt [23] in *Murgantia histrionica* and by Trandaburu [33] [34] in *Graphosoma italicum*.

In spermatids the nebenkern formed by mitochondria clustering at the future posterior pole of the nucleus and their merging as observed by us in *Eurydema ventralis* was also mentioned in other insects. Thus, it has been described in Heteroptera, under light microscope, by Bowen [5], Pollister [22], Gupta et al. [12], Trandaburu [33] [34].

Electron microscopical studies concerning the mitochondria modifications for nebenkern formation have been described in several insects. With regard to Heteroptera we mention the papers of Yasuzumi [18], Pratt [22], Trandaburu [33] [34].

In the nebenkern evolution, the stages observed by us have also been described in Lepidoptera (Pieridae and Macroglossidae) by André [2] as "peloton", "écorce chromophobe", "oignon" specific to spherical mitochondrial body. Pratt [23] showed in Heteroptera (the species *Murgantia histrionica*) the stages: "early,cluster nebenkern, mid-cluster nebenkern and late-cluster nebenkern", by which the nebenkern gradually simplifies its structure, so that eventually, prior to division it becomes bipartite.

In the case of *Eurydema ventralis* we noticed a gradual simplification of the highly complicated structure of the nebenkern which consisted of two formations.

In *Eurydema ventralis* during all development stages of the nebenkern, tubules have been observed in mitochondria matrix. The tubules persisted in mitochondria structure till the two mitochondrial bodies began to taper along the axial filament. This was shown by Trandaburu [33] [34] in *Graphosoma italicum* species, as well.

We deem that by the separation of the two mitochondrial formations constituting the nebenkern, two mitochondrial bodies eventually result, which flank the axial filament, and taper. As mentioned by André [2], Furieri [10], Werner [35], Phillips [20] a.s.o., the two mitochondrial bodies range parallely with the axial filament and an inner rearranging and differentiating process begins, leading to the formation of one or two mitochondrial bodies, the morphology of which is identical in the individuals of the same species and different from one species to another.

In *Eurydema ventralis* the two mitochondrial bodies are equal in size, as described in most insects [2] [4] [11] [13] [32] [33] [34] [36]. In *Eurydema ventralis* lamellary expansions like circular cristae start from the inner membrane of the mitocondrial body. Matrix is fibrilogranular. In *Pieridae*, André [2] showed that within the mitochondrial bodies there existed parallel lamellae like cristae, perpendicularly to the inner membrane and ranged along one side only. Similar infrastructures have also been observed by Furieri [10] in Heteroptera (*Pyrrhocoris apterus*) by Werner [35] in Coleoptera (*Cicindela campestris*), by Yasuzumi and Tanaka [37] in Gasteropoda (*Cipangopaludina malleata*).

CONCLUSIONS

1. In spermatogonia, mitochondria, dictyosomes and centriolus clustered in one pole of the nucleus. In the cytoplasm of these cells annulate lamellae are rendered evident; these are no longer noticed in subsequent stages of spermatogenesis.

2. Mitochondria diffusely spread in spermatocytes, cluster in spermatids on the future posterior pole of the nucleus where they merge and twist in a complicated way, forming the nebenkern.

3. The constituted nebenkern gradually simplifies its structure so that it appears to consist of two mitochondrial formations. Within the nebenkern, during these stages, tubules are noticed.

4. In advanced spermatids and in spermatozoa the two mitochondrial bodies acquire another infrastructure. Lamellary exapansions, circular cristae-like, start from the inner membrane of the mitochondrial body.

REFERENCES

1. André J., Ann. Sci. Zool. Biol. Animale, 1959, **1**, 283–307.
2. — J. Ultrastruct. Res., 1962, **3** supl., 1–185.
3. Baccetti B., Bairati A., Redia, 1965, **49**, 1–29.
4. Baccetti B., Dallai R., and Rosati F., J. Microscopic, 1969, **8**, 249–262.
5. Bowen R., Biol. Bull. 1922, **42**, 53–85.
6. Breland O. P., Gassner G., Riess R. W., and Biesele J. J., Can. J. Genet. Cytol., 1966, **8**, 739–753.
7. Chickering A. M., J. Morph., 1927, **44**, 541–607.
8. Collins H. R., and Richter K. M., Anat. Rec., 1961, **139**, 297.
9. Folliot R., Z. Zellforsch. Mikrosk. Anat., 1968, **92**, 115–129.
10. Furieri P., Redia, 1963, **48**, 179–187.
11. Gatenby J. B., and Tahmisian T., Cellule, 1959, **60**, 105–135.
12. Gupta B., Bedi U., and Nath V., Res. Bull. Panjab Univ. 1960, **11**, 113–119.
13. Herold F., and Munz K., Z. Zellforsch. Mikrosk. Anat. 1967, **83**, 364–374.
14. Kaye J. S., J. Morph., 1958, **102**, 347–399.
15. Kessel R. G., J. Cell Biol., 1963, **19**, 391–414.
16. Merriam R. W., J. Biophys. Biochem. Cytol., 1959, **5**, 117–123.
17. Millonig G., *Further observations on a phosphate buffer for osmium solutions*. S. S. Breese 5 th. Intern. Congr. Electron. Microscopy, London, Acad. Press, 1962, *2*.
18. Payne F., J. Morph. and Physiol., 1927, *43*, 299–345.
19. — J. Morph., 1966, *119*, 357–383.
20. Phillips D. M., J. Cell Biol., 1970 *44*, 243–277.
21. Poisson R., Arch. Zool. Exp. et Gen., 1936, *78*, 133–194.
22. Pollister A., J. Morph. and Physiol., 1930, *49*, 455–507.
23. Pratt S. A., J. Morph., 1968, **126**, 31–66.
24. Rebhun L. I., J. Biophys. Biochem. Cytol., 1956, **2**, 93–105.
25. Reynolds E. S., J. Cell Biol., 1963, **17**, 208–212.
26. Ruthmann A., J. Biophys. Biochem. Cytol., 1958, *4*, 267–275.
27. Sakai A., and Shigenaga M., Cytologia, 1967, **32**, 72–86.
28. — Cytologia, 1968, **33**, 34–45.
29. Steopoe I., C. R. Soc. Biol. Paris, 1928, **99**, 902.
30. Swift H., J. Biophys. Biochem. Cyrol., 1956, **2**, 4 suppl., 415–418.
31. Tahmisian T. N., Powers E. L., and Devine R. L., J. Biophys. Biochem. Cytol., 1956, **2** (2 suppl.), 325–330.
32. Tandler B., and Moriber L. G., J. Ultrastruct. Res., 1966, **14**, 391–404.

33. Trandaburu V., Rev. roum. Biol., série Zool., 1971, **16**, 2, 97—101.
34. — *Cercetări citologice în microscopia optică și electronică asupra spermatogenezei la Eurydema ventialis Kol și Graphosoma italicum Müll (Heteroptera-Pentatomidae)* Doctoral thesis, 1971.
35. Werner G., Z. Zellforsch. Mikroskop. Anat., 1965, **66**, 255—275.
36. — Z. Zellforsch. Mikroskop. Anat., 1966, **73**, 576—599.
37. Yasuzumi G., and Tanaka M. D. M., J. Biophys. Biochem. Cytol., 1958, **4**, 621—633.
38. Yasuzumi G., Ishida H., Nakano S., et Yamamoto H., J. Ultrastruct. Res., 1960, **3**, 484—494.

Received April 24, 1972

Faculty of Biology
Bucharest 35, Splaiul Independenței 91—93

Fig. 1. — Spermatogonia. *N*, nucleus; *AL*, annulate lamellae; *R*, ribosomes. × 9000.

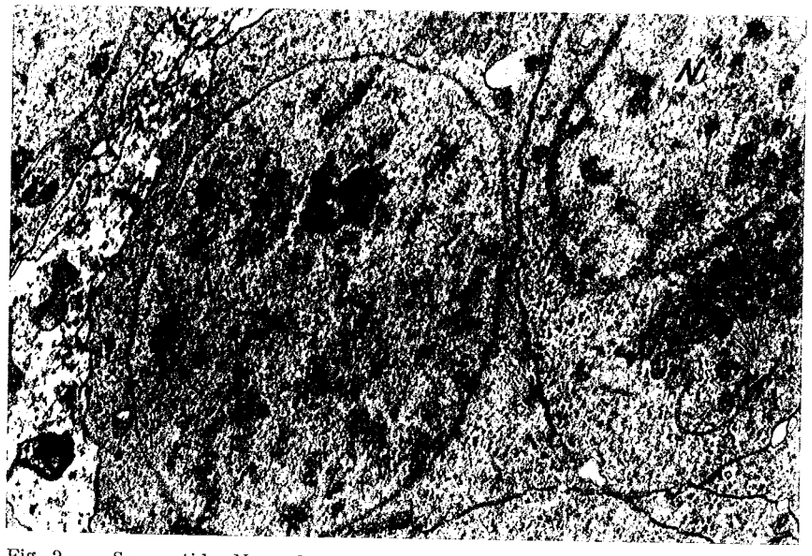


Fig. 2. — Spermatid. *N*, nucleus; *M*, mitochondria clustered at the posterior pole of the nucleus. × 15 000.

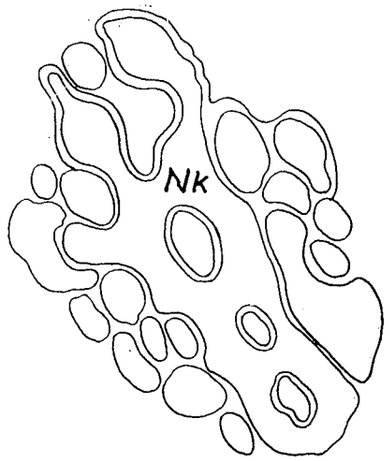


Fig. 3—7. — Nebenkern evolution. Beginning of branching out of peripheral mitochondria. *Nk*, nebenkern. Drawing after an electron microscopical image magnified × 19500.

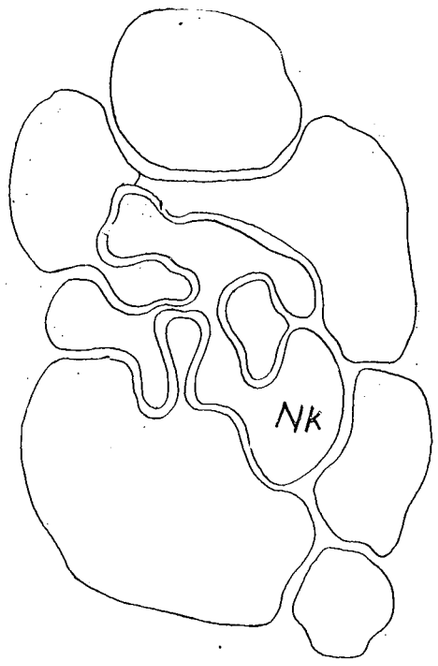


Fig. 5. — Peripheral mitochondria teclusred by groups. Drawing after an electron microscopical image magnified × 13000.

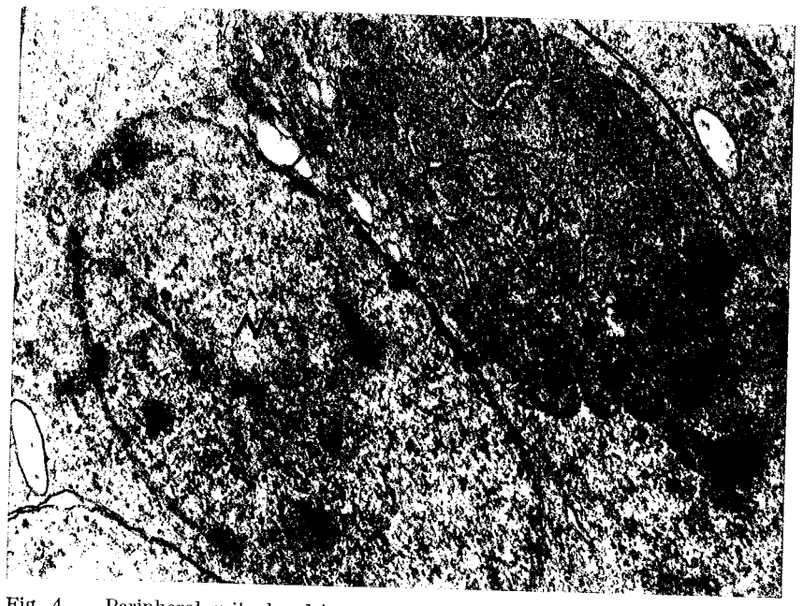


Fig. 4. — Peripheral mitochondria more ramified; among their ramifications, inner mitochondria not yet merged are seen. *Nk*, nebenkern; *N*, nucleus; × 18000.

Fig. 6. — *Nk*, Nebenkern consisting of two external mitochondria surrounding the two inner mitochondria. *N*, nucleus; *Ac*, acrosomic vesicle. × 5000.

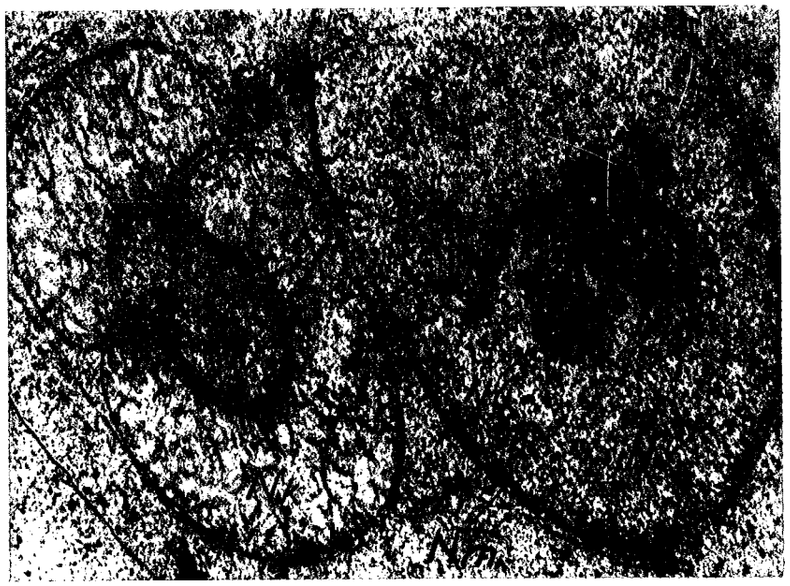


Fig. 7. — *Nk*, nebenkern consisting of two mitochondria with tubules; *N*, nucleus; *Nu*, nucleolus; *Nm*, nuclear membrane. × 18000.

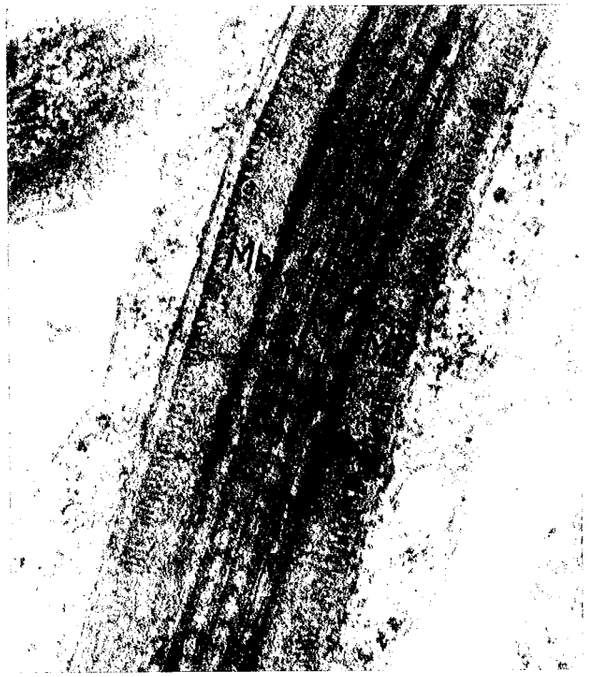


Fig. 8. — Spermatozoon; tail longitudinal sectioned. *Mb*, mitochondrial bodies; *Af*, axial filament. ×48000.

THE DISTRIBUTION OF SOME ENZYMES IN THE MUCOSA OF THE INTESTINAL TRACT FROM LOACH (*MISGURNUS FOSSILIS* L.)

BY

RADU MEȘTER, DRAGOȘ SCRIPCARIU
and LOTUS MEȘTER

Isoenzymic pattern and histochemical localization of some hydrolytic and glycolytic enzymes, in the anterior and posterior part of the intestinal mucosa from loach, have been reported. The histochemical observations indicate, that alkaline phosphatase, acid phosphatase and leucine aminopeptidase are localized only in the anterior part of the intestine. Nonspecific esterases were evenly distributed along the whole length of the epithelium of the intestinal tract. The results of the electrophoretic studies, point toward an increased activity of dehydrogenases (G-3-P DH, LDH, MDH, IDH), in the posterior part of the intestine.

Comparative investigation of the enzymes activities distribution in the intestinal tract is essential for the understanding the mechanism of digestion and absorbtion of food and the physiology of the intestinal mucosa. Histochemical and biochemical studies have shown, the distribution and nature of some enzymes in the mucosa of the intestinal tract on vertebrates [7—10] [21] and fishes [1] [2] [20]. The intestinal mucosa of fishes present a variety of enzymes, some of them with segmental differences, in relation with the character of the food and the feeding habits of the organism. Their metabolic importance correlated with morpho-functional adaptation of the intestinal tract of fishes is unknown.

The intestinal tract of loaches has a well definit morpho functional specialization: anterior part of the gut is adapted for the digestive functions while posterior part of the intestine is specialized for the diffusion of oxygen (intestinal respiration [3] [14] [19]). This poses the question whether or not corresponding functional differences are known.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, No 6, p. 413—418, BUCAREST, 1972

In the present study, histochemical and electrophoretical techniques are used to investigate the distribution of some hydrolytic and glycolytic enzymes of anterior (digestive) and posterior (respirative) mucosa of the intestinal tract, to facilitate the understanding of their relationship to this important differentiation.

MATERIAL AND METHODS

Experiment were carried out on loaches (Misgurnus fossilis L.), of average weight about 50 g. Intestinal tract was obtained from fish and washed with ice-cold 0.65% NaCl. The mucosa from anterior and posterior part of the intestine was removed by scraping, homogenized with cold distilled water. The supernatant fluid, obtained after centrifugation at 5000 g for 15 min, was used for electrophoresis.

Electrophoresis was performed in polyacrylamide gel, at a 7.5% concentration, according to the method of Davis [5], disk-electrophoresis system. The tank buffer was 0.1 M tris-glycine, pH 8.6; the gel buffer contained 0.1 M tris-HCl, pH 8.6. The electrophoresis was carried out for about 2 hr, with current setting of 3 mA/tube.

After the run, the gels were drawn out from the tubes, washed with cold distilled water and then with 0.1 M tris-HCl buffer, pH 7.4. Gels were further incubated in buffers containing substrates adequate for each enzyme.

Lactate dehydrogenase was determined according to L. E. Lush [15], NADP-isocytrate dehydrogenase according to Farber and Bueding [8], malate dehydrogenase according to Dawidson and Cartner [6], glyceraldehide-3-phosphate dehydrogenase after Lush [15] in which, the lactate was replaced by alpha glycerophosphate with the same molarity; nonspecific esterases were determined by the procedure of Pearce [18], with three different substrates: incubating in alpha naphthyl acetate, alpha naphthyl caprylate and naphthol AS laurate. As a coupling reagent was used diazolium salts fast bllue B to localize nonspecific esterases activities in the gels. Leucine aminopeptidase activity was determined according to Nachlas [16], using L-leucyl-4-methoxy naphthylamide as substrate and fast blue B as coupler. Alkaline phosphatase and acid phosphatase have been detected in medium prepared according to Burstone [4], using alpha naphthyl phosphate as substrate and fast Red Violet LB salt as coupler.

Qualitative histochemistry. This was done on fixed frozen intestinal segments, sectioned in a cryostat (10 μ) and stained, using the same substrates and solutions as those employed for electrophoresis. Alkaline phosphatase and acid phosphatase were detected also using naphthol AS TR phosphate as substrate and Red Violet LB salt as coupler, with the same results.

RESULTS

We followed the distribution in the anterior and posterior intestinal tract of the various enzymes activities.

Alkaline phosphatase activity decreased distally the intestinal tract. On the basis of histochemical staining reaction, alkaline phosphatase are localized exclusively in the anterior intestine. An intense activity in the striated or brush border cells and in the crypt epithelium can be seen (Plate I). In the posterior region of the intestinal tract, alkaline phosphatase is not decelable. The electrophoretical study reveal two molecular

forms in anterior region of the intestinal mucosa while posterior region no band with enzymic activity was observed (Fig. 1).

The pattern of acid phosphatase distribution in the intestinal tract of loach is similar to that described for alkaline phosphatase. The histochemical observations show that in the anterior part of the gut, acid phosphatase reaction was positive. The reaction product appeared to be localized in the villus epithelium and in crypt epithelium. Mucosa from the posterior region of the gut is devoide of enzyme activity. Electrophoresis

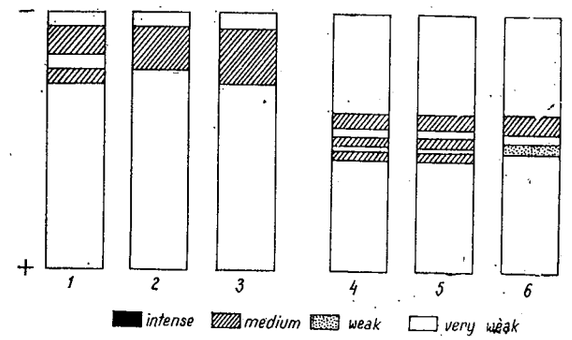


Fig. 1. — Isoenzymic pattern of alkaline phosphatase (1). acid phosphatase (2), leucine aminopeptidase (3), caprylate esterase (4), acethyl esterase (5) and laurate esterase (6), in the mucosa of the anterior part of the intestine.

on polyacrylamide gel of gut mucosa, revealed one enzymic fraction from the anterior region and didn't find any enzymic fraction in the mucosa of posterior part of the intestinal tract (Fig. 1).

Leucine aminopeptidase activity could be demonstrated in the brush border of the epithelial cell layer of anterior region. Except for some weakly staining azo dye precipitate, no enzyme activity was found in the epithelial cells of posterior region. The zymogram of enzyme indicate one band with leucine aminopeptidasic activity, from the mucosa of anterior part of the intestine. Mucosa from the posterior part of the gut is devoid of enzymic activity (Fig. 1).

Following the distribution of nonspecific esterases on histochemical preparations, were observed an enzymatic activity homogenously distributed along the crypt and villus. In the basal part of the crypt, esterases activities were very low or absent while in the villus epithelium, the enzymes were distributed along the length of the villus. The staining reaction of nonspecific esterases is very slight or moderate and did not vary significantly in the anterior or posterior part of the intestine (Plate I). The electrophoretical studies of nonspecific esterases do not exhibit any basic variation in the pattern of esterase zymograms from anterior and posterior part of the intestinal mucosa. The incubation of gels with naphthyl acetate and naphthyl caprylate, reveal electrophoretically three molecular forms with esterase activity, in both anterior and posterior intestinal mucosa (Fig. 1). Similar results are obtained with naphthol

LC laurate as substrate: the electrophoretic pattern is characterized by two enzymic fractions, irrespective of the region of the intestine (Fig. 1).

Glycolytic enzymes. The electrophoretical pattern of glyceraldehide-3-phosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase and isocytrate dehydrogenase from anterior and posterior part of loach intestinal mucosa are presented in figure 2. Lactate dehydrogenase from the intestinal mucosa of loach contains four molecular forms, irrespective of the region of the intestinal tract. The zymograms of the other three dehydrogenases reveal electrophoretically, two molecular forms in both anterior and posterior part of the intestinal mucosa. As show in figure 2, the level of the enzymic activity of the dehydrogenases are slightly increased, in mucosa from the posterior part of the intestine.

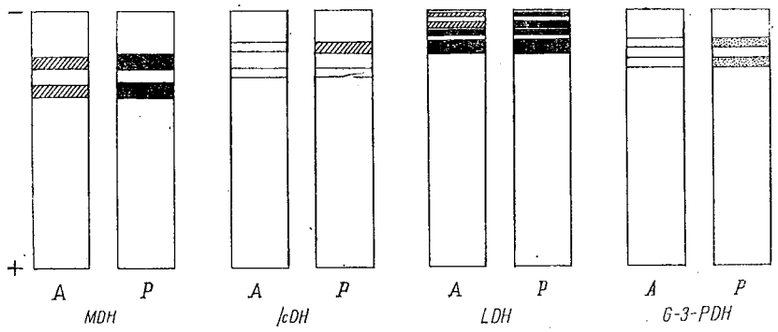


Fig. 2. — Isoenzymic pattern of glyceraldehide-3-phosphate dehydrogenase (G-3-P DH), lactate dehydrogenase (LDH), NADP-isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH) from mucosa of anterior (A) and posterior part (P) of the intestine

DISCUSSION

The results of the histochemical and electrophoretical studies of investigated enzymes show, that the intestinal mucosa of loach not only secrete enzymes, but the various regions of the intestine exhibit this property in different way.

It is well established that the anterior part of the intestinal tract of loach is directly related to digestion and absorbtion. The activity of some enzymes as acid phosphatase, alkaline phosphatase and leucine aminopeptidase change quantitatively and qualitatively as a function of the physiological adaptation of the intestinal tract. Insofar as they can be judged, the findings of the histochemical and electrophoretical

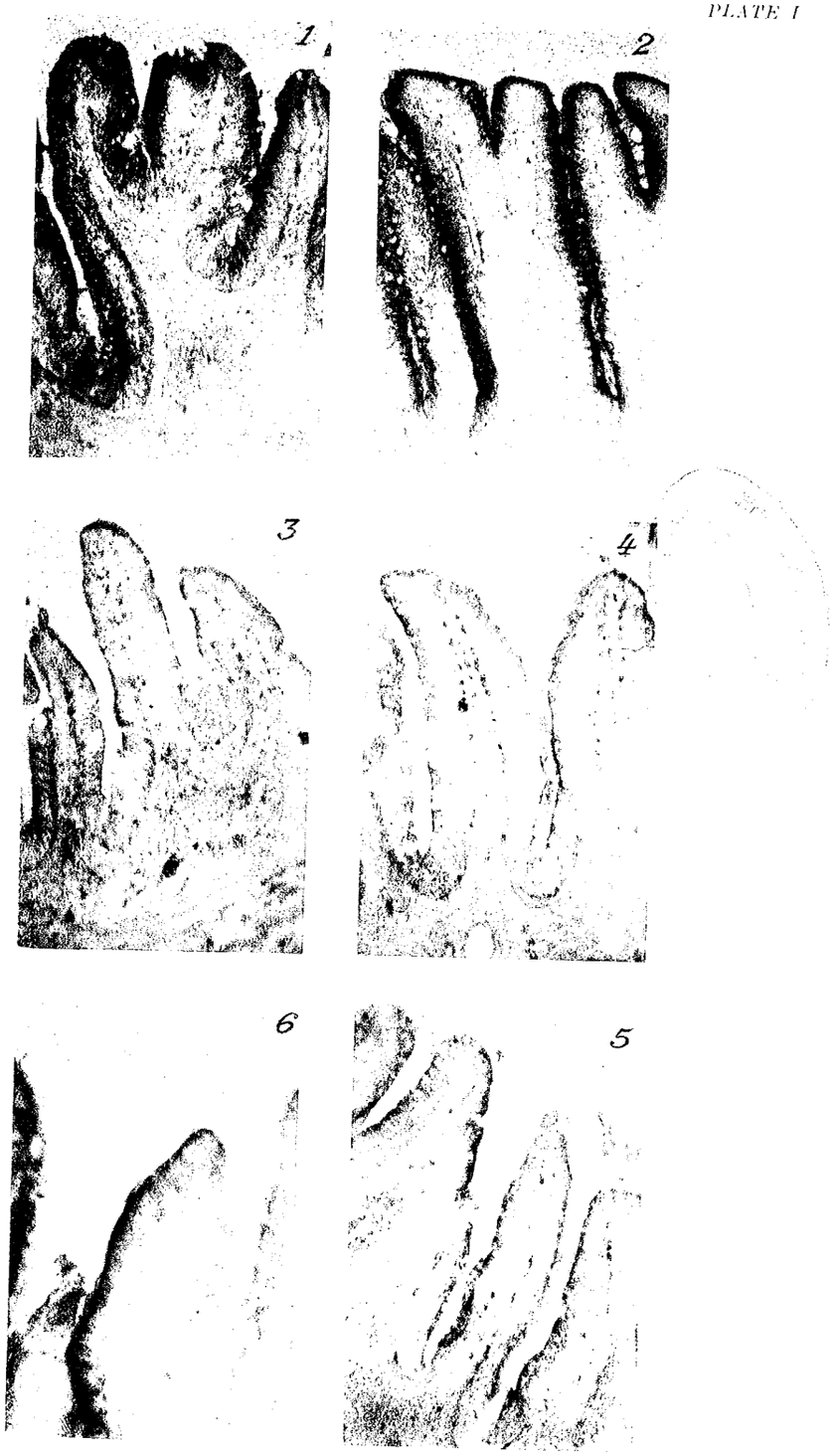


PLATE I. — Histochemical localization of acid phosphatase (1), alkaline phosphatase (2), acetyl esterase (3), laurate esterase (4), caprylate esterase (5) and leucine aminopeptidase (6) in the mucosa of the anterior part of the intestine.

studies appear to correspond, with regard to their specific activity and their significant role, to the function of the intestinal tract. The activity of these enzymes was demonstrated for other fishes [2] [20] and many vertebrates, and was related to the functional state of the mucosa. However the pattern of enzyme distribution along the intestinal tract of fishes and vertebrates are conflicting [17].

The study of nonspecific esterases are of additional interest as these enzymes are known to exist in multiple molecular forms [11]. Nonspecific esterases activities were evenly distributed along the whole length of the epithelium of the intestinal tract. The substrate alpha naphthyl acetate and alpha naphthyl caprylate produced essentially similar results, both as the histochemical localization and intensity of activity of the various isoenzymes. In spite of the hydrolytic function of esterases, their existence in the mucosa cells along the intestinal tract of loach, suggeste that this enzymes may be involved in the esters synthesis, in relation with the structure of the lipido-proteic membranes. Nonspecific esterases found in the intestinal mucosa fulfill multiple functions of secretory, resorptive and possibly synthesizing character.

The electrophoretical studies of dehydrogenases serve as valuable indices for certain physiological states of mucosa. Although sufficient experimental evidence accumulated in recent years shows, the participation of dehydrogenases in metabolism of the cells, the relationship between enzyme activity and various cells, in generally, is uncertain. Experiments related to the existence of the glycolytic and oxidative enzymes in the mucosa of the intestinal tract of fishes are few and restricted [2]. Kubat and Koldovsky [13] examining the distribution of some dehydrogenases in rat intestine found an uniform activity, irrespective of the intestinal part.

Katsumasa and Fujie [12] suggested that the source of energy within the cell is a process which parallels secretory activity. A comparison of dehydrogenase zymograms reveals, that the over-all increase in enzymic activity in the posterior part of the gut is not correlated only with the existence of hydrolytic enzymes. In our case, the biological function of the dehydrogenases from the posterior intestinal segment of loach, may be correlated with the adaptation of this region to the respirative function.

REFERENCES

1. Al-Hussaini A. H., Quart. Jour. Micros. Sci., 1949, **90**, 323.
2. Arvy L., in *Handbuch der Histochemie*, ed. Graumann W. and Neuman K. Enzyme Band VII, Verlag Stuttgart, 1962.
3. Călugăreanu D., Archiv fur die ges. Physiol., 1907, **118**, 42.
4. Burstone M. S., *Enzyme Histochemistry*, Acad. Press, New York, 1962.
5. Davis J. B., Ann. N. Y. Acad. Sci., 1964, **121**, 404.
6. Dawidson G. R., Cartner R., Science, 1967, **157**, 1569.
7. Etzler M. E., Birkenmeter E. H., Mooc F., Histochemie, 1969, **20**, 99.
8. Farber E., Bueding E., J. Histochem. and Cytochem., 1956, **4**, 307.
9. Holt J. H., Miller D., Biochem. Biophys. Acta, 1962, **58**, 239.
10. Hugan J., Borgers M., Acta histochem., 1969, **34**, 349.

11. KASKIN A. P., SHARECKIJ A. N., SURINOV B. P., BOSKOVA D. N., Biohimia, 1971, **36**,335.
12. KATSUMASA I., FUJIE K., Histochemie, 1970, **21**, 314.
13. KUBAT K., KOLDOVSKY O., Acta histochem., 1969, **33**, 75.
14. LUPU H., Ann. Scient. Univ. Iassy, 1928, **15**, 272.
15. LUSH L. E., Comp. Biochem. Physiol., **32**, 23.
16. NACHLAS M. M., MONIS B., ROSENBLATT D., SELIGMAN A. M., J. Biochem., Biophys. Cytol., 1960, **7**, 261.
17. NORDSTROM C., DAHLQUIST A., JOSEFFSSON L., J. Histochem. and Cytochem. 1968, **15**, 713.
18. PEARCE A. G. E., *Histochemistry: Theoretical and Applied*, ed. J. and A. Churchill Ltd, London, 1961.
19. PODUBSKY V., Sbornic Ces. Akad. Zem. Ved., 1955, **28**, 423.
20. PRAKASH A., J. exp. Zool., 1961, **141**, 237.
21. SPENCER R. P., KNOX W. E., Feder Proc., 1960, **19**, 886.

Received April 28, 1972

Faculty of Biology
Bucharest.

Ca^{45} ASSIMILATION FROM FOOD AND ITS DISTRIBUTION IN THE BODY OF SOME SPECIES OF TERRESTRIAL ISOPODS

BY

NICOLAE TOMESCU

This paper presents the results obtained by the researches carried out on Ca^{45} assimilation at gut level in six species of terrestrial isopods. It was found that Ca^{45} assimilation takes place at a high rate in all isopod species used for the experiment. It was also studied Ca^{45} distribution in different organs and parts of the tegument, as well as the rôle of calcium deposited in the sternal plates. Calcium concentration is different in the organs and parts of tegument of one animal, but is similar from one species to another. On the basis of the carried out studies the author arrived at the conclusion that the sternal plates are not used as calcium reserves during moulting, but they are removed with the exuvia.

In a previous work [6] we published the first results concerning Ca^{45} assimilation from food and its distribution in different parts of the body, in one species of terrestrial isopod, *Trachelipus* (= *Tracheoniscus*) *balticus*.

The much controversial question concerning the part played by the calcium deposited on the sternal plates of isopods [1] [3] [4] [9] determined us to continue these investigations on other species, too. We carried out comparative studies, selecting species of different biotypes, remote from a phylogenetic point of view, in order to see to what extent the obtained results can be generalised. The experiments were performed on the following species : *Ligidium hypnorum*, *Porcellium conspersum*, *Protracheoniscus politus*, *Trachelipus affinis*, *Trachelipus balticus* and *Armadillidium versicolor*

REV. ROUM. BIOL.—ZOOLOGIE, TOME 17, No 6, p. 419—425, BUCAREST, 1972

MATERIAL AND METHOD

The isopods used for experiments were collected from the Făget forest and the Galcer hill, both places being located near the town of Cluj.

The animals (10 individuals of each species) were kept in the laboratory in covered glass receptacles, on permanently wet filter paper. During the experiment they had a natural light regime, and the room temperature was between 17°—20° C. Ca^{45} was administered with the food: lime leaves marked by means of the method described in the previous work [6].

In order to calculate the Ca^{45} assimilated from food at the gut level, we daily took samples of excrements and food and measured their radioactivity. We took samples of feces from the leaves in order to avoid the errors caused by a possible Ca^{45} ion migration from food and excrements, both of them being placed on a wet layer.

The samples were dried in the drying closet at 105° C, then their radioactivity was measured. We calculated the Ca^{45} amount assimilated at the gut level, making a difference between the food and faeces radioactivity, relating it to mg. of dry substance. The measurements were made with a G. M. detector having a terminal window, 1.7 mg/cm², connected to a B_2 type recorder.

For the investigation of the Ca^{45} distribution in the isopod body, we fed the animals (4 individuals of each species) with marked leaves, then we dissected them under the stereomicroscope. The obtained samples were worked out according to the same method.

During the period of October 1971 — January 1972 we made two repetitions for both the investigated problems.

The whole experiment was carried out in the radioisotope laboratory of the Zoology Chair, under the direction of Prof. V. Radu.

RESULTS OBTAINED

a) **Ca^{45} assimilation**. In isopods the assimilation of calcium from food takes place both at hepatopancreas and intestine level, from the alimentary particles which pass directly from the stomach into these organs. In our experiments we observed only the calcium assimilation at gut level, the total assimilation in the living organism being more difficult to measure in this radioelement because it needs a highly fine and precise equipment.

So as the data recorded in literature show [5] [6] [7] [10] [11], the assimilation of radionuclides and generally of the substances varies, depending on the physiological state of the animals, their age, the surface and volume of the digestive tract, the passing speed of food, etc. The external temperature has an important rôle, acting directly upon the metabolism intensity [7] [10].

The results of our experiments on the six species of terrestrial isopods are shown in table 1.

It is found that, generally, Ca^{45} assimilation at the gut level takes place at a high rate in all the studied species. The physiological property, preserved by the isopods following their adaptation to the terrestrial life, of extracting calcium from every kind of food in a high amount, allowed them to people also the places which are poor of this element.

Table 1

Radioactivity of food (A) and feces (B) samples expressed in terms of imp. (min.) mg. dry substance.
I = series 1 II = series 2

	Date	SPECIES											
		Ligidium hypnorum		Porcellium conspersum		Protracheoniscus politus		Trachelipus affinis		Trachelipus balticus		Armadillidium versicolor	
		A	B	A	B	A	B	A	B	A	B	A	B
I	19 XII	8 055	241	9 618	1 065	14 640	1 200	5 607	1 140	9 706	2 077	8 395	1 066
	20 XII	8 344	340	11 296	1 720	17 630	2 260	7 593	1 788	7 462	2 170	6 420	1 125
	21 XII	6 209	262	13 182	1 000	17 533	2 300	5 600	1 477	8 052	2 401	5 508	1 330
	22 XII	8 728	700	10 357	1 460	14 314	2 793	5 710	1 840	9 260	2 896	5 168	1 064
	23 XII	8 160	850	13 265	2 550	18 925	3 171	7 752	1 570	9 010	2 770	5 075	1 258
II	19 I	7 490	355	1 835	268	3 392	500	12 690	1 344	8 141	1 070	5 298	530
	20 I	7 500	427	2 340	500	2 720	401	12 600	1 220	9 440	935	6 400	1 270
	21 I	7 588	298	1 984	540	2 953	425	13 920	2 215	7 822	2 200	6 960	1 160
	22 I	8 488	800	2 778	740	2 736	530	12 640	2 160	8 320	2 946	7 296	2 080
	23 I	8 442	605	2 281	560	3 024	786	12 160	2 592	7 527	2 800	6 154	1326

The calcium concentration of food is not an element which might limit the distribution areal of terrestrial isopods. This may be seen in nature in the widely spread species, as for instance: *Ligidium hypnorum*, *Hyloniscus riparius*, *Trachelipus balticus*, *T. affinis*, *Armadillidium vulgaris*, etc. There are other agents which play a much more important part in the isopods' life, particularly the humidity and the temperature.

The results obtained under laboratory conditions may present differences in comparison with the phenomena which take place in natural conditions [5] [7], so that we cannot consider them as being absolute. But they can illustrate the intensity of a biological phenomenon in nature.

b) **Ca^{45} distribution and deposition in the body of some isopod species.** In the previously published work [6] we described these aspects in one species. *Trachelipus* (= *Tracheoniscus*) *balticus*. The results showed that Ca^{45} distribution is different in the organs of an animal and in certain parts of its tegument. In this paper we publish the result of some similar researches performed in 6 isopod species. For experiments we selected only adult animals. The results are graphically shown in figure 1. It is found that in all the studied species, Ca^{45} was deposited in similar rates in the same organs and parts of tegument. The highest concentration of radioactive calcium/dry substance mg. is found in pleopodes, then, in decreasing order, in: sternites, pereiopodes, tergites; it is very low in hepatopancreas and gut. Taking into account the size and volume of these studied parts, the tergites, sternites and pereiopodes represent the principal components of the isopod body, which mobilize a great amount of calcium.

In terrestrial isopods the excess of calcium, which appears in the organism, is deposited on sternites as plates, named sternal calcareous plates [1] [2] [4] [9]. Radu and co-workers [6] found that in young animals the calcium distribution in tegument is uniform; in opposition with the adult animals, the sternal calcareous plates are not formed.

In our opinion, the explanation is that the young animals moult several times a year, their body loosing a great amount of calcium in this way; so that there is no excess which could be deposited. The adult animals moult once a year, therefore the calcium loss by this way is reduced. In this case an excess appears and it is deposited under the form of sternal plates. Many authors [1] [2] [9] think that this deposited calcium is a reserve for the tegument strengthening of the posterior part of the body during the moulting. Mehely [4] and Lagarrigue [3] doubt these assertions, showing that further investigations are still necessary.

During the period of the experiments we surprised several individuals in process of moulting. They were isolated from the marked food, dissected, and then the radioactivity was measured. As it is shown in figure 1 — *B*, after moulting there is a very small amount of Ca^{45} in the tegument. We observed under the stereomicroscope some individuals in process of moulting and found that the sternal plates are removed at the same time with the exuvia. However, during the preexuvial period, a very small amount of calcium is mobilized, and it is diffusely distributed in the new tegument. Lagarrigue [3] shows that the calcareous lamelar layer of the crust begins to form itself only 3—4 days after moulting. A Ligidium *hypnorum* female was dissected at the moment of moulting

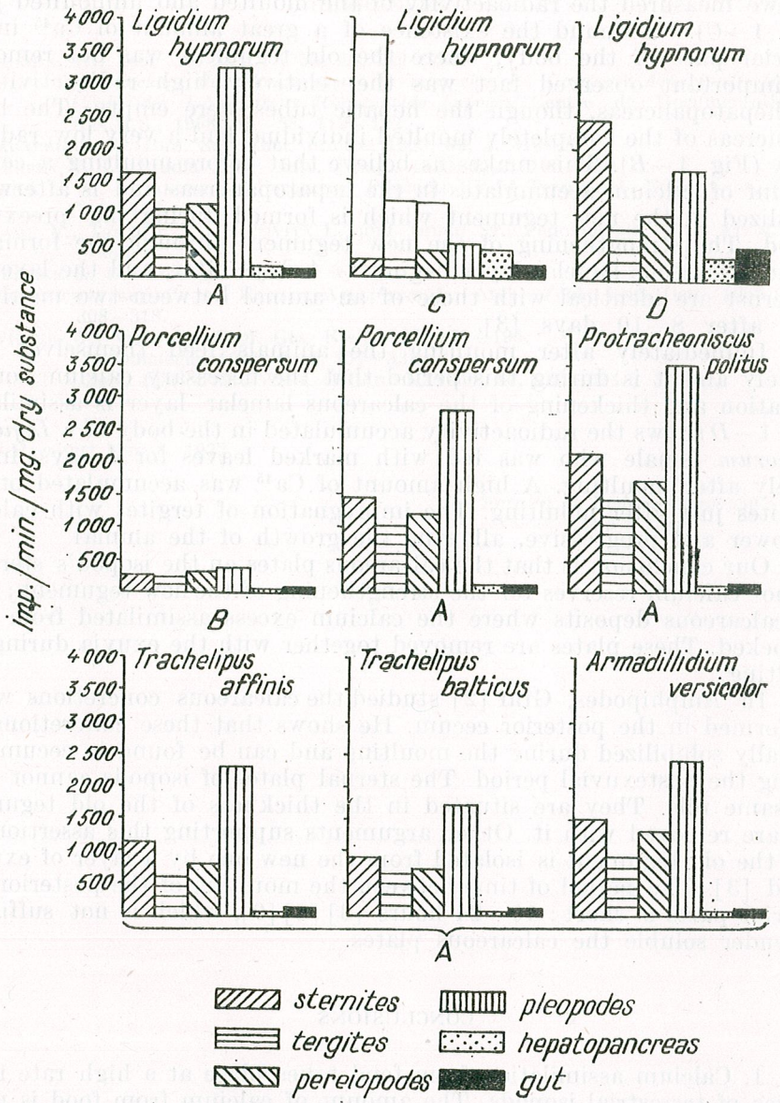


Fig. 1. — Ca^{45} concentration in different organs and parts of tegument in terrestrial isopods species. *A*, animals between two moultings *B*, animal immediately after moulting *C*, animal during moulting (the marked spaces show the radioactivity of the moulted parts, the white ones show the radioactivity of the unmoulted parts) *D*, animal immediately after moulting, fed with marked leaf for four days.

and we measured the radioactivity of the moulted and unmoulted parts (Fig. 1—*C*). We found the existence of a great amount of Ca^{45} in the anterior part of the body, where the old tegument was not removed. An important observed fact was the relatively high radioactivity of the hepatopancreas, though the hepatic tubes were empty. The hepatopancreas of the completely moulted individual had a very low radioactivity (Fig. 1—*B*). This makes us believe that before moulting a certain amount of calcium accumulates in the hepatopancreas and is afterwards mobilized in the new tegument which is formed during the preexuvial period. The strengthening of the new tegument through the formation of the calcareous lamelar layer begins 3—4 days after, and the layers of the crust are identical with those of an animal between two moultings only after 8—10 days [3].

Immediately after moulting the animals feed themselves very actively and it is during this period that the necessary calcium for the formation and thickening of the calcareous lamelar layer is assimilated. Fig. 1—*D* shows the radioactivity accumulated in the body of a *Ligidium hypnorum* female who was fed with marked leaves for 4 days, immediately after moulting. A high amount of Ca^{45} was accumulated on the sternites just after moulting. The impregnation of tergites with calcium is slower and progressive, allowing the growth of the animal.

Our conclusion is that the calcareous plates on the isopod's sternites are not calcium reserves for the strengthening of the new tegument; they are calcareous deposits where the calcium excess assimilated from food is stocked. These plates are removed together with the exuvia during the moulting.

In Amphipodes, Graf [2] studied the calcareous concretions which are formed in the posterior cecum. He shows that these concretions are partially solubilized during the moulting and can be found in cecum also during the postexuvial period. The sternal plates of isopods cannot have the same rôle. They are situated in the thickness of the old tegument and are removed with it. Other arguments supporting this assertion are that the old tegument is isolated from the new one by a layer of exuvial liquid [3]. The period of time between the moulting of the posterior and anterior parts is short: 10—24 hours [3] [8] [9], which is not sufficient to render soluble the calcareous plates.

CONCLUSIONS

1. Calcium assimilation from food takes place at a high rate in all species of terrestrial isopods. The amount of calcium from food is not a factor which might limit the distribution of species in the areal.

2. Calcium concentration in different organs and parts of the tegument is different in an animal, but very similar from one species to another. The calcium excess which appears in the organism and is removed with the exuvia is deposited on the sternites.

3. The calcareous sternal plates do not serve for the strengthening of the new tegument. Its strengthening is slow and progressive, on the basis of the calcium directly assimilated from food after moulting.

REFRENCES

1. Dunger W., Zool. Jb. Syst., 1958, **86**, 139—180.
2. Graf F., *Le stockage de calcium avant la mue chez les Crustacés Amphipodes Orchestia (Talitridé) et Niphargus (Gammaride hypogé)*. Centre de Documentation du C.N.R.S., 1968, 45—58, 119—122.
3. Lagarrigue J., Bull. Soc. Zool. France, 1965, **89**, *4*, 310—316.
4. Mehely V. L., Studia Zool. Univ. Hung., 1932, **2**, *2*, 103—120.
5. Paris H. O. a. Sikora A., Secondary Productivity of Terrestrial Ecosystems, 1967, **2**, 741—766.
6. Radu V., Tomescu N., Racoviță L. and Imreh St., Pedobiologia, 1971, **11**, *4*, 277—285.
7. Reichle E. D., Ecology, 1967, **48**, *3*, 351—366.
8. Tchernigovtzeff C. et Ragage-Willigens J., Arch. Zool. Exp. Gen., 1968, **109**, *2*, 308—318.
9. Wieser W., Verh. Dt. Zool. Ges. Kiel, 1964, 178—195.
10. — Pedobiologia, 1965, **5**, *4*, 304—331.
11. — Zool. Inst. Univ. Wien, Österreich, 1966, **14**, 326—334.

Received May 8, 1972

Faculty of Biology-Geography,
Zoology Chair
Cluj

KARYOTYPE EVOLUTION DURING FOUR TRANSPLANT PASSAGES OF A 3,4-BENZPYREN-INDUCED TUMOUR IN GOLDEN HAMSTER

BY

AGRIPINA LUNGEANU and ADRIANA STANA

Chromosomal analysis of four transfer generations in tumor induced by 3,4-benzpyren on the golden hamster, showed that the transplantable tumor cells have a high diversity. The presence in a high proportion of metaphases with abnormal chromosomes, is considered as being linked to the genetic dereglation of cell division.

Karyotype evolution in tumors is one of the main problems in oncology to which cytogenetics is supposed to give an answer. The present paper describes an attempt to follow such a karyotype evolution during isologous transplantations of a 3,4-benzpyren-induced tumour in the golden hamster. The tumour under study has been propagated in vivo for ten transplant generations, the cytogenetic examinations being performing in the 7th, 8th, 9th and 10th passages.

MATERIAL AND METHODS

The tumours were prepared for chromosome studies by usual techniques, as soon as they reached suitable dimension to become palpable. A tumour piece was graft dorsally, the transplantation pattern being as follows :

Passage : 6—7—8—9—10

Sex : ♀—♀—♀—♂—♀

The tumours displayed high growth rates, becoming visible after two weeks from the graft Karyotypes were constructed according to Lehman [12] and Ishihara [10].

REV. ROUM. BIOL.—ZOOLOGIE, TOME 17, No 6, p. 427—429, BUCAREST, 1972

RESULTS

The cytogenetic constitution in four tumours of the 7th passage was analysed, the corresponding karyotype being constructed for each of them. A high karyotype instability was found, with chromosome numbers ranging from 41 to 155, with no significant maximum, thus with no stem line.

A similar instability was shown by the 8th passage, though the variation of chromosome numbers was confined in this case to a smaller interval (43—127), while the yield of polyploid cells decreased from 28.7 p. c. in the previous passage, to 16.6 p.c.

One of the five tumours studied in the 9th passage displayed a stem line with chromosome numbers between 60 and 64. The remaining four tumours in this passage showed about 60 p.c. polyploid cells with ploid levels around 6n and 40 p. c. pseudodiploid cells.

At the 10th passage, one of the three tumours examined showed a chromosome distribution indicating a marked decrease in the heteroploid cells occurrence, 50 p. c. of the cells being subsequently pseudodiploid and 50 p. c. aneuploid. In one of the tumours in this passage, hexaploidy in a p. c. 87 p. c., was found to predominate over the 13 p. c. diploid cells.

On the other hand, during the successive transplantations of the tumour investigated it was observed a reduction or even a lack of chromosome aberrations occurring with high frequency in the first passage examined, such as dicentric chromosomes, secondary constrictions, deletions, steaky chromosomes.

DISCUSSION

Cytogenetic studies of the tumours induced by polycyclic hydrocarbons in laboratory animals revealed in a significant number of them, chromosome alterations consisting in large variations of chromosome numbers, as compared to the high constancy shown by the normal somatic cells [1] [6] [9] [15] [17]. In agreement with these data, the passages analysed by us in benzpyren-induced tumours, display a noticeable karyotype diversity, indicating the tumoural selection processes as not yet accomplished. This karyotype instability is taken into account by Levan (1967) as one of the indispensable stages in the cytogenetic evolution of the tumour [14]. In the case of the tumour analysed by us, pseudodiploid cells seem to have a certain selective advantage, similarly to the results obtained by Huang and Strong (1963) with dimethyl-benzanthracene-induced tumours in Mastomys [9] and by Cassingena and Suarez with 20 methylcholanthrene-induced tumours in syrian hamster [2]. The results should not be considered as definitive, as no indication is available whether the same chromosome constitution occurs in subsequent transfer generations.

The late stabilization of the tumour karyotype in a stem line does not seem surprising, being not an unique case. Haemmerli et al (1966)

reported a reticulosarcoma in the golden hamster which reached a modal number after 68 transplantations only [3]. Several authors, like Huang and Strong (1963), Cassingena and Suarez (1967), Nowell and Ferry (1963), observed the maintainance of the diploid modal number during successive transplantations of certain chemically induced tumours and methylcholanthrene-induced chloroleucemia [9] [2] [16].

As to the mechanisms involved in the production of the number aberrations observed, these probably consist in selective non-disjunction or endoreduplication of single chromosomes, which might result in the acquisition and duplication of structurally normal chromosomes or markers. It should be noted that polyploidization preferentially involves the pairs of subtelocentric and metacentric autosomes.

CONCLUSIONS

1. The study of cytogenetic evolution of 3,4-benzpyren-induced tumours during four successive passages, revealed a marked karyotype diversity.

2. As the genotypic heterogeneity in the tumours of the 10 th passage is still quite marked, no statement is to be made concerning the chromosome constitution of the line to be stabilized by selection, to survive the competition and to become adapted to the neoplasic environment

REFERENCES

1. Biedler J. L., Proc. Amer. Assoc. Cancer Res., 1962, **3**, 304.
2. Cassingena R. and Suarez H., Int. J. Cancer, 1967, **2**, 5.
3. Haemmerli G., Zweidler et Sträulirr. H., Int. J. cancer, 1966, **1**, 599.
4. Hauschka T. S., J. Cell and Comp. Physiol., 1958, **52**, (suppl. 1), 197.
5. — , Cancer Research, 1961, **21**, 8.
6. Hellström K. E., J. Nat. Cancer Inst., 1959, **23**, 1019—1033.
7. Homburger F., Cancer, 1969, **23**, No. 2, 313—337.
8. Hsu T. C., Intern. Rev. Cytol., 1961, **12**, 69.
9. Huang C. C. and Strong L. C., Cancer Research, 1963, **23**, 1800—1807.
10. Ishihara T., Moore G. E. and Sandberg A. A., J. Nat. Cancer Inst. 1962, **29**, 161—165.
11. Jean P. et Bois P., Rev. Can. Biol., 1967, **26** 207—227.
12. Lehman J. M. Mac Pearson I. and Moorhead P. S., J. Nat. Cancer Inst., 1963, **31**, 635—650.
13. Lejeune J., Bulletin U.I.C.C., 1966, **4**, 4.
14. Levan A., Hereditas, 1967, **57**.
15. Nachtigal M. and al., J. Nat. Cancer Inst., 1967, **38**, 697—721.
16. Nowell P., J. Nat. Cancer Inst., 1963, **30**, 687—703.
17. Popescu N. C. and al., Int. C. Cancer, 1969, **4**, 185—792.

Received April 8, 1972

The "Traian Săvulescu" Institute
of Biology
Department of Genetics

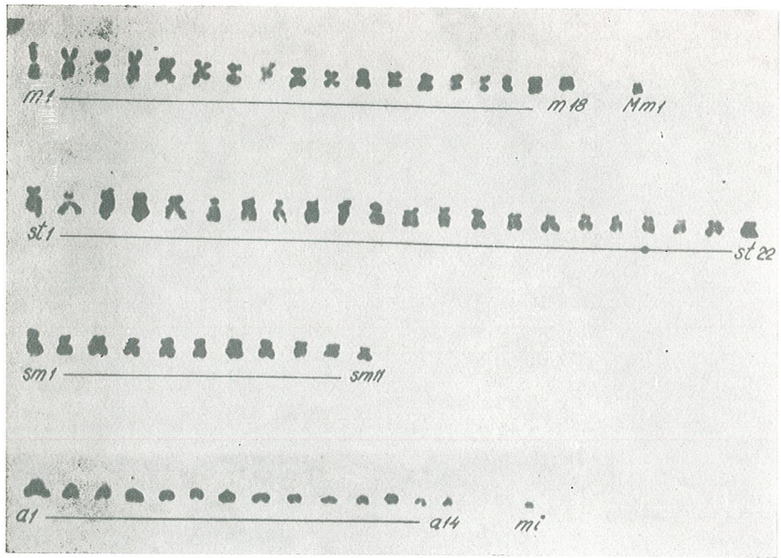


Fig. 1. — Karyotype of a hypertriploid cell with 67 chromosomes (7th passages): the subtelocentrics show values greatly higher than the ploidy level.

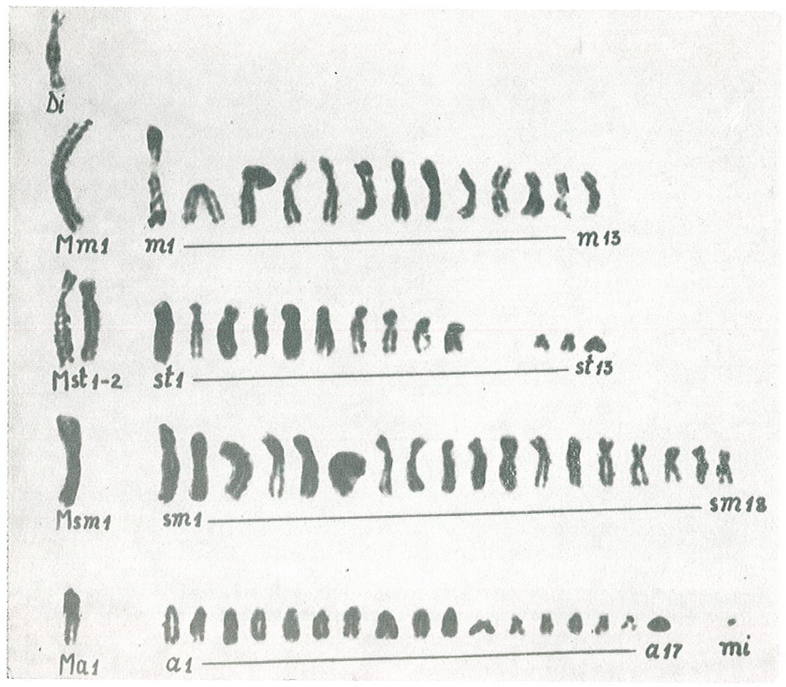


Fig. 2. — Karyotype of a hypertriploid cell with 68 chromosomes from a 7th passage tumour: note the occurrence of a dicentric chromosome, of the markers Mml, Mst1, Mst2, Msml, and Ma1 as well as of a minute chromosome.

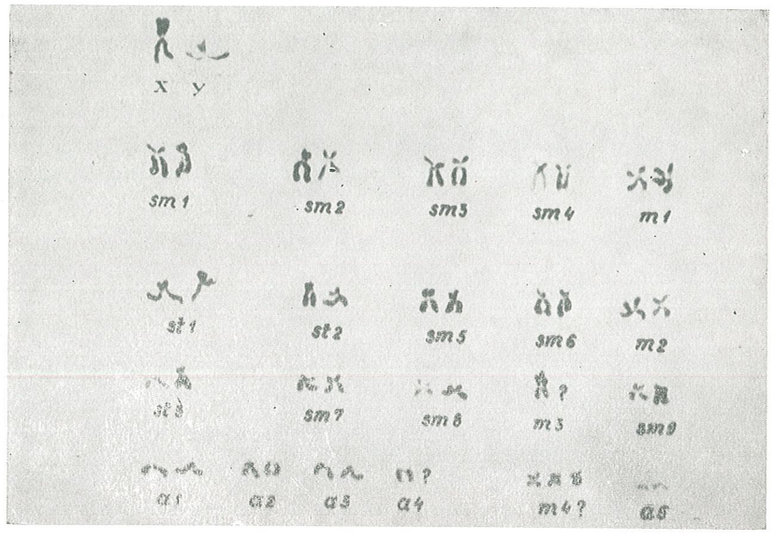


Fig. 3. — Karyotype of a hypodilpoid cell in a 7th passage tumour : monosomy for m3 and a4, as well as trisomy for m4 are to be observed.

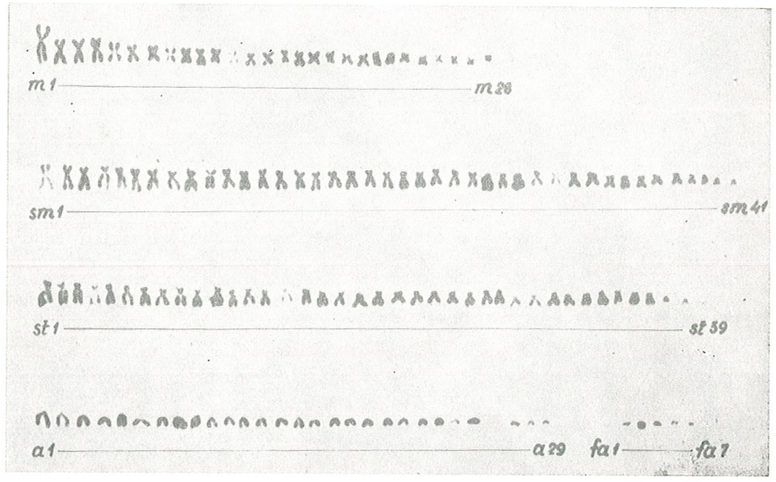


Fig. 4. — Karyotype with 137 chromosomes and 7 acentric fragments of a 9th passage tumour : intensive multiplication of the st1, st2, and st3 chromosomes (from 3 pairs to 19 pairs) is striking.

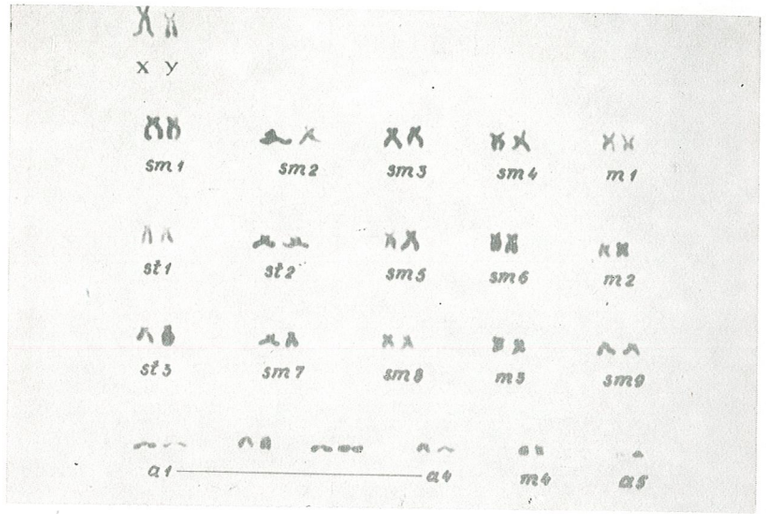


Fig. 5. — Diploid karyotype of a 9th passage tumour.

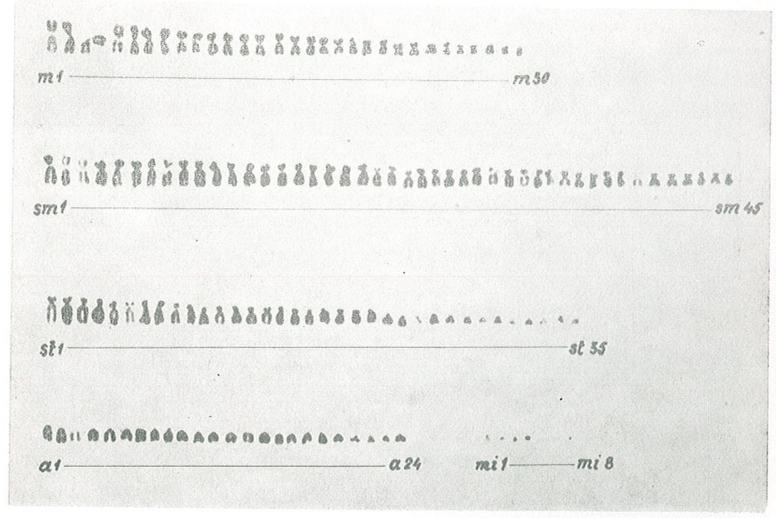


Fig. 6. — Hexaploid karyotype of a 9th passage tumour : the occurrence of eight minute chromosomes and preferential polyploidization of the metacentric and subtelocentric groups are to be observed.

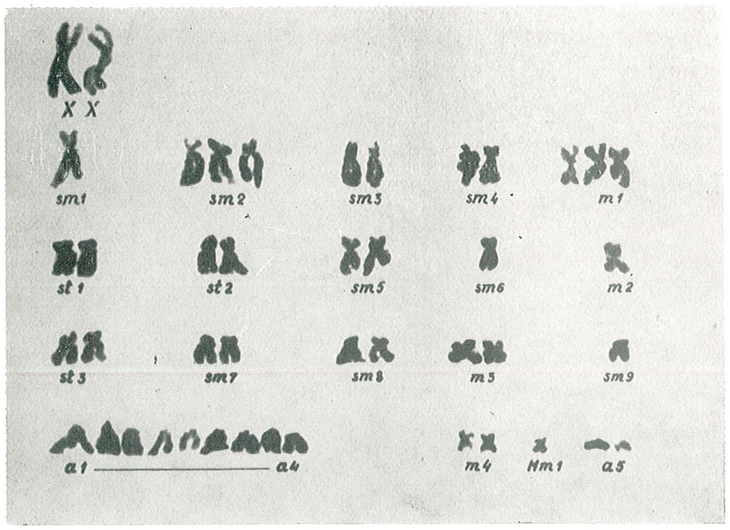


Fig. 7. — Karyotype of a pseudodiploid cell in the 10th passage tumour : it should be noted the trisomy for the m1 pair, the monosomy at the sm1, sm6, m2 sm9 pairs, as well as the occurrence of the Mm1 marker.

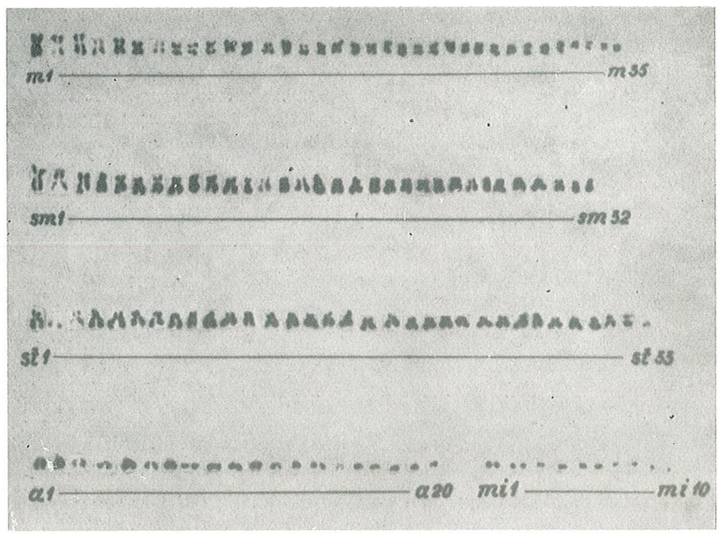


Fig. 8. — Karyotype with 120 chromosomes of a 10th passage tumour : observe the preferential polyploidization of metacentric pairs (from 4 pairs to 17 pairs) and subtelocentric pairs (from 3 pairs to 16 pairs).

REVUE ROUMAINE DE BIOLOGIE

— SÉRIE DE ZOOLOGIE —

TOME 17 1972

INDEX ALPHABÉTIQUE

AVIS AUX AUTEURS

La « Revue Roumaine de Biologie — Série de Zoologie » publie des travaux 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 trois 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 du prénom, le titre de la revue, abrégé, conformément aux usances internationales, l'année, 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 Independenţei, Bucarest.

PRINTED IN ROMANIA