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SOMMAIRE

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CHIROCEPHALUS DIAPHANUS ROMANICUS n. ssp. *(PHYLLOPODA, ANOSTRACA)*

A. STOICESCU

The specimens of *Chirocephalus diaphanus* from Romania (temporary pools from the south-east to the south-west of the country and alpine pools in the Bucegi Mountains) differ from the western European nominal subspecies described by Daday (4) and even from the population from Slovakia described by Brtek (2, 3) in several characteristics described in the French text and illustrated in the paper, being ascribed to a new subspecies, *Chirocephalus diaphanus romanicus*.

L'espèce est répandue en particulier dans les pays situés à proximité de la Méditerranée et de la mer Noire : le Maroc, l'Algérie, la Tunisie, la Syrie, la C.E.I. (le Caucase), la Grèce, l'Albanie, la Yougoslavie, la Bulgarie, la Roumanie et la Hongrie. Vers l'ouest cette espèce se rencontre en Suisse, en France, en Espagne, jusqu'en Angleterre et vers le nord en Allemagne et en Pologne (Gdansk).

Chirocephalus diaphanus (Prevost, 1803) forme des populations bien isolées au point de vue géographique, chacune d'entre elles correspondant à une race géographique à part, décrites initialement en tant que variétés : *salinus* Daday, 1910, *carinatus* Daday, 1910 *pentheri* Pesta, 1921.

Les recherches entreprises sur le territoire de la Roumanie par N. Botnariuc et T. Orghidan (1), ont mis en évidence la présence de cette espèce dans les eaux temporaires de printemps et d'automne dans la plaine et dans les creux alpins et les lacs glaciaires, l'été. À la suite des investigations des années 1967—1991 sur la faune des eaux temporaires de la plaine du Banat, d'Olténie, de Valachie et dans les creux alpins des Monts Bucegi, ont été identifiées des populations de *Chirocephalus diaphanus* formées d'exemplaires qui présentaient certaines différences par rapport à la sous-espèce ouest-européenne et qui appartenaient à une nouvelle race, dont nous allons présenter en ce qui suit la description sous le nom de *Chirocephalus diaphanus romanicus* n. ssp.

Matériels : des centaines d'exemplaires dans les localités suivantes : Oltenița, département de Călărași, le 13. 4. 1967, le 3. 5. 1988, le 15. 4. 1991 ; Comana, département de Giurgiu, le 28. 4. 1977 ; Chirnogi, le 3.5. 1988 et Călărași, le 28. 4. 1988, département de Călărași (leg. A. Stoicescu) ; Ghencea, le 3. 5. 1961 et le 16 février, le 6. 5. 1988 autour de Bucarest ; Uberland, marais situé au N—E de la ville de Timișoara, le 10. 4. 1985, le 18. 4. 1990 département de Timiș ; Recas, le 12. 4. 1988 et Sinnicolaul Mare, le 18. 4. 1987, département de Timiș ; Romula au nord de Caracal, le 9. 4. 1986, le 6. 5. 1988, département d'Olt ; Cernica, le 10. 4. 1988 et Malul Spart, le 23. 4. 1988, le Secteur agricole Ilfov (leg. P. Bănărescu) ; Piatra Arsă dans les Monts Bucegi, le 15. 7. 1991 (leg. D. Cogălniceanu).

Le mâle. Le corps est robuste. La longueur du corps, du front jusqu'à l'extrémité des cercopodes est comprise entre 15—30 m.m. La couleur du corps est blanchâtre avec des nuances verdâtres.

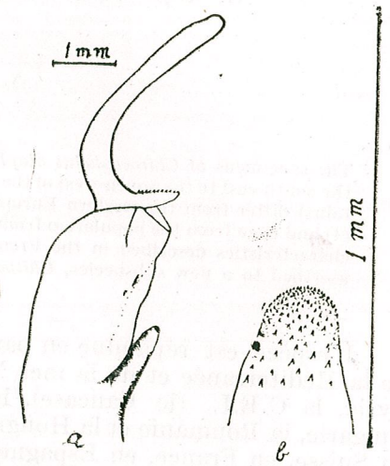

Fig. 1. a. — L'antenne II du mâle ; b. — — Le bout distal du prolongement digitiforme de l'article basal de l'antenne II.

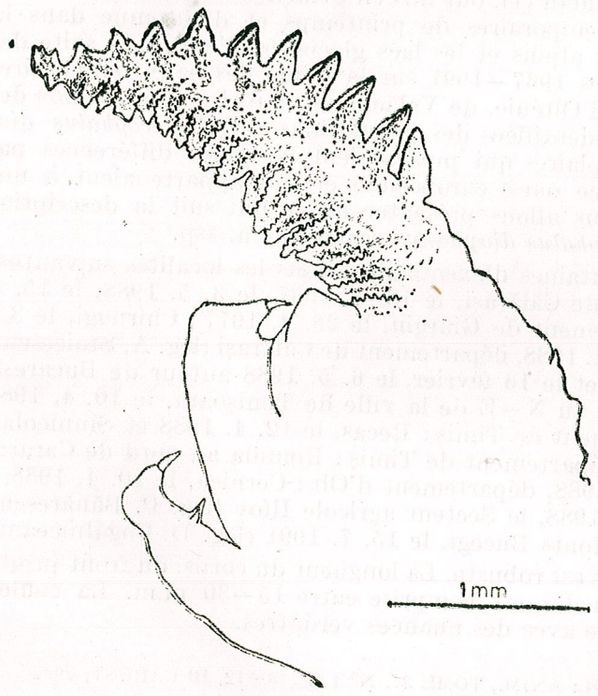

Fig. 2.—L'appendice antennal dorsal (serriforme).

Le bouclier céphalique est arrondi. Le pédoncule oculaire a la longueur entre 0,5—1 m. m. L'antenne I n'est pas plus longue que l'article basal de l'antenne II. L'antenne II est biarticulée (fig. 1a).

L'article basal de l'antenne II atteint la longueur de 4,5 m. m. aux exemplaires les plus grands. Dans la partie inférieure, l'article basal a un prolongement digitiforme au bout distal arrondi et couvert de petites épines (fig. 1 b). Près de ce prolongement digitiforme il y a sur la partie interne de l'article basal, l'appendice antennal dorsal (serriforme), tordu en spirale et plus long que l'article basal. L'appendice antennal dorsal est comme une lame au bout distal aminci et sur les bords des prolongements coniques pourvus de rangées médiales de denticulées (fig. 2). L'appendice antennal dorsal a dans la partie basale et externe 3—5 prolongements digitiformes qui ont au bout et sur les bords des épines (fig. 3a, b). Sur le total des exemplaires étudiés, 56% avaient 3 prolongements digitiformes ; 20% avaient 4 prolongements digitiformes ; 23% avaient 3 prolongements digitiformes sur un appendice antennal dorsal et 4 sur l'autre ; 1% avait 5 prolongements digitiformes sur chaque appendice antennal dorsal ou 5 prolongements sur un appendice et 4 sur l'autre.

Nous ne trouvons aucun exemplaire à deux prolongements digitiformes. Au cas où il y a 3 ou 4 prolongements digitiformes, alors le premier prolongement est plus long (fig. 3 b), lorsqu'il y a 5 prolongements digitiformes, le deuxième est plus long (fig. 3 a). Par le nombre des prolongements digitiformes la race *romanicus* se distingue de celle ouest-européenne décrite par Daday (4) (fig. 4) et Brtek (2, 3) qui avait 2—4 prolon-

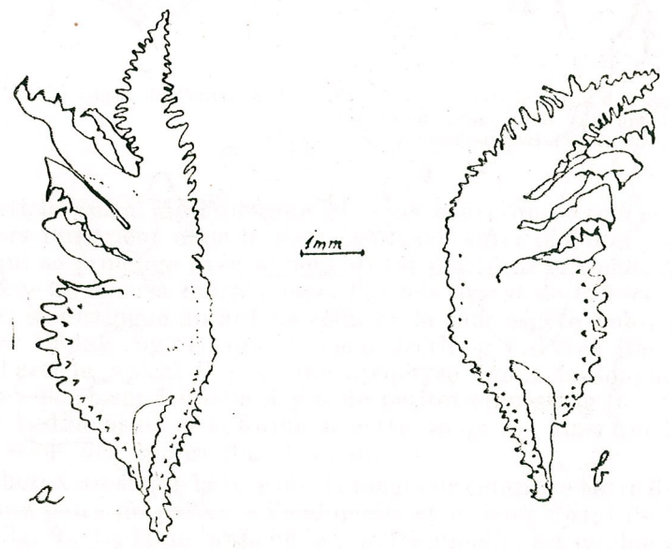

Fig. 3. a. — L'appendice antennal dorsal à 5 prolongements, de pair avec l'appendice antennal ventral ; 3. b. — L'appendice antennal dorsal à 4 prolongements, de pair avec l'appendice antennal ventral.

gements. L'appendice antennal ventral (fig. 5) présente deux parties. La partie externe est à peu près triangulaire ayant 10—11 prolongements sur le bord, qui diminue vers la partie interne dans de simples monticules ; à la base de chaque prolongement il y a un petit monticule qui se termine par une épine orientée vers l'intérieur. La partie interne de l'appendice antennal ventral a un pli caractéristique et un prolongement pourvu, sur les bords, de petits monticules et denticules.

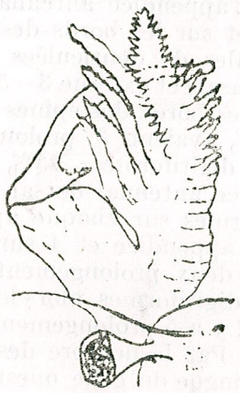

Fig. 4. — L'appendice antennal dorsal et l'antenne II du mâle d'après Daday.

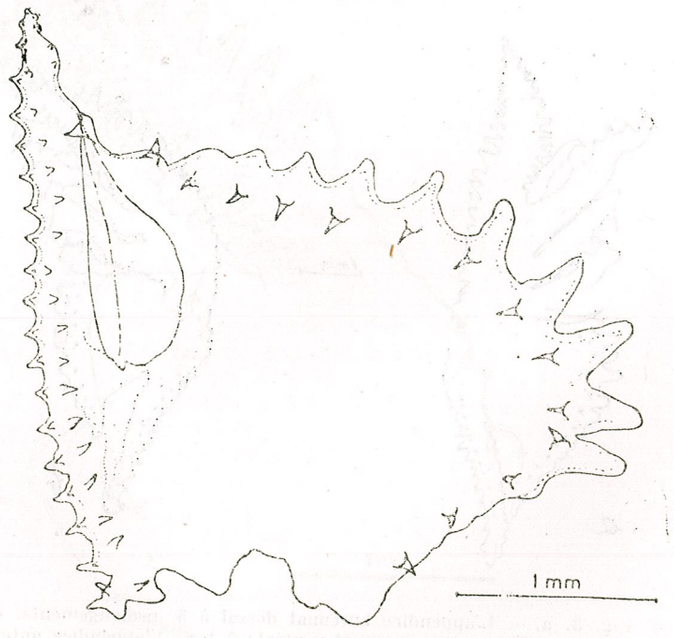

Fig. 5. — L'appendice antennal ventral.

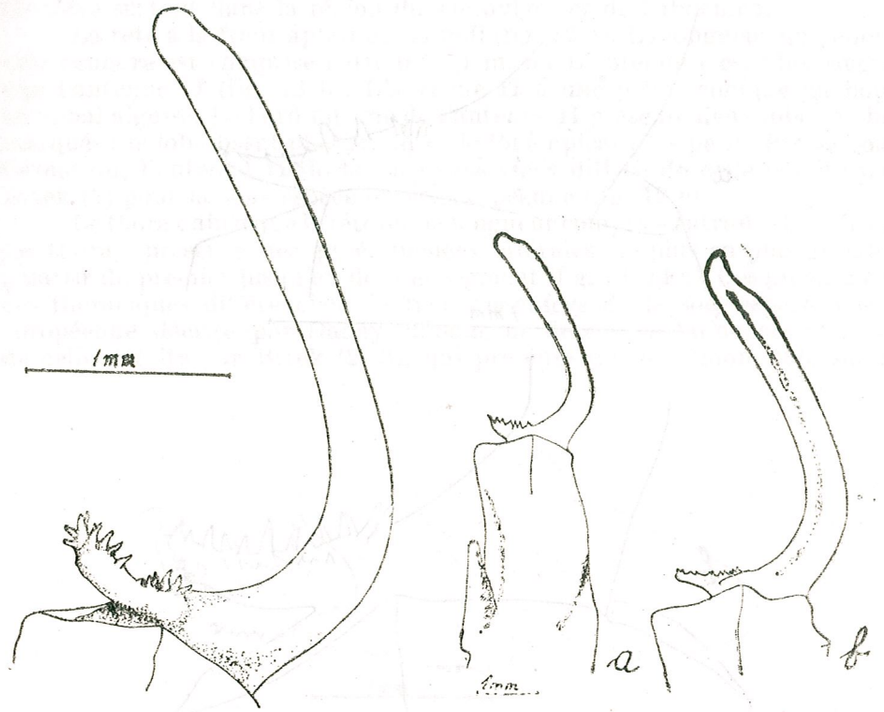

Fig. 6. — L'article apical de l'antenne II.

Fig. 7. a. — L'article apical de l'antenne II à la race romanicus ; 7. b. — L'article apical de l'antenne II d'après J. Brtek.

L'article apical de l'antenne II, plus court que l'article basal, est courbé vers l'intérieur dans le tiers proximal, suivi par une portion plus amincie qui se prolonge avec le bout distal plus gros et aplati sur la face externe (fig. 6). Par sa morphologie, l'article apical de la race *romanicus* (fig. 6, 7a) se distingue autant de celui de la sous-espèce ouest-européene décrite par Daday (fig. 4), que de celui décrit par Brtek (fig. 7b). À la base de l'article apical il y a une apophyse ayant la longueur entre 0,2—0,7 m. m. et sur laquelle il y a de petites épines (fig. 6). Très variables sont la dimension et la forme de cette apophyse ainsi que le nombre et l'orientation des épines (fig. 8 a, b).

Le thorax ainsi que la tête ont la longueur comprise entre 6—14 m.m. La septième paire de pattes a l'endopodit et le bout distal de l'exopodit arrondis (fig. 9). La lame branchiale (le préépipodit) est double aux bords dentelés ; le sac branchial (l'épipodit) est allongé ayant les bords lisses (fig. 9, 10).

La longueur de l'abdomen est entre 5—11 m. m. Le dernier segment abdominal est plus court que les autres. Les cercopodes sont allongés,

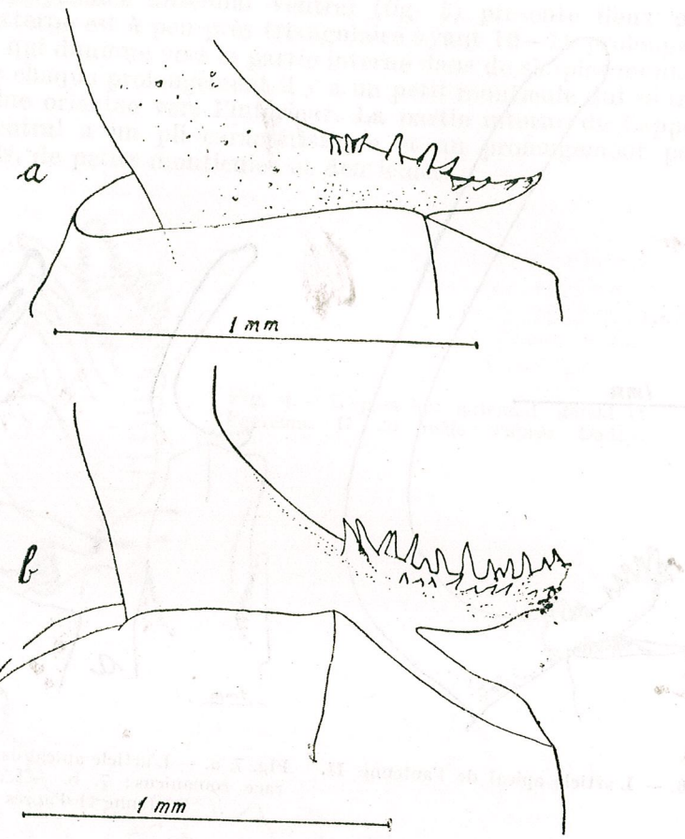

Fig. 8. a, b. — Diverses formes de l'apophyse d'article apical de l'antenne II.

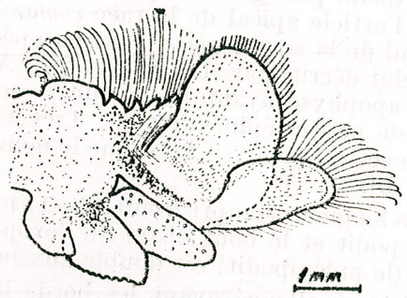

Fig. 9. — La septième paire de pattes du mâle.

atteignant une longueur entre 2—5 m. m., ayant plus de 80 poils chacun. Le pénis (fig. 12a) a la pièce basale avec de nombreuses épines sur les bords (fig. 11 a, b).

La femelle. La longueur du corps est plus grande que celle du mâle étant comprise entre 13—34 m. m. La couleur du corps est verdâtre ou bleuâtre surtout dans la région du sac oviger et de l'abdomen.

La tête a la front aplati et arrondi (fig. 12 a). La longueur du pédoncule oculaire est comprise entre 0,5—1 m. m. L'antenne I est plus longue que l'antenne II (fig. 12 b). L'antenne II a une forme conique au bout terminal aiguisé. Le bord interne de l'antenne II présente deux lobes moins marqués ; le lobe basal plus grand et le lobe apical plus petit. Par sa conformation, l'antenne II de la race *romanicus* diffère de celle décrite par Brtek (2) pour la sous-espèce ouest-européenne (fig. 12 c).

Le thorax ainsi que la tête ont la longueur comprise entre 6—15,5 m.m. Le thorax présente des proéminences latérales de plus en plus grandes à partir du premier jusqu'au dernier segment (fig. 13, 14 a). Ces proéminences thoraciques différencient la race *romanicus* de la sous-espèce ouest-européenne décrite par Daday, dépourvue de ces proéminences et aussi de celle décrite par Brtek (2, 3), qui présénte un petit monticule sur le

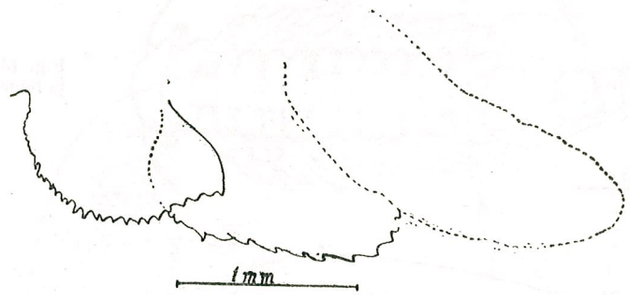

Fig. 10. — La lame branchiale et le sac branchial de la septième paire de pattes.

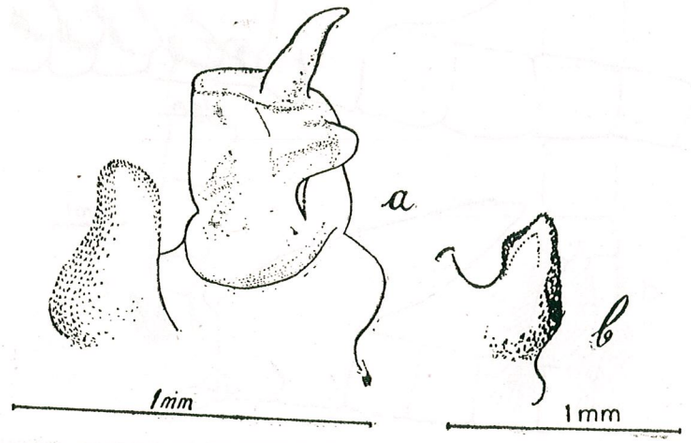

Fig. 11. a. — Le pénis ; 11. b. — La pièce basale du pénis (vue latérale).

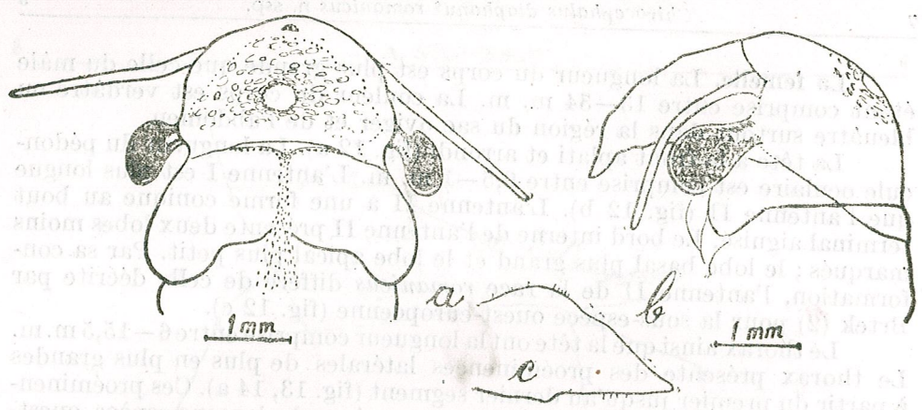

Fig.12. a. — La tête de la femelle (vue de haut); 12 b. — La tête de la femelle (vue latérale); 12. c. — L'antenne II de la femelle d'apres J. Brtek.

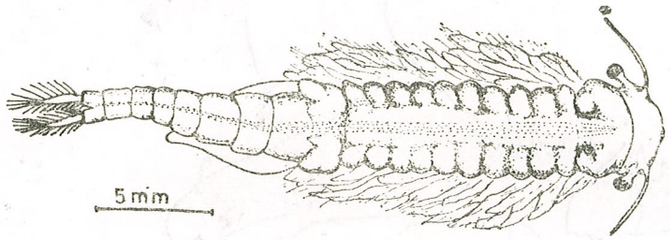

Fig. 13. — Le corps de la femelle (vue de haut).

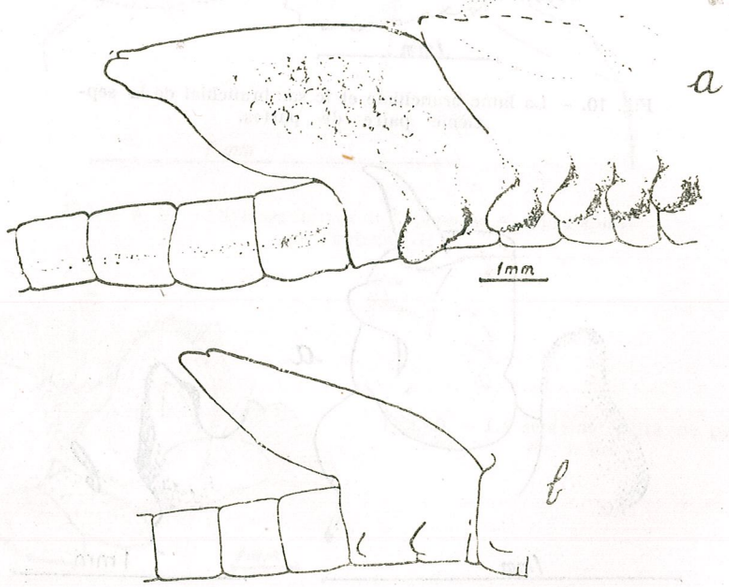

Fig. 14. a. — Les derniers segments thoraciques et les premiers segments abdominaux de la femelle de la race romanicus. 14. b. — Le dernier segment thoracique et les premiers segments abdominaux d'après J. Brtek.

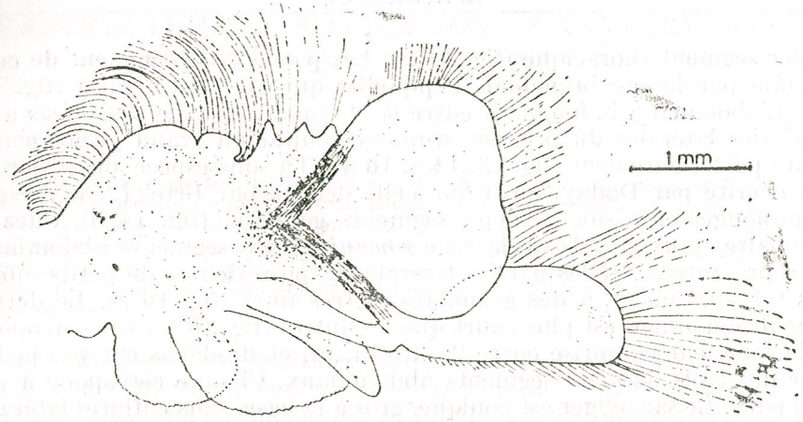

Fig. 15. — La septième paire de pattes de la femelle.

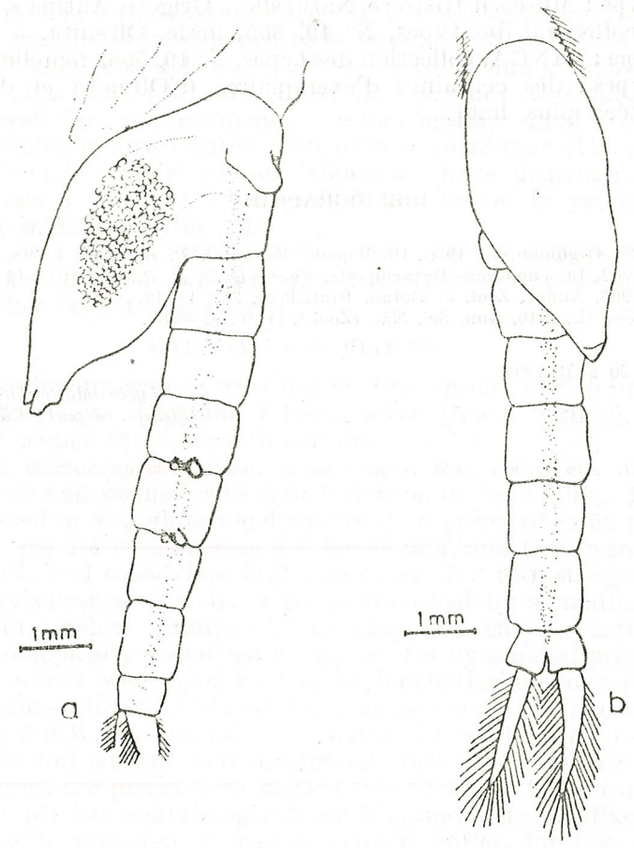

Fig. 16. a. — L'abdomen et le sac oviger (vue latérale). 16. b. — L'abdomen et le sac oviger (vue de haut).

dernier segment thoracique (fig. 14 b). Les pattes se distinguent de celles du mâle par le sac branchial (l'épipodit) qui est plus aiguisé (fig. 15).

L'abdomen a la longueur entre 6—14 m. m. La race *romanicus* a sur les parties latérales du premier segment génital un grand prolongement orienté postérieurement (fig. 13, 14 a, 16 a). La sous-espèce ouest-européenne décrite par Daday, ainsi que celle décrite par Brtek (2, 3) présente des prolongements sur les deux segments génitaux (fig. 14 b). Chez les exemplaires plus grands de la race *romanicus*, les segments abdominaux 5 et 6 présentent sur les parties latérales et postérieures de petits monticules 0,1—0,2 m. m. à des granulations très fines (fig. 16 a). Le dernier segment abdominal est plus court que les autres (fig. 16 b). Les cercopodes ont la longueur comprise entre 2—4,5 m. m. et ne dépassent pas la longueur des trois derniers segments abdominaux. Chaque cercopode a plus de 80 poils. Le sac oviger est conique, gros à la base, sans enflures latérales, ayant la longueur entre 3—9 m. m. et ne dépasse pas le segment 5 abdominal.

Holotype : Musée d'Histoire Naturelle « Grigore Antipa », Bucarest (MINGA), collection des types, N° 49. 505, mâle, Oltenița.

Allotype : MINGA, collection des types, N° 49. 506, femelle, Oltenița.

Paratypes : des centaines d'exemplaires d'Oltenița et des autres localités citées plus haut.

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Lycée industriel nº 2
Oltenița, départ. Călărași

ULTRASTRUCTURAL LOCALIZATION OF ARYLSULFATASE ACTIVITY IN FISH OOCYTES

R. MEȘTER, D. SCRIPCARIU and LOTUS MEȘTER

Ultrastructural cytochemistry of fish oocytes showed them to possess arylsulfatase. The enzyme was located at the periphery of yolk platelets, at the points of contact between lipid droplets and yolk platelets, at the level of zona radiata, in lysosomes like structures and in multivesicular bodies. This heterogeneous localization suggests that arylsulfatase may function in controlling the metabolism of glycoconjugates and sulfated glycolipids during the process of oocyte maturation.

Arylsulfatases or arylsulfate sulfohydrolases (EC 3. 1. 6. 1) are a group of enzymes which catalyse the hydrolysis of the sulfate group from many compounds, including sulfated glycoconjugates, sulfatides and tyrosine sulfated proteins. Mammalian arylsulfatases were classified into three types (A, B and C). Arylsulfatases A and B are considered lysosomal enzymes, while arylsulfatase C is a microsomal enzyme, whose physiological substrates are believed to be steroid sulfates (4), (5), (7), (10).

Arylsulfatases A and B were demonstrated in many organs of vertebrates (3), (12), (15), (16). Moreover, the enzymes were identified in several intracellular compartments : mitochondria, small vesicles of the Golgi complex and associated with plasma membrane (11), (12), (13), (18), (22). Previous studies of our laboratory have demonstrated that the enzyme has a different cytochemical localization in young oocytes of fish and in mature oocytes (14).

In order to gain additional information on the cytochemical distribution of arylsulfatase, we carried out a study of the localization of enzyme in other teleost fish oocytes.

MATERIALS AND METHODS

Our experiments were carried out on two species of fish : *Ctenopharyngodon idella* (Grass carp) and *Ictiobus niger* (Black buffalo), supplied by the Nucet piscicultural research station.

Electron microscopical study. The ovary was removed, minced in small fragments and washed with saline solution of NaCl 0.6%. The small pieces were fixed in 2% glutaraldehyde solution prepared in 50 mM cacodylate buffer, pH 7.4 with sucrose 3% for 30 min, and then were washed four times with cold cacodylate buffer solution. The ultrastructural localization of arylsulfatase activity was accomplished by a modification of the method of Zucher—Franklin et al. (22), and enzyme activity was electron microscopically visualized in the oocytes by a metal precipitation method. The pieces were incubated in an incubation medium consisting of sodium acetate buffer 0.1 M, pH 5.0, lead acetate 1 mM and p-nitrocatechol sulfate 5 mM. Incubation was carried out for 1—2 hours at 37°C. The specimens for control were incubated with substrate-free medium. After incubation, the pieces were washed five times for 10 min in veronal acetate buffer pH 5.0, containing sucrose 3%, and were postfixed in 1% osmium tetroxide prepared in acetate-veronal buffer, for two hours at cold. The pieces were dehydrated and embedded in Epon 812 resin. Ultra-

thin sections were examined either unstained or stained with uranyl acetate and lead citrate in a Philips EM 201 electron microscope.

RESULTS AND DISCUSSION

In figure 1 B two lysosomes and a multivesicular body are seen showing the presence of the arylsulfatase reaction product. The membrane boundary, the lysosomes like structures and the moderately electron--dense black foci can be readily seen. A multivesicular body containing lamellar lipid rich inclusions with numerous reaction products of enzyme which appear evenly distributed over the entire surface is also observed. The presence and the frequency with which multivesicular like bodies arise in the oocyte is difficult to estimate. Their formation probably contributes to the regulation of the sizes of endocytotic structures and related lysosomes by providing a sort of microautophagic mechanism for disposing of excess membrane from the cell surface. Such a process could endow cells to metabolize materials acquired from their own cytoplasm as well as those picked up by endocytosis. For example, Drosophila eggs at about the time of ovulation form many multivesicular bodies that may be involved in degrading of receptors no longer required for the uptake of yolk proteins (8).

Apart from this localization, the reaction product of arylsulfatase was also identified at the periphery of yolk platelets (Fig. 1 A and 2 C), and at the points of contact between platelets or between platelets and some lipid droplets (Fig. 2 A and B). The relationship of aryl sulfatase activity and yolk platelets is unclear, in part because the major natural substrates for enzyme are still uncertain. However, the presence of arylsulfatase at the periphery of yolk platelets suggests the existence of a functional relationship between the enzyme and the sulfated compounds in the formation of platelets. In this context, it has been shown that in *D. melanogaster*, the major tyrosine sulfated proteins were found to be yolk proteins, suggesting the essential role of tyrosin sulfation in the functioning of yolk proteins (2). Chemically, yolk platelets consist of proteins, phospholipids and neutral lipids, but the presence of sulfated compounds was not detected. Fatty yolks in the oocytes occur in the form of droplets of various size which coalesce to form large fatty droplets (19). Their relationship with yolk platelets is not clear. The enzyme probably contributes to the desulfation of glycolipids. The information presently available does not allow us to distinguish between these possibilities. Relationships are also conceivable that sulfated glycolipids may serve to supply the developing embryo with sulfate (2).

In a previous paper (14) we have identified, at the light microscopic level, small foci of arylsulfatase reaction product on the surface of growing oocytes. Now, arylsulfatase has been demonstrated ultrastructurally, as electron-dense precipitates at the level of microvillous-like projections of zona radiata (Fig. 1 D). This particular localization of the enzyme is very intriguing and its role can only be speculative. It has been reported the localization of arylsulfatase in the plasma membrane of lymphocite killer cells (22), and its role was correlated with the degradation of cerebroside sulfate esters. The presence of arylsulfatase in zona radiata may be necessary for the uptake of sulfated compounds into the oocyte. It is

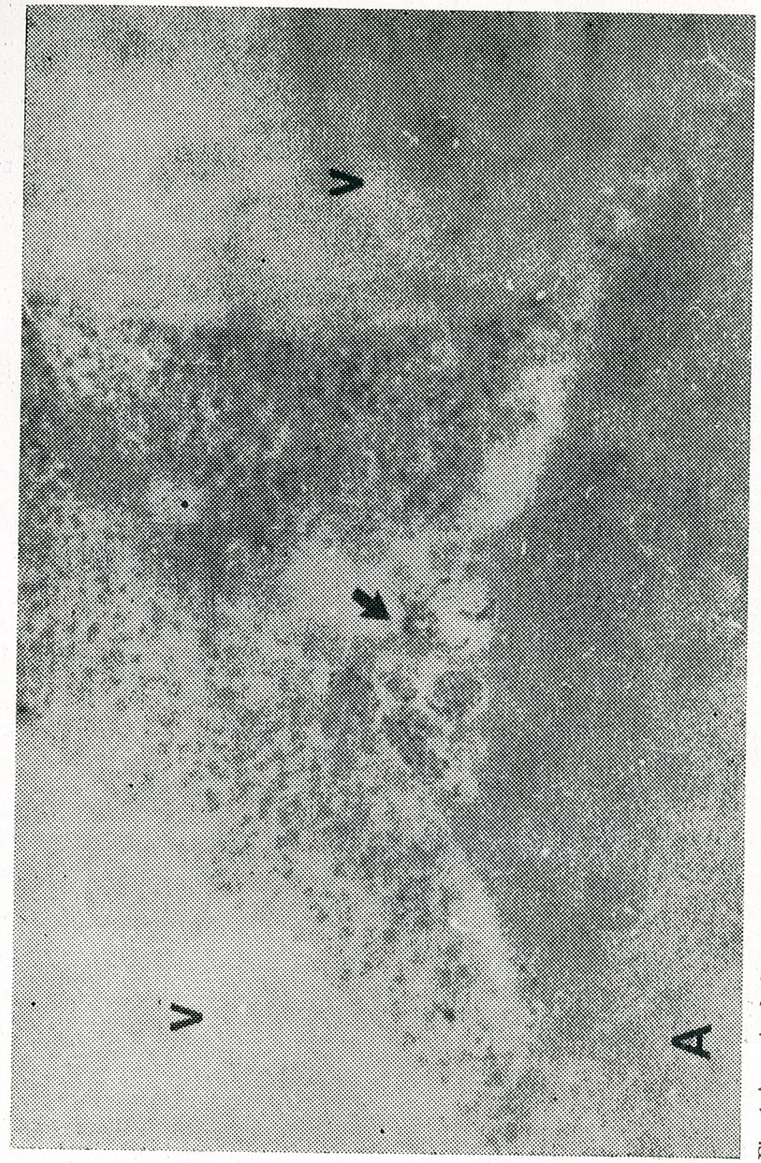

Fig. 1 A. — Arylsulfatase activity in fish oocyte showing numerous small foci of reaction products in the vicinity of yolk platelets (arrow). V — cortical vacuoles. Unstained preparation of Grass carp.

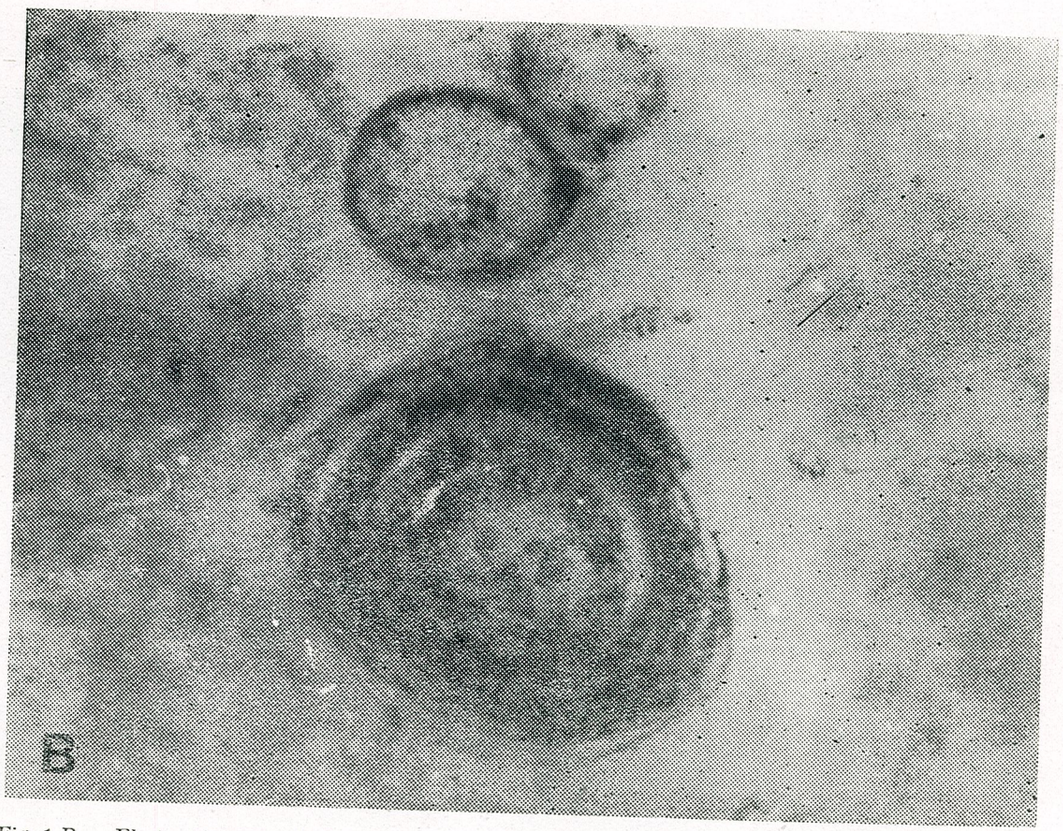

Fig. 1 B. — Electron micrograph showing two lysosomes and a multivesicular body with aryl sulfatase reaction product. Unstained preparation of Grass carp.

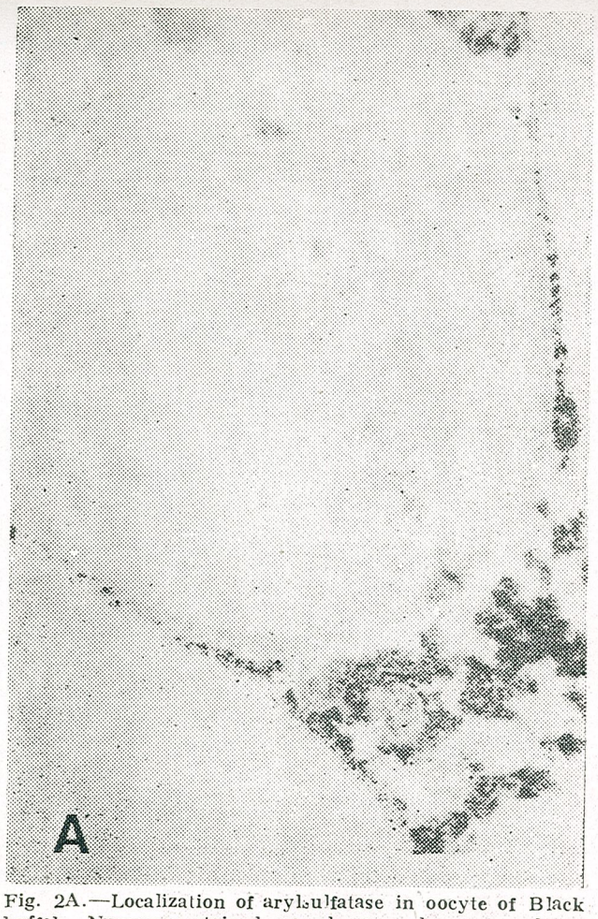

Fig. 2A.—Localization of arylsulfatase in oocyte of Black buffalo. Numerous stained granules are demonstrated to limited zones between yolk platelets.

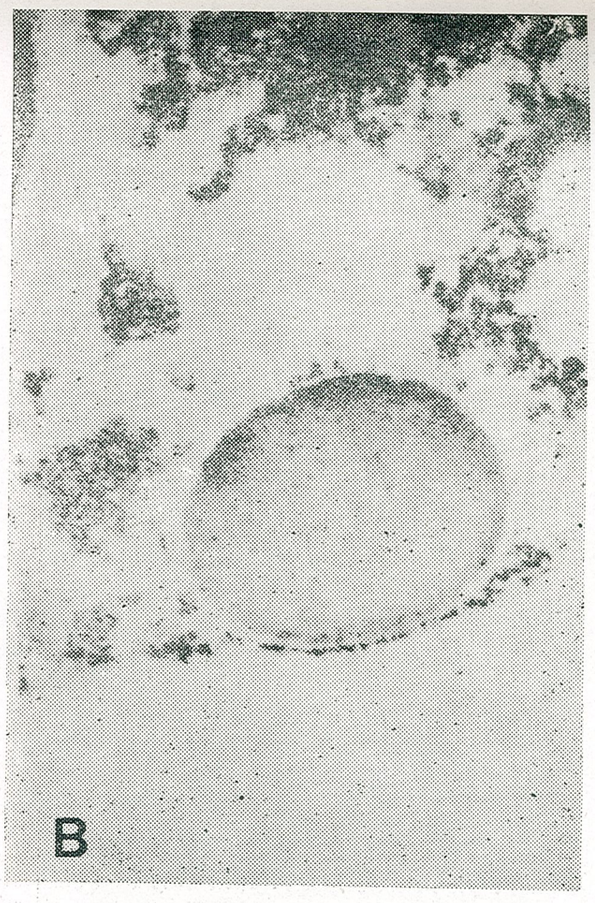

Fig. 2 B. — Arylsulfatase activity identified in fish oocyte localized between lipid droplet and a cortical vacuole.

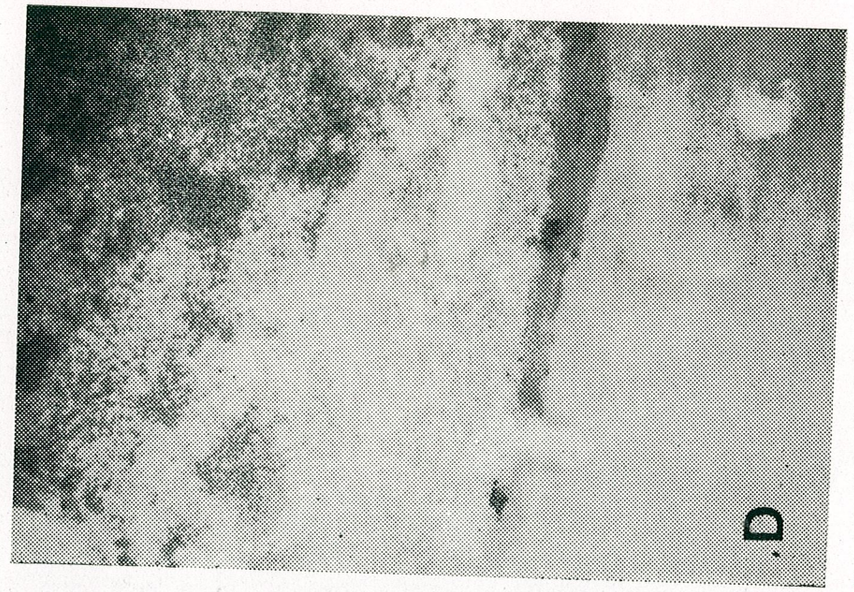

Fig. 2 D. — Small foci of arylsulfatase reaction product identified in zona radiata of fish oocyte.

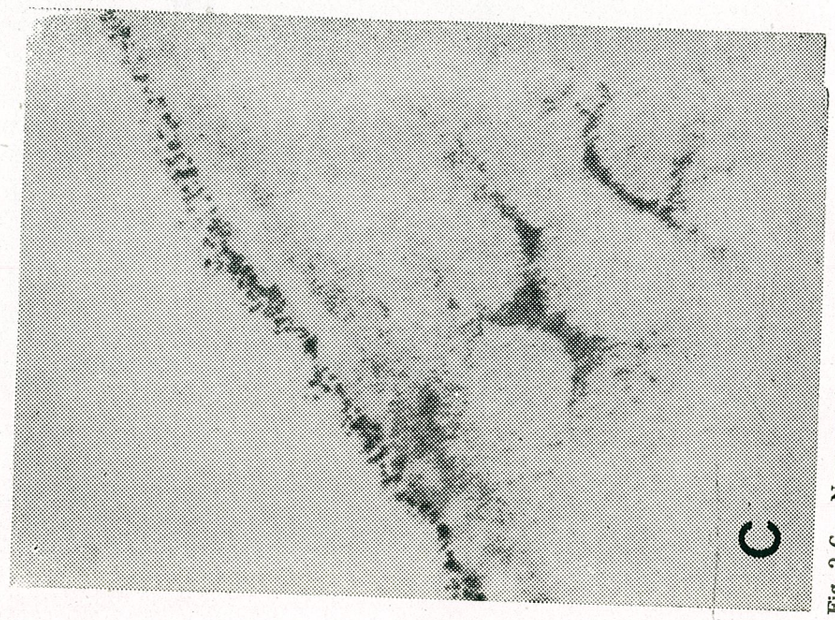

Fig. 2 C — Numerous foci of arylsulfatase reaction product are seen associated with the surface of yolk platelet.

not excluded the possibility that the enzyme play also a role in the degradation of cerebroside sulfate esters and the formation of membrane lipids of mature oocyte (17). Zona radiata lies between egg plasma membrane and surrounding follicle cells, with egg microvilli and follicle cell processes traversing zona radiata (1). This extracellular material is composed of sulfated glycoproteins and the participation of the enzyme in the formation of oocyte glycocalyx is not excluded.

Although arylsulfatase is known to play a role in intracellular digestion, its heterogenous distribution in oocytes may suggest several functions. Vitaioli et al. (21) studying the arylsulfatase A activity in the frog oviduct indicated that oestradiol increases the synthesis process of sulfoglycoconjugates as well as the arylsulfatase A activity, which regulate their intracellular storage. The authors also showed that sulfatides is a natural substrate for arylsulfatase A. Various reports suggest that some natural substrates are hydrolyzed by different enzymes (9), (20). In addition, it has been shown that structural changes caused by the degree of aggregation of enzyme can change the substrate specificities (6).

The functional role of arylsulfatases and of their natural substrates in fish oocytes should be further investigated.

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Faculty of Biology
Bucharest, Splaiul Independenţei 91—95

THYMO-ADRENAL MODIFICATIONS IN WISTAR WHITE RATS CONSECUTIVE TO TIMOLINFOTROPINA TREATMENT

RODICA GIURGEA, CORINA ROȘIORU, D. COPREAN, Z. URAY and IOANA ROMAN

The effects of a calf thymic extract (Timolinfotropina) on the thymus and adrenals were studied. The applied dose was 0.1 ml/100 g b. w. daily, for 10 days. The results showed a slight stress state, which did not affect the thymic function. Moreover, an increase of the organ weight, together with increased levels of nucleic acids, AlAT and AspAT activities were noticed.

Numerous studies investigated the effects that thymic extracts of different nature have in mammals (2), (6), (16) and birds (8). These data emphasize a specific stimulation of the organs implied in humoral and cellular immunity, and also more general protective effects against different stress agents.

This experiment pursued our previous studies on this problem (5), (8), (9) and investigated the effects of a thymic extract — Timolinfotropina — on the thymus and adrenal glands. The thymus is one of the most important target organs for the action of immunostimulating agents, and adrenals can provide important data concerning the stress status of the organisms treated with the thymic extract.

MATERIAL AND METHODS

Experiments have been conducted on adult, male Wistar rats, weighing 200 $\pm$ 10 g and kept in standardised conditions; they received food containing carbohydrates, proteins and lipids in the appropriate proportion required for a normal development, and water *ad libitum*.

Rats were randomly distributed in 2 experimental groups of 8 animals each : the control group (C), and the Timolinfotropina-treated group (T). Timolinfotropina (a calf thymus extract — Ellem Milano, Italy) was injected into the hip muscle, each rat in the T group receiving daily 0.1 ml thymic extract/100 g body weight (which corresponds to 0.37 mg peptide mixture), for 10 days. The rats in the C group were injected with the corresponding quantity of physiologic saline. The animals were sacrificed by beheading, in the morning of the 6th day after the end of the treatment, with a previous fasting period of 16 hours.

The thymus, previously weighed on a torsion balance, was used to determine : total protein concentration (10), nucleic acids concentrations — DNA and RNA (15) and the activities of alanine-aminotransferase (AlAT) and aspartate-aminotransferase (AspAT) (12). The adrenals were weighed and used for the determination of ascorbic acid (4) and glycogen content (11).

Statistical processing of the results included the control of homogeneity of mean values by Chauvenet's criterion, aberrant values being

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eliminated, and comparison of the means using Student's "t" test. Percentage modifications versus control values (± D%) were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

Thymic extracts showed positive effects on lymphatic organs (3), (7), (8), (16), stimulated phagocytosis in the reticulo-endothelial system and the differentiation of bone marrow stem cells (13). There were also emphasized the protective effects of thymic extracts on the irradiated organism (17) and the thymotrope and spleenotrope action of thymic peptides used as clinical immunomodulators (14), (16).

In our experiment, the Timolinfotropina treatment induced a slight stress state in the organism, emphasized as a decrease of the ascorbic acid level and an increase in the adrenal glycogen content (Table 1). This

Table 1

Ascorbic acid – Aa. – (μg/mg) and glycogen-G.-(μg/mg) content in adrenals after Timolinfotropina treatment

Groups :		Control	Timolinfotropina
Aa.	$\bar{x} \pm SE(n)$	3.09±0.12(7)	2.17±0.08(5)
	D %	—	−29.78
	p	—	< 0.001
G		3.06±0.20(5)	3.74±0.48(7)
		—	+22.22
		—	< 0.05

$\bar{x}$ = mean values; ± SE = standard error; D% = percentage differences versus control; p = statistical significance; (n) = = number of individual values.

fact usually reflects an increased synthesis of glucocorticoids; it is well-known that the thymus possesses receptors for these hormones and that high glucocorticoid levels have a negative influence on the thymus function (1).

Table 2 shows the modifications induced by Timolinfotropina treatment in the thymus. The DNA concentration in this organ was significantly increased, together with the tested enzymatic activities (AlAT, AspAT), which could suggest an increased protein synthesis, Meanwhile, the significant increase of the thymus weight may be interpreted as a tentative to counteract the negative effect of the slight stress state induced by Timolinfotropina. The fact that we did not notice a rise in the total protein content in the thymus may be due to our experimental model, which introduced a delay of 5 days between the end of the treatment and the moment when the animals were killed. The investigation of the thymic and adrenal functions at the very end of a 10 days Timolinfotropina administration could provide valuable evidence to support this hypothesis.

Table 2

Total protein (mg%) – TP, RNA, DNA (mg/g) and transaminase activities (ALAT and AspAT), (μg/mg/h) in the thymus and the weight of this organ (mg – TW) in Wistar rats after administration of Timolinfotropina

Groups :		Control	Timolinfotropina
TP	$\bar{x} \pm SE$	175.26±7.70 (8)	170.09±7,51(9)
	D%	—	−2.95
	p	—	NS
RNA		1.10±0.20 (10)	1.13±0.44(5)
		—	+2.72
		—	NS
DNA		1.38±0.28(10)	3,00±0,74(5)
		—	+117,39
		—	< 0.02
AspAT		14.49±1.82 (5)	18,79±3.36(8)
		—	+29.67
		—	< 0.05
ALAT		20.74±10.74 (5)	23.83±3.98(8)
		—	+14.85
		—	NS
TW		256.00±33.49 (8)	299.42±39.37(7)
		—	+16.96
		—	<0,05

Explanations in Table 1

We may conclude that, even if Timolinfotropina treatment induced a slight stress state in the organism, emphasized by the modification of adrenal parameters, that one did not disturb the normal thymic function; moreover, Timolinfotropina influenced positively some parameters of the protein metabolism in the thymus.

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The Biological Research Centre
Cluj-Napoca, Clinicilor 5–7

METABOLIC MODIFICATIONS INDUCED IN NORMO-AND HYPERTENSIVE RATS BY OXPRENOLOL TREATMENT

D. COPREAN, CORINA ROȘIORU, RODICA GIURGEA and DINA COPREAN

The glucose and glycogen contents, glycogen-phosphorylase *a* and ATP-ase activities, as well as glucose, glycogen and oxygen consumption of the cardiac muscle were determined in hypertensive male Wistar rats (the high blood pressure was induced by DOCA-salt administration) and after treatment with oxprenolol against the background of DOCA-salt administration. We concluded that hypertensive status induced metabolic modifications in the cardiac muscle: tissue glycogen content and glycogen-phosphorylase *a* activity were elevated; *in vitro* glycogen consumption was increased while *in vitro* glucose consumption was decreased. The treatment with oxprenolol did not change metabolic modifications induced by hypertensive status.

It is well known that salt and mineralocorticoid excess in rat elicit arterial hypertension (21), (29), (30), (32), (34) and systemic hypertension significantly affects the glucose and glycogen metabolism in the smooth muscle (7), (26), (36). On the other hand, it is established that beta-adrenoceptor mediated influences of catecholamines are involved in the pathogenesis of arterial hypertension (10), (12), (33) and systemic hypertension modifies the contractile activity (4), (6), (9).

In the present study we tested the dynamics of some of cardiac metabolic parameters in normo-and hypertensive rats subjected to an oxprenolol treatment.

MATERIALS AND METHODS

Our experiments were carried out on heart isolated from male Wistar rats of 200 ± 10 g b. w., reared in the stockfarm of our laboratory and kept under standardized feeding and bioclimatic laboratory conditions. The animals were divided into four groups: control group (C); group treated with DOCA and sodium chloride (H); group treated with oxprenolol (O); group treated as group H + oxprenolol (HO).

To obtain moderate arterial hypertension (36), in the first period, natrium chloride (30% solution) was administered by gastric tubage in daily doses of 300 mg/100 g b. w. for 30 days, and DOCA (desoxicorticosterone acetate, "Mincortid") was injected s. c. in daily doses of 1.4 mg/100 g animal during this period. For maintaining the evoked hypertension, in the second period 1% NaCl was added to the drinking water and the treatment with DOCA was continued for 30 days more. In the second period, against the background of DOCA-salt administration the animals were treated with oxprenolol (1—/2—(allyloxy) phenoxy/—3—/ isopropylamino/—2—propanol) — hydrochloride), administered intragastrically in daily doses of 2.88 mg/100 g b. w. DOCA was a commercial product of "Terapia" Cluj-Napoca, and oxprenolol of I.C.C.F. București.

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The animals were sacrificed by decapitation after a fasting period of 16 hours, and 24 hours after cessation of the above treatments; drinking water being allowed *ad libitum*.

The hearts were quickly excised and put on filter paper dipped in ice-cold Krebs-Henseleit saline. From each heart rings of 30—35 mg were used for testing the initial glycogen (18) and glucose contents (20; with unimportant modifications for our conditions). Rings of 200 mg from hearts were used for glycogen phosphorylase *a* (11) and ATP-ase (13), (14) activities determinations. For oxygen, glucose and glycogen consumptions we used tissue slices incubated for an hour in a Warburg apparatus. The medium of incubation was sanguine plasma, with 20 mM glucose, and gas phase was air (for other explanation see 5).

The statistical analysis included: calculation of the mean values and standard error for each mean value. When comparison was made between the mean values the differences were checked with Student's test (31) in order to establish their statistical significance. $P < 0.05$ was considered significant (31).

RESULTS

From the data summarized in Table 1 it is obvious that the mean value of the initial glycogen content in the heart muscle of normal rats is 3.49 ± 0.22 mg/g tissue while in the hypertensive (H) and hypertensive + oxprenolol (HO) groups this metabolic parameter is markedly increased ($+ 31.44\%$; $p < 0.01$ and $+ 71.89\%$; $p < 0.001$ respectively) versus the corresponding normal level.

When the treatment with oxprenolol (group O) was applied upon the normal rats glycogen content was not significantly modified (-14.07%).

GPa activity modifications paralleled those of glycogen content: with increases in hypertensive ($+ 33.71\%$; $p < 0.05$) and hypertensive + oxprenolol ($+ 42.94\%$; $p < 0.01$) groups and with an insignificant modification in group O ($+ 0.91$ %) versus the corresponding normal level (33.02 ± 3.62 nM of P_i liberated/mg of protein).

Table 1 shows that the mean value of the initial glucose content in the heart muscle of normal rats is 1.52 ± 0.25 mg/g fresh tissue. Glucose contents in group H (1.41 ± 0.05 mg/g), O (1.54 ± 0.12 mg/g) and HO (1.50 ± 0.11 mg/g) were not statistically significantly modified versus the normal level.

Myofibrillar ATP-ase activity was significantly modified in hypertensive rats receiving oxprenolol ($- 46.67\%$; $p < 0.01$) versus the normal rats (3.45 ± 0.24 micromoles of P_i liberated/mg of myofibrillar protein).

The data presented in Table 2 show very important modifications in glucose and glycogen consumptions in hypertensive, hypertensive + oxprenolol and normotensive + oxprenolol rats versus the control rats (53.01 ± 3.33 micromoles / g/h for glucose and 2.84 ± 0.27 milligrams/ g/h for glycogen). Hypertensive status caused a decrease in glucose (-55.35 %; $p < 0.01$) and an increase in glycogen consumptions ($+ 32.75\%$; $p < 0.05$). The treatment with oxprenolol did not modify the increased capacity of hypertensive rat heart muscle in glycogen consumption ($+ + 71.48\%$; $p < 0.01$) as well as its decreased capacity in glucose utili-

Table 1

Glucose and glycogen content (mg/g) as well as ATP-ase (micromoles of P_i liberated/ mg of myofibrillar protein/ minute) and GPa (nanomoles of P_i liberated/ mg of protein/ minute) activities in heart muscle of control rats (C), hypertensive rats (H), normotensive rats treated with oxprenolol (O) and hypertensive rats treated with oxprenolol (HO)

Group		Glucose	Glycogen	ATP-ase	GPa
C	$\bar{X} \pm SE$	1.52 ± 0.25	3.43 ± 0.22	3.45 ± 0.24	33.02 ± 3.62
	n	7	6	6	9
H	$\bar{X} \pm SE$	1.41 ± 0.05	4.39 ± 0.01	3.52 ± 0.34	44.15 ± 3.38
	n	5	6	6	6
	p <	NS	0.01	NS	0.05
	$\pm C\%$	-7.24	$+31.44$	$+2.02$	$+33.71$
O	$\bar{X} \pm SE$	1.54 ± 0.12	2.87 ± 0.20	2.75 ± 0.33	33.32 ± 5.20
	n	6	6	6	7
	p <	NS	NS	NS	NS
	$\pm C\%$	$+1.32$	-14.07	-20.29	$+0.91$
HO	$\bar{X} \pm SE$	1.50 ± 0.11	5.74 ± 0.36	1.84 ± 0.42	47.20 ± 4.69
	n	6	6	6	6
	p <	NS	0.001	0.01	0.02
	$\pm C\%$	-1.32	$+71.86$	-46.67	$+42.94$

Note: $\bar{X}$ = mean; $\pm SE$ = standard error; n = number of experiments; p = significant threshold, $\pm C\%$ = percentage differences calculated versus the control values; NS = not significant.

Table 2

Glucose (micromoles/ g/h), glycogen (milligrams /g/h) and oxygen (micromoles/ g/h) consumptions in the heart muscle of control rats (C), hypertensive rats (H), normotensive rats treated with oxprenolol (O) and hypertensive rats treated with oxprenolol (HO)

Group		Glucose	Glycogen	Oxygen
C	$\bar{X} \pm SE$	53.01 ± 3.33	2.84 ± 0.27	7.76 ± 1.01
	n	6	6	6
H	$\bar{X} \pm SE$	23.67 ± 6.56	3.77 ± 0.18	8.80 ± 1.08
	n	6	6	6
	p <	0.01	0.05	NS
	$\pm C\%$	-55.35	$+32.75$	$+13.14$
O	$\bar{X} \pm SE$	26.98 ± 6.31	1.55 ± 0.44	8.48 ± 1.57
	n	6	6	6
	p <	0.01	0.05	NS
	$\pm C\%$	-49.10	-45.42	$+8.5$
HO	$\bar{X} \pm SE$	38.40 ± 3.53	4.87 ± 0.41	7.77 ± 2.74
	n	6	6	6
	p <	0.05	0.01	NS
	$\pm C\%$	-27.57	$+71.48$	$+0.10$

Note: For explanation see Table 1

zation (− 27.57%; $p < 0.05$) versus heart muscle of normotensive rat. The treatment with oxprenolol in normotensive rats caused a decrease in both glucose (−49.10%; $p < 0.01$) and glycogen (− 45.42%; $p < 0.05$) consumptions.

As we can see in table 2 the mean of the oxygen consumption in the heart muscle of normal rats is 7.76 ± 1.01 micromoles/g/h. Hypertensive status did not significantly modify this consumption (+ 13.4%). The treatment with oxprenolol in both normo-and hypertensive rats also did not significantly change heart muscle oxygen consumption (+ 8.5% and + 0.1% respectively).

DISCUSSIONS

As we can see in Table 1 hypertensive status caused a correlated increase în glycogen content with an elevated GPa activity. It is obvious that heart slices from hypertensive rats preferred to utilize its glycogen as carbohydrate energy source instead of added glucose to incubation medium. It is clear that heart muscle from hypertensive rats became more glycogenolytic in comparison with normotensive rats. It is well known that 50 % of the heart muscle energetical necessity is supplied by a carbohydrate source (3), (8), (24), (38).

Then it is well established that in systemic arterial hypertension the activation of beta-adrenoceptors by catecholamines at the level of cardiac myocytes is mainly involved, and that under this condition the cytosolic cAMP production significantly increases (27). On the other hand it is well known that the activation of beta-adrenergic cAMP—dependent cytosolic protein-kinase in the cardiac myocytes leads to the increase of the disposable channels for Ca^{++} penetration (1), (2), (17), (27), (35) and the increased cytosolic cAMP and Ca^{++} lead to the activation of phosphorylase system involved in glycogen breakdown and hexose-monophosphate formation, with an important role in cell energy (37). It is obvious that an increased glycogen consumption required an intensification of glycogen synthesis. It is known too that beta-blocking agents are widely used in the treatment of heart diseases, because they have some properties such as : chronotrope-negative, inotrope-negative, dromotrope-negative (19). For those actions, beta-blocking agents, such as oxprenolol are used in the treatment of hypertension (16), (22), (23), (25), (28). The treatment with oxprenolol in normotensive rats caused a decrease in both glucose and glycogen consumptions without modifications in glucose and glycogen content, GPa and ATP-ase activities, and oxygen consumption. Kaiser (15) did not find modifications in glucose, glycogen and glucose-6-phosphate content of rat heart muscle after the treatment with propranolol. In our experiment it is possible that the decrease in glucose and glycogen consumptions, under the treatment with oxprenolol of normotensive rats, to be a consequence of a reduction in cardiac activity induced by beta-adrenergic blockade. It is very interesting that the treatment with oxprenolol did not change the metabolic modifications induced by hypertensive status.

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Biological Research Institute
Cluj-Napoca, Republicii 48

BIOELECTRICAL EFFECTS OF PROCAINE INCORPORATED INTO LIPOSOMES

I. NEACȘU, C. V. UGLEA, C. V. ZĂNOAGĂ and I. Ī. BĂRA

The action of procaine, free or incorporated into lecithin liposomes, on the membrane resting potential of the frog sartorius muscle fibre was studied. The effects of free procaine were found to be concentration dependent: 0.50, 1, 2 and 2.50 mM caused dose dependent hyperpolarization, almost no effect was observed with 3 mM and depolarization at 5 mM. Upon incorporation into liposomes, 0.50, 1 and 2 mM procaine induced increased hyperpolarization which was removed at 2.50 mM, and depolarization was accentuated at 3 and 5 mM. Liposomes alone, in the absence of procaine, led to slight hyperpolarization. The concentration-effect plot is bell shaped (Gaussian), that for the incorporated procaine is shifted to the left (toward smaller concentrations) and is wider, suggesting a stronger and more persistent effect of the incorporated drug as compared to the same concentration of the free drug.

The properties of procaine as a local anesthetic and a membrane stabilizer (20), (21), (23), as well as its positive effects on ageing phenomena (3) have been extensively studied. Several aspects of the interactions of local anesthetics with membrane proteins and lipids have been discussed (1), (19), (20), (24), (25), including the effects on membrane fluidity (7), (20), the action of the cationic and neutral forms of anesthetics at the level of the external and internal membrane layer (1), (2), (13), (20), as well as their effects on ionic channels and on the ion action at the cell membrane level (5), (16), (21), (23). However, the detailed mechanism of the action of local anesthetics has remained unexplained.

At the same time, the action of local anesthetics on liposomes as membrane models was studied (5), (17). Also, the interactions of liposomes with living cells have been investigated, in order to check their possible importance for biomedical research (5), (12) and the possibility of using liposomes to introduce certain pharmacological agents into the cells (4), (5), (10), (12).

In the present work, the effects of various concentrations of free procaine on the resting membrane potential (RMP) were studied. The effects of the same concentrations of procaine incorporated into lecithin liposomes, and the effects of liposomes alone were compared with those of free anesthetic.

MATERIALS AND METHODS

The experiments were performed on frog (*Rana ridibunda*, Pall.) sartorius muscles (4—6 muscles taken from different individuals in each experimental series), in normal Ringer (NR) solution, pH = 7.20 (bicarbonate buffer). Procaine. HCl (Merck) (0.50, 1, 2, 2.50, 3 or 5 mM) was dissolved in NR. The liposomes were prepared from lecithin solution in diethylether, in NR medium with or without procaine thermostated at 55°C (8). Lecithin was obtained according to the method of Singleton et

al. (1965) (22) from the yolk of fresh eggs. The technique of intracellular glass microelectrodes was applied to measure the membrane potential (MP). The data were statistically processed using the Chauvenet and the Student tests.

RESULTS

The effects of free procaine in NR on MP were concentration dependent and were easily reversible upon changing into NR (Fig. 1, curves A—F, left). Concentration of 0.50 mM procaine induced very weak membrane hyperpolarization, by 0.84 mV ($p > 0.25$) (Fig. 1, curve A, left) and 1 mM procaine hyperpolarized the membranes after 60 minutes by 1.92 mV ($p > 0.1$) (Fig. 1, curve B, left). A hyperpolarization by 2.46 mV ($p < 0.001$) (Fig. 1, curve C, left) was recorded in the presence of 2 mM, and by 4.57 mV ($p < 0.001$) (Fig. 1, curve D, left) in the presence

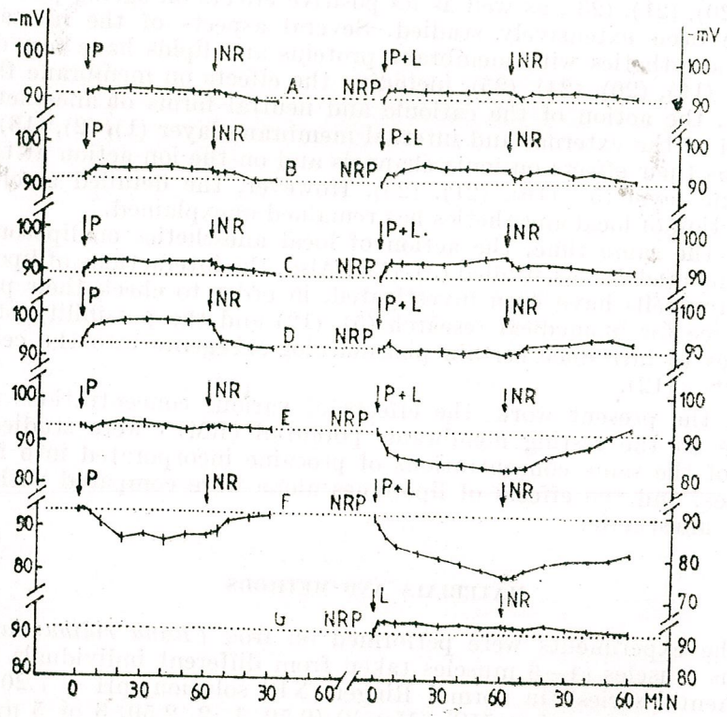

Fig. 1. — The effect of free procaine (P, left), of procaine incorporated into liposomes (P + L, right) and of the liposomes without anesthetic (L, right) on the resting membrane potential: A — 0.50 mM procaine; B — 1 Mm; C — 2 mM; D — 2.50 mM; E — 3 mM; F — 5 mM. (NRP Normal resting potential; NR = normal Ringer). The data are mean ± SEM values from 25—50 measurements; p = see the text.

of 2.50 mM free procaine. A drop was recorded with 3 mM (hyperpolarization by 0.74 mV, $p > 0.25$) (Fig. 1, curve E, left), and with 5 mM depolarization by 6.93 mV ($p < 0.001$) (Fig. 1, curve F, left) was recorded, as an expression of the sublytic effect of the agent on the membrane (1), (20).

Incorporation into liposomes did not change the typical effect of the agent on MP, the action being generally stronger and prolonged (Fig. 1, curves A—F, right). After 60 minutes of action, 0.50 mM procaine incorporated into liposomes hyperpolarized the membrane by 1.96 mV ($p = 0.05$), 1 mM by 3.49 mV ($p < 0.002$), and 2 mM by 4.26 mV ($p < 0.001$). A slight depolarization was seen with 2.50 mM, after 60 minutes (by 0.92 mV, $p > 0.25$), and a weak membrane hyperpolarization appearing on washing the muscles with NR (1.94 mV, after 60 minutes, $p < 0.005$). With 3 mM a depolarization of 8.28 mV ($p < 0.001$) appeared, while 5 mM caused a depolarization by 14.40 mV ($p < 0.001$), hardly reversible in NR.

DISCUSSIONS AND CONCLUSIONS

The hyperpolarizing effects of local anesthetics at blocking concentration have been reported by other authors, too (11), (13), (20), (25), who considered them insignificant for the impulse blocking. However, in agreement with other investigators (9), we also consider that hyperpolarization reduces membrane excitability and increases the excitation threshold, thus contributing to the blocking action.

The specific effects of local anesthetics have been shown to be mainly achieved by drug interactions with membrane proteins, with the resulting inhibition of the Na^+ channel and consequent blocking of the action potential propagation (11), (20), (21), (24), (25). Local anesthetics have also been reported as affecting both the K^+ and Ca^{2+} channel, yet with a much stronger affinity for Na^+ channel (23). Also, it has been shown that anesthetics interact with the $Na^+—K^+$ pump, stimulate the Na^+ active transport and determine an increase of membrane resistance, which can result in hyperpolarization (2), (6). As a matter of fact, in our previous experiments, procaine was able to abolish the action of ouabain in inhibiting the activity of the $Na^+—K^+$ pump (15).

A series of studies have also pointed to the importance of the interactions of local anesthetics with membrane phospholipids with respect to blockage of the Na^+ channel (19), (23), to the control of the $Na^+—K^+$ pump and ATP-ase activities (18), and to the determination of membrane hyperpolarization and its stabilization (1), (16), (21). Based on these observations it may be concluded that the mechanisms underlying the blocking action of local anesthetics are quite complex (1).

Procaine incorporation into lecithin liposomes results in an enhancement of the specific effects of the anesthetics on MP: small concentrations of incorporated procaine can induce similar effects to those induced by higher concentrations of the free agent (Fig. 1, left and right). Considering that liposomes without the agent (Fig. 1, curve G) induced slight but stable membrane hyperpolarization (by 1.83 mV, $p \geqslant 0.1$), the final

effect of incorporated procaine is not just a sum of the separate effects of the two agents.

The graphic representation of the concentration dependence of free (P) and incorporated procaine (P + L) (Fig. 2) yields bell-shaped Gaussian curves. The curve for the incorporated procaine is shifted to

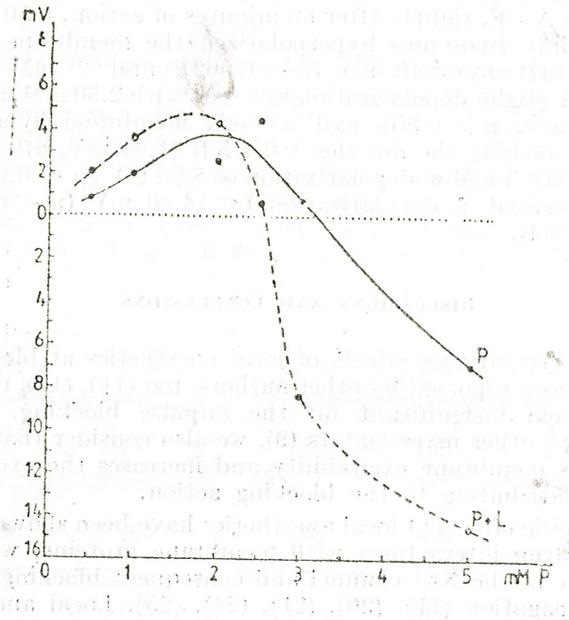

Fig. 2. — Concentration dependence of the effect of free (P) or incorporated procaine into liposomes (P + L).

the left, towards low concentrations, and it is larger, as compared to that for free procaine; this suggests a stronger (and more persistent) effect of the incorporated drug as compared to the same concentration of free drug.

Lecithin-consisting liposomes determine a change of the membrane phospholipids ratio and, implicitly, of the membrane properties and of the procaine action mode, too (7). Also, liposomes assure the protection over procaine, maintaining a certain ratio between its cationic and neutral forms with specific effects at the membrane level (2), which leads to enhanced and prolonged effects of the liposome-incorporated agent.

It has also been shown that some interactions take place between the phospholipidic bilayer of liposomes and the incorporated anesthetic, thus resulting a certain liposome-procaine system with characteristic properties, including its own electric charge, that interferes with MP (7), (14). At the same time, this can contribute to the enhancement and persistence of the effects of the liposome-incorporated procaine.

Some positive medical effects of the drug incorporation into liposomes are suggested, too. Thus, different researches have revealed the importance of some local anesthetics and of liposomes in connection with the possibility of correcting the membrane fluidity that appears modified in the case of some affections (5), (7).

The data point out a series of characteristics of the action of liposome-incorporated procaine which suggests the possibility of diminishing the useful concentration of anesthetic, of facilitating the penetration of substance into the cell and of prolonging the duration of its action. Some other researches are however necessary in order to make all these aspects clear.

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Institute of Biological Researches
Iași, Bd. Copou 20 A

ECOLOGICAL STUDIES ON DESERT MESOFAUNA OF KUWAIT

A. M. KHEIRALLAH

A survey of desert mesofauna of the state of Kuwait was carried out in three sites : Umm Al-Rimam in the north and Al-Wafrah and Al-Khiran in the south. The mesofauna groups in the three sites arranged in order of dominance as indicated from the relative importance value (R. I. V.) and absolute importance value (A. I. V.) index were Coleoptera, Formicidae, Arachnida, Neuroptera, Isoptera and an unidentified insect nymph in Umm Al-Rimam ; Coleoptera, Arachnida, Formicidae and Isoptera in Al-Wafrah ; and Coleoptera, Arachnida, Isopoda, Isoptera and Formicidae in Al-Khiran. The dominant mesofauna group in the three sites was Coleoptera. Other groups associated with the three sites were Arachnida, Isoptera and Formicidae. Changes in the abundance of these groups were apparant between the three sites. An increase indicated from the absolute importance value (A. I. V.) index of Coleoptera, Arachnida and Isoptera was noticeable in Al-Khiran site than in the other two sites. Similar changes in Formicidae were apparant where Umm Al-Rimam supported more populations than Al-Wafrah and Al-Khiran. Whilst Al-Khiran site supported more Coleoptera, an increase in species diversity for this group was found in Al-Wafrah. Factors responsible for these changes were discussed.
The ecological function of the taxa in the three sites was considered. Three trophic groups were recognised : a) Detritivores, b) Grazers, and c) Predators. The predators in Umm Al-Rimam, Al-Wafrah and Al-Khiran constituted 9.4%, 3.5% and 1.7% of the other groups by density respectively and 0.71%, 3.52% and 1.7% by weight respectively.

INTRODUCTION

In many parts of the World, natural ecosystems are in serious dangers of being partially or completely destroyed as a result of human activities of one sort or another. This is particularly obvious in Kuwait where tremendous economic development has been achieved in recent years. The economic development and unrivalled urbanisation in a small country like Kuwait should have an enormous detrimental effect on naturally existing fauna and flora. Plants and animals in these systems live in a situation of precarious balance between their ability to survive perturbations and the harsh resources of the arid ecosystems to which they belong.

Bearing this in mind, it is aimed to study the desert mesofauna in a number of sites of the state of Kuwait. This type of study becomes essential to monitor the impact of man on the ecosystems. Some species may disappear or decline, some increase, while new species may be introduced. Thus, studies on the naturally existing soil mesofauna will serve a base-line study.

The term "mesofauna" used in this study is in accordance with the definitions given by Rapoport and Tschapek [13] and Ghilarov and Arnoldi [5]. It is meant to distinguish soil animals obtained by sieving method of sampling from soil microfauna (less than 1 mm in length), and from soil macrofauna (soil vertebrates). This consideration was collaborated by Ghabbour and Shakir [3], Ghabbour et al. [4] and Kheirallah [10].

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Climate

The climate of Kuwait is typically arid. The summer temperature is above 40°C and the mean yearly precipitation is a mere 100 mm, daily temperature changes of up to 18°C are common in winter. The highest temperature recorded was 51°C, in July 1978 and the lowest was —4°C, in January 1964. Rainfall fluctuations are less obvious. The summer is almost completely dry with most of the rain falling in winter and spring. Annual rainfall also varies greatly, in 1964 a meagre 24 mm of rain was measured whereas 336 mm fell in 1954.

Heat and aridity are not the only characteristics of the climate; strong dry winds are also a feature. The north-westerly Tawz winds prevail for nearly fourty days during June and July. Together with heat of the sun, these winds are responsible for the high rate of water evaporation during summer. In addition, dust and sand storms occur predominantly during winter and in June and July at the time of Tawz. The dust storms are produced by a strong wind lifting thick clouds of dust particles thousands of feet into the air thus blocking out the sun. Sand being much heavier, is rarely blown more than several feet off the ground.

MATERIAL AND METHODS

a) Site description

The state of Kuwait (Fig. 1) lies at the northern part of the Arabian peninsula. It occupies 18,000 km² of desert land and ten offshore islands distributed in the Arabian Gulf. It is limited from the north and north west by Iraq and from the east by the Arabian Gulf. This Gulf covers an area of about 239,000 km².

The sandy coastal line of Kuwait has a stretch of about 300 km and its formation is oolitic limestone (Fuchs et al. [2]). The northern part of the coast as well as the bottom of Gulf are covered by a layer of sility mud brought down by the floor of shatt Al-Arab in the south of Iraq. The significant features of the monotonous topography of the coast of Kuwait are Khor Al-Khiran in the southern part and Kuwait Bay in the northern part.

The investigations reported here were made in three sites : At Umm Al-Rimam in the north near an irrigated farmland (plate la) where the vegetation was represented by the perennial shrub *(Aellenia supaphylla)*; at Al-Wafrah in the south (Plate 1b) where the vegetation is represented by the trees *(Tamarix aphylla)*; and at Al-Khiran in the south (Plate 1c) where the shrubs in the site were recently overgrazed by sheep and goats. Grazers dung accumulate on the surface of sand, thus provide food for dung feeder mesofauna. This site is also covered by scattered discarded sheets of wood and corregated iron where they provide habitat for the desert mesofauna.

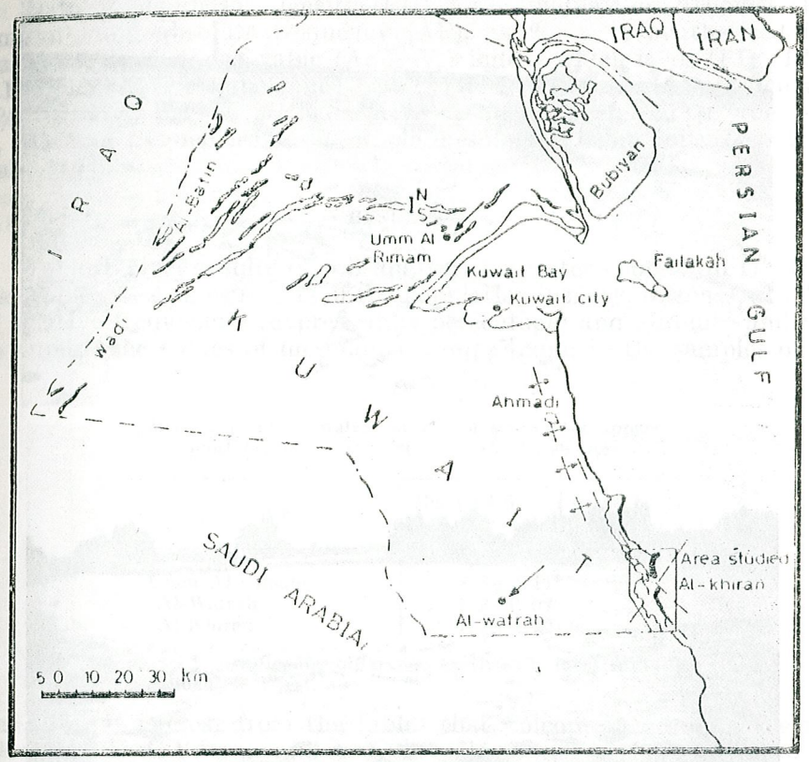

Fig. 1. — Map of the state of Kuwait.

b) Sampling, analysis and data treatment

Sampling was carried out during the end of February and early March 1989. A large sized area of about 1 km² was chosen in each site. Ten sampiing units, each consisted of 0.09 m² of soil together with overlying debris to a depth of 30 cm were collected from each site on a stratified random basis. The sampling units were collected either from under shrubs as in Umm Al-Rimam or from under the discarded sheets of wood and corregated iron as in Al-Khiran or from under tree branches and heaps of leaf litter as in Al-Wafrah. The sample units were processed in 1 mm mesh sieve as generated by Kheirallah [10]. All the debris and animals remaining in the sieve were collected and kept in tight bags and examined in the laboratory. Each animal was then picked, stored in 70% ethanol and later weighed. The weights given here as biomass refer to ethanol preserved specimens which for practical purposes are considered equivalent to fresh weights. This consideration was corroborated by Ghabbour and Shakir [3], Ghabbour et al. [4] and Kheirallah [10].

Identification of taxa in some cases was possible to the species level.

Soil samples were taken from each sampling unit in different sites and analysed for water content in the usual way (Jackson, [9]).

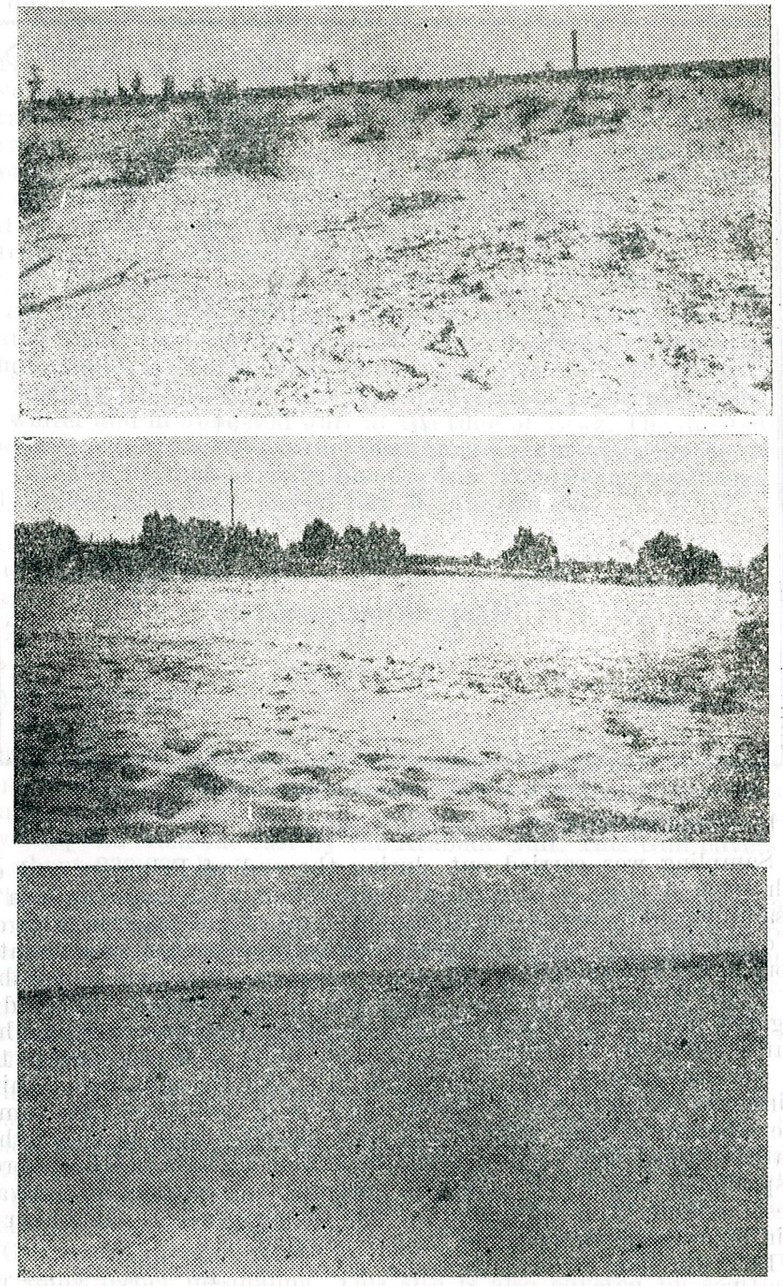

Plate 1. — Physiographic features of the sites : a, Umm Al-Rimam ; b, Al-Wafrah ; c, Al-Khiran.

Data of population density (PD), as individuals/m², biomass (BM) as mg/m², and absolute frequency (AF), as %, were synthetized into an absolute importance value (A. I. V.) index, which is log (PD×BM× ×AF). The relative importance value (R. I. V.) index was calculated as % PD + % BM + % RF (relative frequency) referred to the total PD, BM and frequencies of the whole mesofaunal community (see Kheirallah, [10]).

RESULTS

Table 1 gives results of soil analysis for water content in the three sites. Tables 2—4 show results of the population density, biomass, absolute and relative frequencies (expressed as percentage) and absolute and relative importance values of mesofauna groups found in the samples of the

Table 1

Mean percentage water content of soil in the uppermost layers (30 cm depth) in the three sites

Site	Mean (±S. E.) percentage water content of soil
Umm Al-Rimam	2.8±0.11*
Al-Wafrah	1.2±0.07
Al-Khiran	1.7±0.10

* Significance difference at the 5% level using Student's t-test.

three sites. It is clear from the tables that Coleoptera occupies the first rank on the R. I. V. scale and A. I. V. scale as well in the three sites. The value of R. I. V. and A. I. V. for this group in Umm AL-Rimam is (157.9) and (6.89) ; in Al-Wafrah is (262.5) and (8.3) ; and in Al-Khiran is (234.9) and (8.6). The Coleoptera in Umm AL-Rimam is followed by Formicidae (93.6) and (5.4), Arachnida (20.9) and (3.6), Neuroptera (8.6) and (2.5), Isoptera (7.6) and (0.87), unidentified insect nymph (6.9) and (1.4) and Thysanura (3.8) and (—0.2). In Al-Wafrah, the Coleoptera is followed by Arachnida (12.5) and (4.3), Formicidae (12.3) and (3.0) and Isoptera (11.7) and (3.0). In AL-Khiran, the Coleoptera is followed by Arachnida (19.3) and (4.82), Isopoda (19.2) and (4.68), Isoptera (19.0) and (4.6), Formicidae (5.3) and (2.3) and Diptera (2.3) and (1.8). Thus, the two importance values give the same order of dominance for all taxa in the three sites except in the case of Isoptera and unidentified insect nymph of Umm Al-Rimam where the two groups exchange positions on the percentage scale.

It is remarkable from the tables that Coleoptera in Al-Wafrah is represented by six families (Tenebrionidae, Carabidae, Mycetophagidae, Staphylinidae, Nitidulidae and Cryptophagidae) whereas in Umm Al- -Rimam and Al-Khiran it is represented by two families : Tenebrionidae and Coccinellidae in Umm Al-Rimam and Tenebrionidae and Scarabidae in Al-Khiran.

The tables also indicate that the total mean population density of mesofauna groups in Al-Khiran, Umm Al-Rimam and Al-Wafrah si

263.2/m², 142.1/m² and 129.2/m² respectively, while the total mean bio-
mass is 53.3 gm/m², 20.82 gm/m² and 55.0 gm/m² respectively. Thus,
the three sites exchange positions with respect to total population den-
sity and biomass. This is true in Umm Al-Rimam where a wide variation
of density and biomass of Formicidae (high density and low biomass)
is obvious.

Figure 2 shows variations in the absolute importance value (A.I.V.)
index of Coleoptera, Arachnida, Isoptera and Formicidae associated with
the three sites. Coleoptera, Arachnida and Isoptera are more important

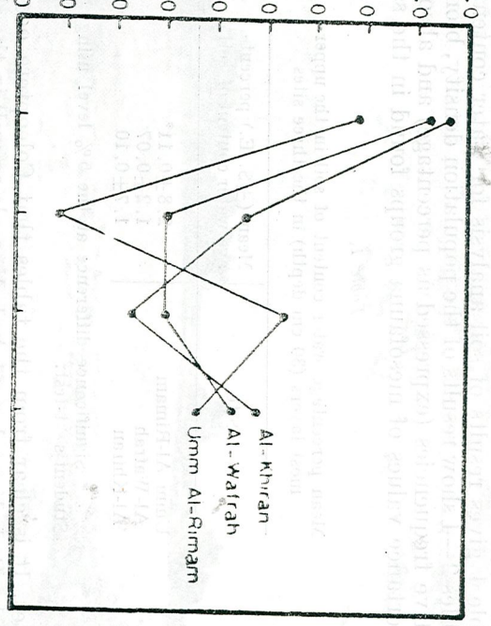

Fig. 2. — Variations in the absolute importance value (A. I. V.) index
of the mesofauna groups associated with the three sites.

in Al-Khiran than Al-Wafrah and Umm Al-Rimam, whereas Formicidae
is more important in Umm Al-Rimam than Al-Wafrah and Al-Khiran.

The last column of Tables 2—4 shows the separation of taxa into
trophic groups. Three groups are considered : a) Detritivores, b) Grazers,
and c) Predators. For the total mean, the three groups are distributed
as regards density and biomass for the three sites in the following manner :

Trophic level	Site	
	Umm Al-Rimam	Al-Wafrah	Al-Khiran
Detritivores	122.1/m² and 20.67 gm/m²	116.3/m² and 52.56 gm/m²	239.9/m² and 46.2 gm/m²
Grazers	7.77/m² and 0.006 gm/m²	8.5/m² and 0.056 gm/m²	18.9/m² and 0.34 gm/m²
Predators	12.21/m² and 0.146 gm/m²	4.4/m² and 1.40 gm/m²	4.4/m² and 6.8 gm/m²

Table 2

Population density (PD) per m² Population biomass (mg fresh weight/m², BM), percentage (%) of population density and biomass, absolute and relative frequencies (AF and RF) expressed as percentage and absolute and relative importance values (A. I. V. and R. I. V.) of mesofauna groups of UMM AL — RIMAM

Animal group	Stage	PD	%	BM	%	AF	RF	A.I.V.	R.I.V.	Ecological function
Coleoptera		*29·97*	*21·1*	*20·34·97*	*97·7*	*3·0*	*39·1*	*6·89*	*157·9*	
Tenebrionidae										
Adesmia cancellata	A	6·66	4·7	17069·58	82·0	3·0	9·0	5·5	95·7	D
Pimelia arabica	A	4·44	3·1	2804·53	13·5	2·0	6·1	4·4	22·7	D
Ammogiton sp.	A	4·44	3·1	30·19	0·14	3·0	9·0	2·4	12·2	D
Mesostena sp.	A	12·21	8·6	349·98	1·7	3·0	9·0	4·1	19·3	D
Prochoma sp.	A	1·11	0·8	82·03	0·4	1·0	3·0	1·9	4·2	D
Coccinellidae										
Phyllobius sp.	A	1·11	0·8	7·66	0·04	1·0	3·0	0·9	3·8	P
Formicidae		*92·13*	*64·9*	*334·66*	*1·6*	*9·0*	*27·1*	*5·4*	*93·6*	
Messor sp.	A	89·91	63·3	317·24	1·5	7·0	21·0	5·3	85·8	D
Cataglyphis sp.	A	2·22	1·6	17·43	0·1	2·0	6·1	1·8	7·8	D
Isoptera		*2·22*	*1·6*	*1·665*	$8·2\times10^{-3}$	*2·0*	*6·0*	*0·87*	*7·6*	
Psammotermes sp.	A	1·11	0·8	0·999	5×10^{-3}	1·0	3·0	0·04	3·8	G
Anacanthotermes sp.	A	1·11	0·8	0·666	$3·2\times10^{-3}$	1·0	3·0	—0·10	3·8	G
Thysanura	A	1·11	0·8	0·555	$2·7\times10^{-3}$	1·0	3·0	—0·20	3·8	D
Neuroptera (antlions)	L	3·33	2·3	44·73	0·2	2·0	6·1	2·5	8·6	P
Arachnida										
Araneidae	A	7·77	5·5	93·57	0·45	5·0	15·0	3·6	20·9	P
Unidentified insect nymph	N	5·55	3·9	4·22	0·02	1·0	3·0	1·4	6·9	G
Total=		142·1	100·1	20823·4	99·9					
Number of sampling units =						10·0	—			
Total number of occurrences							33·0			

A= adult, N = nymph, L = larva, D= detritivores, G = grazers, P = predators

Table 3

Population density (PD) per m², Population biomass (mg fresh weight/m², BM), percentage (%) of population density and biomass, absolute and relative frequencies (AF and RF) expressed as percentage and absolute and relative importance values (A. I. V. and R. I. V.) of mesofauna groups of AL—WAFRAH

Animal group	Stage	PD	%	BM	%	AF	RF	A.I.V.	R. I. V.	Ecological function
Coleoptera		*115·2*	*88·7*	*53059·5*	*96·45*	*35·0*	*77·5*	*8·3*	*262·5*	
Tenebrionidae										
Gonocephalum sp.	A	18·9	14·6	154·2	0·3	4·0	8·9	4·1	23·8	D
Adesmia cancellata	A	1·1	0·8	420·7	0·8	1·0	2·2	2·7	3·8	D
Pimelia arabica	A	23·3	18·0	31097·3	56·5	4·0	8·9	6·5	83·4	D
Ammogiton sp.	A	4·4	3·4	34·3	0·06	1·0	2·2	2·2	5·7	D
Mesostena sp.	A	35·5	27·4	1538·5	2·8	8·0	17·8	5·6	48·0	D
Trachyaerma sp.	A	2·2	1·6	2691·8	4·9	2·0	4·4	4·1	10·9	D
Triptera sp.	A	15·5	12·0	16024·7	29·1	5·0	11·1	6·1	52·2	D
Clitobicus sp.	L	1·1	0·8	506·5	0·9	1·0	2·2	2·8	3·9	D
Anemia sp.	A	3·3	2·5	14·4	0·03	2·0	4·4	2·0	6·9	D
Oxycara sp.	A	1·1	0·8	7·9	0·01	1·0	2·2	0·9	3·0	D
Carabidae										
Calosoma sp.	A	1·1	0·8	552·9	1·0	1·0	2·2	2·8	4·0	P
Mycetophagidae										
Drasterius sp.	A	1·1	0·8	5·1	0·01	1·0	2·2	0·7	3.0	D
Staphylinidae										
Unidentified insect	A	3·3	2·5	4·1	0·01	2·0	4·4	1·4	6·9	D
Nitidulidae										
Nitidula ciliata	A	1·1	0·8	5·8	0·01	1·0	2·2	0·8	3·0	D
Cryptophagidae										
Cryptophagus sp.	A	2·2	1·7	1·3	0·002	1·0	2·2	0·5	3·9	G
Formicidae		*4·4*	*3·4*	*52·3*	*0·11*	*4·0*	*8·8*	*3·0*	*12·3*	
Messor sp.	A	2·2	1·7	2·6	0·01	2·0	4·4	1·2	6·1	D
Cataglyphis sp.	A	2·2	1·7	49·7	0·1	2·0	4·4	2·3	6·2	D
Isoptera										
Psammotermes sp.	A	6·3	4·7	55·1	0·1	3·0	6·7	3·0	11·7	G
Arachnida		*3·3*	*2·5*	*1847·4*	*3·4*	*3·0*	*6·6*	*4·3*	*12·5*	
Araneida	A	2·2	1·7	100·9	0·2	2·0	4·4	2·7	6·3	P
Scorpionida										
Androctomus crassicauda	A	1·1	0·8	1746·5	3·2	1·0	2·2	3·6	6·2	P
Total =		129·2	100·2	55014·3	100·0					
Number of sampling units =						10·0	—			
Total number of occurrences						—	45·0			

A = adult, L = larva, D = detritivores, G = grazers, P = predators

Table 4

Population density (PD) per m², population biomass (mg fresh weight/m², BM), percentage (%) of population density and biomass, absolute and relative frequencies (AF and RF) expressed as percentage and absolute and relative importance values (A. I. V. and R. I. V. of mesofauna groups of AL—KHIRAN

Animal group	Stage	PD	%	BM	%	AF	RF	A.I.V.	R.I.V.	Ecological function
Coleoptera		*210·7*	*77·7*	*45756·7*	*86·0*	*38·0*	*67·0*	*8·6*	*234·9*	
Tenebrionidae										
Gonocephalum sp.	A	1·1	0·4	14·0	0·03	1·0	1·8	1·2	2·2	D
Adesmia cancellata	A	2·2	0·8	965·5	1·8	2·0	3·6	3·6	6·2	D
Ammogiton sp.	A	17·8	6·7	97·7	0·2	5·0	9·1	3·9	16·0	D
Mesostena sp.	A	148·7	56·5	6839·9	12·8	10·0	18·0	7·0	87·4	D
Triptera sp.	A	4·4	1·7	3202·9	6·0	3·0	5·5	4·6	13·1	D
Oxycara sp.	A	1·1	0·4	7·9	0·02	1·0	1·8	0·9	2·2	D
Clitobicus sp.	L	1·1	0·4	104·7	0·2	1·0	1·8	2·1	2·4	D
Tentyrina sp.	A	1·1	0·4	108·2	0·2	1·0	1·8	2·1	2·4	D
Scurus sp.	A	5·5	2·1	8076·2	15·2	3·0	5·5	5·1	22·8	D
Cyphostethe	A	2·2	0·8	157·2	0·3	2·0	3·6	2·8	4·7	D
Pimelia arabica	A	22·2	8·4	21937·6	41·2	6·0	10·9	6·5	60·5	D
Scarabidae										
Scarabaeus sacer	A	3·3	1·3	4245·4	8·0	3·0	5·5	4·6	14·8	D
Formicidae										
Messor sp.	A	4·4	1·7	22·0	0·04	2·0	3·6	2·3	5·3	D
Isoptera										
Diptera										
Psammotermes sp.	A	18·9	7·2	342·0	0·6	6·0	10·9	4·6	19·0	G
Asilidae sp.	A	1·1	0·4	55.6	0·1	1·0	1·8	1·8	2·3	P
Arachnida		*3·3*	*1·2*	*6740.1*	*12·64*	*3·0*	*5·4*	*4·82*	*19·3*	
Araneida	A	1·1	0·4	18.4	0·04	1·0	1·8	1·3	2·3	P
Scorpionida										
Androctomus crassicauda	A	1·1	0·4	6675·1	12·5	1·0	1·8	3·9	14·7	P
Compsobuthus arabicus	A	1·1	0·4	46·6	0·1	1·0	1·8	1·7	2·3	P
Isopoda (Oniscoidea)	A&J	24·8	9·4	386·1	0·7	5·0	9·1	4·68	19·2	D
Total =		263·2	99·8	53302·5	100·1					
Number of sampling units =						10·0	—			
Total number of occurrences						—	55·0			

A= adult, L = larva, J = juvenile, D = detritivores, G = grazers, P = predator

The predators in Umm Al-Riman, Al-Wafran constitute 9.4%, and 1.7% of the number of detritivores and grazers respectively and 0.71%, 3.52% and 1.7% of thier biomass respectively.

DISSCUSSION

The major components of the desert mesofauna in the surveyed areas of the state of Kuwait as indicated from the relative and absolute importance values (R. I. V. and A. I. V.) index is Coleoptera. It is important to note that this group is the dominant mesofauna in most arid soils of the world (Kolkaila et al., [11]; Rodin, [14]; Seely, [15]; Hammed, [8]; Ghabbour and Shakir, [3]). This gives an idea that this group shows a specially high adaptation to the harsh environment, possibly by physiological means. Hadley [6], [7] found that epicuticular permeability to water loss in the desert Tenebrionid beetle *Eleodes armata* decreases as a function of long chain branched hydrocarbons and/or long chain fatty acids.

Variations in the absolute importance value (A. I. V.) index of Coleoptera, Arachnida, Isoptera and Formicidae were apparent between the sites. Al-Khiran site supported more populations of Coleoptera, Arachnida and Isoptera than Umm Al-Rimam and Al-Wafrah sites, whereas more populations of Formicidae were associated with Umm Al-Rimam than Al-Wafrah and Al-Khiran sites. The abundance of Coleoptera in Al-Khiran site may be due to the fact that the surveyed area was recently heavily grazed by sheep and goats. Grazer's dung were accumulated on the surface of sand, thus favouring dung feeder mesofauna such as *Scarabaeus sacer* to flourish. Similarly, the increase in (A. I. V.) index of Arachnida may have resulted from the variation in the dominance of heavier individuals such as Scorpionidae which were totally absent from Umm Al-Rimam. Further, the increase in Isoptera is most probably related to the discarded sheets of rotten wood found in the site which provide food for this group of mesofauna.

Whilst Al-Khiran supported more populations of Coleoptera, an increase in species diversity was found in Al-Wafrah site. This increase may be the result of the physiographic features of the site. The area surveyed in Al-Wafrah was surrounded by the trees *Tamarix aphylla*. In addition, heaps of leaf litter and tree branches were also found. These conditions could create different habitat niches for varieties of species. Odum [12] mentioned that the enlargement of the organic structure of an ecosystem increases species diversity.

Again, the increase in the absolute importance value (A. I. V.) index of the thin cuticle insect (Formicidae) in Umm Al-Rimam may be in accordance with the general moisture content of the soil. The area surveyed in Umm Al-Rimam was nearby an irrigated farmland where irrigation is heavily applied to counterbalance the harsh climate. Soil water content of Umm Al-Rimam was significantly higher than the other two sites (Table 1) and this is likely to have been the case. Moreover,

the farmland nearby Umm Al-Rimam supports plant seeds which can attract more individuals of seed harvesting ants. Crawford [1] suggests that different ecological categories of desert ants are relating to their food resources.

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University of Kuwait
Kuwait
Present address:
Faculty of Science, Zoology Department
Moharrem Bey
University of Alexandria
Alexandria, Egypt

THE ROLE OF EARTHWORMS IN DECOMPOSITION PROCESSES

M. FALCĂ

Two forest ecosystem soils have been analysed in order to establish the characteristics of decomposition and to emphasize the role of earthworms in these processes. Using nylon mash bags with 1/1 mm and 7/7 mm openings it was shown that the decomposition rate of timberline spruce and dwarf pine needles was nearly the same in both sets of bags. Because of acid soils and other structural properties of these soils, earthworms, as an important decomposing animal group, presented small numerical densities and did not play an important role in decomposition, even they could penetrate the bags with 7/7 mm openings.

There are many studies on the role of soil animals in decomposition processes. Heath and Edwards (1964), Madge (1965), Heath, Arnold and Edwards (1966), King, Heath (1966), Masson (1977) Heal and Dighton (1986) are only some of the names that emphasized the subject. But no one tried to establish the correlation between the earthworm number and litter decomposition. It is well known that in acid soils of timberline spruce forests and dwarf pine ecosystems as well as in some other acid soils, the earthworm species composition and numerical densities are smaller than those of soils with a higher pH in some deciduous forests. The slower rate of decomposition of timberline spruce needles is closely related, among the other factors (the chemical structure of organic material being a fundamental one), with the numerical structure of earthworm communities.

MATERIAL AND METHODS

Decomposition of timberline spruce (*Picea abies*) and dwarf pine (*Pinus mugo*) needles from the high altitude of the Bucegi Mountains was studied for 9 years until this process was finished. 60 nylon mesh bags with 1/1 mm and 7/7 mm openings (7/10 cm the dimension of a bag), containing 1 g dry needles each, were buried 2.5 cm deep into the soil. Three of them from each of the two areas were yearly analyzed. For the evaluation of earthworm numerical densities each plot was randomly sampled by a metal frame of 25/25 cm. Seven soil samples were taken from each plot, each month, from June till October.

Timberline spruce forest is located on the Cocora Mountains, on the Ialomitean side of the Bucegi Massive. The altitude is between 1770 m and 1810 m, North-West exposure, 30° slope. Yearly mean temperatures are around 0°. Mean precipitations are around 700 ml in a year. The soil is regosoil skeleton-like, profound, oligobasic. Strong bioaccumulation and strong acid with pH = 4.7 in A_0 horizon. The humus content is between 53.5 in A_0 horizon and 2. 1 in (B_s) D horizon. The C/N ratio shows a weak mineralization, that is, the transformation in humus of organic

materials. Nitrogen and phosphorous content is middle rich to rich. Th
accompanying flora comprises *Soldanella hungarica, Homogyne alpina* an
rare individuals of *Vaccinium myrtillus*.

Dwarf pine ecosystem is located on the Cocora Mountains at the
lower limit of subalpine level. The altitude is between 1850 m 1870 m
West-North-West exposure and 20° slope. The soil is typically brown
strongly acidified with pH = 4.15 in upper level. The soil is rich in nit
high (39.2 in the litter level). The ratio C/N is very
Temperature, soil humus content and precipitat
to timberline spruce forest.

RESULTS

Timberline spruce forest

The quantity of decomposed needles in th
introduction of plastic bags with 7/7 mm openings
sented 40 %, nearly half from the entire quant
soil (Table 1). The decomposition was slow in t
between 8 and 20 %. The process was finished af
position has followed nearly the same curve in
openings, with some differences from one year to
ficant. It is to show that decomposition was re
the first year compared with the bags with 7/7 mm
nearly one third from the entire litter introduced
In both 7/7 mm and 1/1 mm opening bags the big
through decomposition took place in the first year
into the soil. In order to establish the role of earth
processes, their species composition and numeri
blished (Table 3). The small number of species an
were in concordance with the structural and
of the soil, with pH around 4.7. The dominant
Dendrobaena byblica, accompanying species bein
baena species and *Aporrectodea rosea rosea*. In t
introduction of the bags into the soil the earthw
was doubled, compared with that of the following
litter was 40 % in the first year in the bags of 7/7
earthworms could enter, even if they were scarc
that they have participated in this process, along
Collembola, which took over the decomposition
of soil. The decomposition was slow in the bags
the first year, because the earthworms could not er
posing animals with big size like Diplopoda, Isopo
identified, the decomposing role of soil fauna was
rina and Collembola. Owing to that fact the de
timberline spruce needles in the bags of 7/7 mm a
tures were nearly similar, with insignificant num
decomposition process of organic material represen
of physical, chemical and biological nature, in
special emphasis on earthworms, represents one o
multiple components.

Table 1

Decomposition rate of timberline spruce and dwarf pine needles in the nylon mesh bags of 7/7 mm mesh apertures.

Tree species	The variant	The litter quantity introduced in each bag (mg)	The weight of needles lost through decomposition during the time: IV 1982 — X 1983	VI 1982 — XI 1984	VI 1982 — X 1985	VI 1982 — XI 1986	VI 1982 — VIII 1987	VI 1982 — X 1988	VI 1982 — X 1989	Losing weight of needles (%) through decomposition during the time: VI 1982 — X 1983	IX 1983 — IX 1984	X 1984 — X 1985	IX 1985 — XI 1986	X 1986 — VIII 1987	IX 1987 — X 1988	IX 1988 — X 1989
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Picea	1	1000	0·428	0·638	0·733	0·821	0·898	0·963		43	21	10	9	8	7	
	2	1000	0·408	0·588	0·683	0·775	0·878	0·947		41	18	10	9	10	7	
abies	3	1000	0·359	0·569	0·652	0·760	0·865	0·966		36	21	9	11	11	10	
	Mean		0·398	0·598	0·689	0·785	0·880	0·959		40	20	10	10	10	8	
Pinus	1	1000	0·357	0·501	0·598	0·703	0·798	0·884	0·995	36	15	10	10	9	9	11
	2	1000	0·301	0·474	0·583	0·696	0·782	0·891	0·998	30	17	11	10	9	11	11
mugo	3	1000	0·309	0·459	0·541	0·671	0·763	0·878	0·979	31	15	8	13	9	12	10
	Mean		0·322	0·478	0·574	0·690	0·781	0·884	0·990	32	16	10	12	9	10	11

Table 2

Decomposition rate of timberline spruce and dwarf pine needles in the nylon mesh bags of 1/1 mm mesh apertures

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Picea	1	1000	0·413	0·611	0·683	0·762	0·857	0·927	0·981	41	20	7	8	10	7	5
	2	1000	0·335	0·707	0·735	0·808	0·880	0·935	0·995	33	37	3	8	7	6	6
abies	3	1000	0·353	0·66	0·695	0·759	0·825	0·916	0·979	35	31	4	7	7	9	6
	Mean		0·367	0·659	0·704	0·776	0·854	0·926	0·985	36	29	5	8	8	7	6
Pinus	1	1000	0·342	0·488	0·560	0·626	0·741	0·862	0·980	34	15	7	7	12	12	12
	2	1000	0·288	0·407	0·537	0·623	0·720	0·822	0·921	29	12	13	9	10	11	10
mugo	3	1000	0·296	0·447	0·547	0·635	0·737	0·841	0·952	30	15	10	9	11	11	11
	Mean		0·309	0·447	0·548	0·628	0·732	0·841	0·951	31	14	10	8	11	11	11

Table 3

Numerical densities of earthworms from the soils of timberline spruce and dwarf pine forests (mean number/square meter) — Confidence level 95 %

Earthworm species	Timberline spruce forest						Dwarf pine ecosystem					
	1		2		3		1		2		3	
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Aporrectodea rosea rosea	3·36	0·26	0·57	0·14	2·28	0·14	3·42	0·26	7·86	0·75	2·28	0·14
Dendrobaena alpina alpina	2·28	0·14	5·13	0·21	2·85	0·42	18·07	1·93	6·85	0·65	2·28	0·14
Dendrobaena octaedra	1·14	0·16	1·71	0·14	2·28	0·18	1·71	0·18	1·14	0·16	2·28	0·14
Dendrobaena byblica	12·52	1·71	0·57	0·14	2·28	0·14	4·00	0·36	1·14	0·16	0·57	0·13
Dendrodrilus rubidus rubidus	—	—	1·71	—	—	—	2·85	0·14	—	—	—	—
	19·30		9·69		9·69		30·05		16·99		7·41	

1, 2, 3 = years of researches

The relation between the yearly rate of this process and the numerical density of earthworms must be analyzed taking into account this complexity. The chemical content of the organic material and the ratio C/N play a fundamental role in this process. The mineral content of timberline spruce needles emphasizes calcium with 0.71 g/100 g soil, the other mineral elements (total nitrogen = 0.32 g/100 g soil; phosphorus = 0.25/100 g soil and potassium = 0.46 g/100 g soil) representing smaller values, compared with the other similar ecosystems. C/N ratio by its high values (32.91 in litter horizon, 28.16 in fermentation horizon and 27.19 in humus horizon) emphasizes a slow mineralization of litter confirming the slow rate of decomposition established by the nylon mesh bags. It is known that decomposer fauna actively participates in decomposition processes when the C/N ration is smaller than 20. In timberline spruce forest the value of C/N ratio is much higher than 20, especially in the horizon litter, which underline the same conclusion, that is, a slow mineralization because of a small contribution of earthworms in the processes of litter decomposition.

Dwarf pine ecosystem

The decomposition of dwarf pine needles has followed nearly the same curve like that of timberline spruce needles. In the nylon bags of 7/7 mm mesh apertures, 0.322 g of needles were decomposed in the first year, representing 32 % from the entire quantity of needles introduced into the bags (Table 1). In the second year the decomposition was half compared with the first year and a relatively uniform decomposition followed in the next years. The entire quantity was decomposed in 7 years. In the bags of 1/1 mm apertures, 31 % from the entire litter introduced into the bags were decomposed, the curve of decomposition being nearly similar to the bags of 7/7 mm mesh apertures (Table 2).

Earthworm fauna was poor, the numerical density values being very low with the exception of the first year when the mean number of individuals was 30.05/m^{-2} (Table 3). The dominant species was *Dendrobaena alpina alpina* followed by *Aporrectodea rosea rosea* with much smaller values. Comparative aspects regarding the important role of mesofauna (Acarina and Collembola) in decomposition processes showed in timberline spruce forest are maintained in this ecosystem. Soil acidity was stronger than in timberline spruce forest (pH = 4.5) and C/N ratio was higher than the established ratio for optimal activity of earthworms (39.12 in litter horizon, 31.15 in the fermentation horizon and 29.14 in humus horizon). In these conditions few earthworms identified in this ecosystem could not play an important role in decomposition processes.

CONCLUSIONS

The rate of litter breakdown depends on many factors. Among them plant species, chemical composition of the leaves, local climate and type of soil fauna are most important. In timberline spruce forest and dwarf pine ecosystem from the Bucegi Mountains (Romanian Carpathians) earthworms did not play a significant role on the decomposition

processes. Using the bags of different openings it was possible to demonstrate that the rate of litter breakdown was nearly the same in both types of nylon mesh bags even if the earthworms could or could not penetrate the openings. Because of the physical and chemical structure of the soil, with pH around 4.5, the earthworms presented small numerical densities. The C/N ratio was very high in these both ecosystems (32.9 in timberline spruce forest and 39.2 in dwarf pine ecosystem), much higher than 20, considered the maximum limit for an optimal decomposing activity of soil invertebrates.

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Institute of Biology
Bucharest, Splaiul Independenţei 296

OCCURRENCE OF OIL MOBILIZING BACTERIA IN DIFFERENT ECOLOGICAL SOURCES IN PAKISTAN

S. F. CHOUDRI and A. HAMEED

The present studies were undertaken to evaluate the status of high oil mobilizing bacteria in different ecological sources in Pakistan. Among 61 samples studied for isolation and selection of bacteria for residual oil release from porous media, 54 samples proved to be potential as hydrocarbon utilizing. Total viable count, gas production, change in pH, and oil utilization influenced by the type of ecological source and bacteria. Sugar waste found to be the best source for isolation of such bacteria.

Enhanced oil recovery to leech out the "iceberger" oil from reservoirs remains an ever target to promote the crude oil production because it is common knowledge in the oil industry that in many oil fields about 70 percent of the original oil steel remains in place (1). A number of enhanced recovery methods, in common practice are helpful up to a certain degree after which they loose their effectiveness (15). The microbial enhanced oil recovery (MEOR) method though not so commonly used appears to be one of the least expensive of the enhanced recovery processes and with proper research and development could become a cost effective process (17). A wide range of metabolic by-products that are biosurfactants, emulsifiers and biopolymers produced by microorganisms can be useful in crude oil technology (5, 6, 19). MEOR can also involve the injection of microorganisms into a reservoir that has the ability to produce gases, acids, alcohols, biosurfactants, biopolymers to improve the rate and amount of oil recovery (11, 14, 18). Several reports are found in the literature of field tests using the in situ MEOR technology (9, 10, 12, 14, 18).

Environmental parameters in a reservoir such as pH, salinity, temperature, oxygen and nutrients greatly influence the growth of these microorganisms and their ability to biodegrade hydrocarbons.

MEOR operations depend upon finding microorganisms that can survive and produce the desired metabolic products in reservoirs containing hydrocarbons and saline water.

Hydrocarbon utilizing microorganisms are ubiquitous in nature and can be isolated from a variety of ecological sources (20).

Microbes suitable for in situ operations have been reported to be isolated from various natural and industrial materials such as oil-soaked soil, soil from filled in slush pits, soil around an oil well, sewage sludge and effluents from the formation water, well bottom mud, salt marsh and marine sediments, waste effluents from food industry, biogas stations and municipal or pharmaceutical plants. The best source ot EOR bacteria is the formation water of the reservoir considered for microbial treatment, because the microbes have already adapted to reservoir's conditions (11, 13). Bacterial populations have been isolated from formation waters and mud from the bottom of recreational lakes. Several of these types of bacterial populations were combined and subjected to simulate reservoir conditions. The adapted populations which developed a capacity to use molasses as a substrate and yield large amounts of gases and acids were selected for further experimentation (13, 14).

Pakistan, being a net importer of energy, is a substantial amount of foreign exchange resources on import of petroleum and petroleum products. In view of ever increasing requirements, this burden will increase manifold unless a significant breakthrough is made through petroleum exploration and improving the amount of recovery from existing oil fields. The microbial enhanced oil recovery (MEOR) is such a technique that is less complex, involves minimum capital resources and has given promising results in countries wherever applied.

The present study was undertaken to isolate and identify hydrocarbon utilizing bacteria from various ecological sources and to study their potential to utilize hydrocarbons. Attempts were made to opt the best sources rich in oil mobilizing bacteria. Emphasis was laid on those sources having microbes with ability of high gas and acid production.

MATERIALS AND METHODS

A number of samples were collected from different ecological sources that were suspected of supporting hydrocarbon utilizing bacterial populations. Initial screening of the samples was performed in order to select samples containing most suitable microflora. Parameters taken into consideration were high gas and acid production. The method used by Lazar (14) was followed. Total viable counts of bacteria in samples that passed initial screening were found out by the method of Sharpley (16). Inoculum of bacteria from each sample was prepared by the enrichment culture technique, Bennett (3). Universal gear oil (Caltex) was used as a sole carbon source. After enrichment, their potential to utilise hydrocarbons from EOR point of view was determined in a medium that contained: Molasses 4%, Inoculum 10%, Oil 2%, Distilled water 100 ml, with pH 7.0.

Medium, inoculum and oil was added to 100 ml Erlenmeyer flasks. Flasks were sealed with rubber corks. 50 ml syringes were injected in each flask to measure gas production. Three controls, one without molasses, one without oil and one without inoculum were also installed to check the role of molasses as substrate, fermentative capacity of inoculum and experimental error in extraction procedure respectively. Flasks were incubated at 37°C. A daily record of gas production was kept in each case.

After two weeks when gas production fell to zero the pH of medium in each flask was recorded. The oil from each flask was recovered with 20 ml of 1.3 v/v ether solvent and benzene mixture. 0.9% NaCl solution was used for back extraction (of water soluble component). The amount of oil degraded was calculated by the method of Bhosle and Mavinkurve (4).

Bacteria were isolated from experimental flasks and identified on the basis of Gram staining, catalase test, oxidase test, motility, gel liquefaction and oxidation and/or fermentation of sugars with and without acid and/or gas production.

RESULTS AND DISCUSSIONS

A total of 61 samples were tested for isolation and selection of bacteria suitable for EOR and 54 samples showed substantial amount of

acid and gas production (Table 1). Lazăr (14) reported that bacteria which have a high capacity to ferment molasses and produce gases and acids were most suitable for EOR.

Table 1

Sources of suitable bacteria

	Name of the Ecological Source	No. of samples	The intensity of fermentation on medium with distilled water, inoculum, molasses and 10—15% of ecological source
1.	Fermented scum from sugar industry	3	++ — +++
2.	Waste from Petrochemical Industry as well as from other industries	22	− — ++++
3.	Soil intensively contamined with crude oil	32	− — ++++
4.	Water from Ihsan Yousaf Textile Mills Ltd. FD.	1	++
5.	Mud from a sulphur lake, Kalarkahar	1	+++
6.	Water sample from a sulphur lake, Kalarkahar	1	++
7.	Lyallpur Chemicals and Fertilizers Ltd. FD.	1	++

Results of total viable counts in each sample showed that samples of sugar waste support the maximum number of bacteria (14×10) with a capacity to ferment molasses. Whereas samples of oil contaminated soil had a lesser number of bacteria as compared to sugar waste samples (Table 2).

Gas production was considered a parameter of bacterial activity. Bacteria in samples of sugar waste proved to be most efficient as their capacity to produce gases was maximum (850 ml) (Table 2) as compared to other samples. The same samples showed a maximum fall in pH (3.1) as compared to other samples (Table 2).

Results of percentage oil utilization indicate that sugar waste samples that produced maximum acid and gases also utilized a maximum percentage of oil (32.5%), meaning that microflora with ability to ferment sugars and to produce acids and gases is most suitable for EOR. Most of MEOR field tests conduced to date involved use of bacterial populations that among certain other abilities had high acid and gas production, as these factors play a major role in an efficient and speedy recovery of oil from reservoir (7, 13, 14).

A mixed bacterial population was used for experimentation as bacteria are found in mixed cultures in nature and it is difficult to main-

tain a pure culture where field tests are involved. Better results are obtained when bacterial populations are used in combination for field trials (9).

Bacterial isolates were identified as *Pseudomonas*, *E. coli*, *Arthrobacter*, *Bacillus*, *Sarcina*. It was inferred from the results that gas production,

Table 2

Some performances in crude oil utilisation and in acid and gas production of the bacteria occurring in different ecological sources

	Ecological source	Total count	pH		Gas product (ml)	% of oil utilization
			Initial	Final		
1.	Oil contaminated soil	11.3×10^4 -22.4×10^5	7	4.3–4.7	170–658	10.25–21.23
2.	Waste from edible oil plant; chemical plant; sugar industry; a soap factory; municipal; textile factory	$3\times10^3-15\times10^6$	7	3.1–4.7	110–850	6–34
3.	Sulphar contaminated soil	3×10^4	7	4.5	484	10.5
4.	Municipal sewage	3×10^5	7	4.5	484	10.75
5.	Mud from sulphur lake	4×10^5	7	4.1	140	13
	CONTROLS					
1.	Oil		7	5.7	246	—
2.	Inoculum		7	5.7	—	5
3.	Molasses		7	6.6	—	6.5

change in pH, and oil utilization were influenced by the type of ecological source and type of microbes along with viable counts. However, microorganisms chosen for EOR will need a careful screening and adaptation procedure for the type of recovery process desired for a particular application, as each reservoir environment differs from the other.

CONCLUSION

1. Among 61 samples collected from several ecological sources in Pakistan, a number of 54 proved to be potential and contain bacteria of interest for residual oil recovery from porous media.

2. It was found that total viable count, gas production, change in pH and oil utilisation influenced by the type of ecological source and bacteria.

3. The wastes from sugar processing plants found to be the best source for isolation of such bacteria.

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Quaid-i-Azam University
Department of Biological Sciences
Islamabad, Pakistan

THE EVOLUTION OF THE GENUS *TRITURUS (AMPHIBIA, URODELA)*. ZOOGEOGRAPHICAL AND BEHAVIOURAL DATA

D. COGĂLNICEANU and M. VENCZEL

The present geographical distribution and the evolution of the genus *Triturus*, in particular of the *Triturus vulgaris* species-group, can be partly understood in the absence of fossil records through a morphological and behavioural study. Some hypotheses on the relationships and evolution of this genus are presented. Most of speciation and subspeciation was caused by geographical isolation due to glaciations. Reproductive behaviour played an important part also in speciation. Reproductive isolation maintained by different courtship behaviours allowed for the further evolution among this genus.

1. INTRODUCTION

The genus *Triturus* comprises 12 species of newts that are widespread in the western part of the Palearctic. It is a monophyletic group, being one of the most developed genus among the family Salamandridae, characterized by a number of unique characters. Their breeding behaviour is extremely important in understanding their evolution. The courtship behaviour is complex; does not imply amplex and consists of a series of stereotype, repetitive and rhythmic movements, done by the male in front of the female. Fecundation is internal, sperm transfer being realized through a spermatophore. Reproduction takes place in spring when newts pass from a terrestrial to an aquatic life. This change is accompanied by a series of morphological adaptations, some of which give rise to sexual dimorphism, more pronounced than in any other Urodela. Males develop during the breeding season a set of secondary sexual characters. The energetic cost for the development of these characters is high, but it might be covered by some immediate sexual adaptative advantages. The secondary sexual characters have evolved species-specific. The basic function of sexual behaviour is to ensure reproduction and sexual isolation. This explains why newt courtship behaviour has proved to be a very useful taxonomic tool (2).

Because of their rarity, fossils give very little direct information on the evolution of the genus *Triturus* in time and space (Table 1). That is why any attempt to construct a zoogeographical model must take into account almost exclusively only the present species. The study of male secondary sexual characters and courtship behaviour correlated with the present distribution of the species allows for some hypothesis on the evolution of this genus.

2. ZOOGEOGRAPHICAL DATA

The extreme diversity and variability of the secondary sexual characters and of the courtship behaviour, the present distribution and

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the evolution of the genus *Triturus* can be better understood by studying the effects of the climatic changes during the Quaternary. In Europe, more than in any other continent, the distribution of the fauna and flora is marked by the effect of glaciations. During the last 1.8 million years at least 4 main glaciations took place in Europe. It is quite improbable that we will ever know in detail the effects of each glaciation, but we can concentrate on their total effects, which were the speeding of the rate of evolution of most species. The climatic changes were very dramatic. During glaciations the glaciers reached southwards till 50 North latitude. Almost all the mountains in the southern part of Europe were covered with ice. Most of the species could survive only in isolated southern refuges. During interglaciations, enormous amounts of water became available causing flooding and rising of the sea level. Since amphibians cannot survive in salt waters this was also an important restrictive factor.

Since only some of the individuals of a population could survive, the overall populational genetic pool was poor. The drastic climatic changes were inducing higher evolutionary rates, thus increasing the differences between the various populations of the same species with fragmented distribution faster than genetic drift only. Some populations belonging to various species and subspecies did not leave the refuge where they survived glaciations. They remained as local species and/or subspecies in the southern part of Europe, in the three great refuges: the Iberian, the Apennine and the Balkan Peninsula (e. g. *T. boscai*, *T. marmoratus pygmaeus*, *T. helveticus sequerai* and *T. alpestris cyreni* in the Iberian Peninsula, *T. italicus*, *T. vulgaris meridionalis*, *T. carnifex* and *T. alpestris inexpectatus* in the Apennine Peninsula and more than 10 species and subspecies in the Balkan Peninsula). Despite these facts there is evidence that in interglaciations some populations spread northwards and in favourable microclimatic conditions survived there during glaciations (Venczel unpubl.). In Holocene started a slow postglacial invasion of northern Europe. The colonization was more successful from the southwestern (Iberian Peninsula) and the southeastern (Balkan Peninsula) refuges. In many cases zones of postglacial secondary contact are found (1). The invasion was "wave-like" and it was certainly favorized by heavy rainfall, by the extending of forests and by the lack of competitors.

Geist (16) showed for mammals that geographic variation is not necessarily genetic. Body size and shape change greatly in allometric fashion with the net income in material resources of the individual during ontogeny. Genomes can thus give rise to extremes in phenotype development; with resource shortages arising an environmental paedomorph (maintenance phenotype) and with resource abundance a hypermorph (dispersal phenotype). These extremes differ diametrically in morphology, behaviour and reproduction output so that maintenance phenotypes emphasize efficiency in utilizing the resources and in competing for them. Dispersal phenotypes maximize reproduction, exploration and dispersal. The same might be true for newts, like for example the paedogenic subspecies of *T. alpestris* from Yugoslavia that were synonymized with the nominal subspecies by Breuil and Guillaume (8); the differences in body size between the southern forms *T. marmoratus pygmaeus* and *T. helveticus sequerai* and the nominal subspecies (43), (44); and the case of the southern subspecies of *T. vulgaris* and the nominal subspecies, case that will be discussed in the next section.

3. EVOLUTION OF THE *T. VULGARIS* SPECIES-GROUP

An interesting case concerning speciation and evolution among the genus *Triturus* is the *T. vulgaris* species-group, that includes *T. vulgaris*, *T. helveticus* and *T. montandoni*. Although *T. montandoni* is very similar to *T. helveticus*, as shown by morphological and behavioural data (19), (34), biochemical and cytogenetical data (2), (30), (36), (37) point out clearly that *T. vulgaris* and *T. montandoni* are the most closely related species. The distribution of *T. montandoni* and *T. helveticus* is clearly allopatric. *T. vulgaris* is the most widely distributed newt species and is sympatric over quite a large area with *T. helveticus* and on a narrow contact zone with *T. montandoni*. Ten subspecies have been described in *T. vulgaris*, that differ mostly in male secondary sexual characters. Five subspecies (*graecus*, *dalmaticus*, *ampelensis*, *meridionalis* and *borealis*) share many characters in common: low and smooth-edged crests, well developed dorso-lateral ridges and tail filaments (38), (43), (44) so that they more closely resemble *T. helveticus* and *T. montandoni*, than the nominal subspecies *T. v. vulgaris*. *T. helveticus* and *T. montandoni* are sympatric only with *T. v. vulgaris*, the morphological differences among them being maximized (Table 2).

T. v. vulgaris hybridises quite easily in nature with *T. montandoni*. In places where both species breed together, hybrid individuals, which are mainly recombinants, may constitute up to 60% of a population (15), (34), (35). Only one true hybrid was signalled in nature between *T. vulgaris* and *T. helveticus* (17), despite the fact that in captivity they hybridise quite easily, mostly with the allopatric *vulgaris* subspecies (28), (Cogălniceanu, unpubl.). Experimental cross fertilization indicated a percentage of hybrid cleavage of 95.5% between the pair I. *vulgaris*-*T. montandoni* and only of 11% for the pair *T. helveticus*-*T. vulgaris* (37). In captivity hybrids between *T. helveticus* and *T. montandoni* were easily obtained (Cogălniceanu, unpubl.). The fact that two related species hybridise in captivity but not in nature demonstrates that they have elaborated effective barriers for reproductive isolation.

Based on electrophoretical data, the divergence between the most similar species, *T. vulgaris* and *T. montandoni*, was estimated as having occurred 6 ± 2 million years ago (36).

Quaternary glaciations found the 3 species of newts already formed, but we do not know their original distribution. *T. montandoni* and *T. helveticus* must have occupied an area near or similar to the present ones. *T. vulgaris* was probably distributed in Central Europe and the Balkan Peninsula. The Balkan Peninsula rather than Western Europe is considered to be the centre of dispersal of *Triturus* (1). Yugoslavia especially appears to be an important centre of evolutionary divergence of *T. vulgaris* subspecies. Four subspecies are found on the Adriatic Coast, on the Southern limit of the areal. Glaciations were here restricted to high mountains

Table 1

Fossil records of *Triturus* from some localities of Europe

m.y.	Age	Fossil records/x/. 1.	2.	3.	4.	5.	Localities	References
	late Holocene				x		Duza Sova Cave	Młynarskis Szyndlar, 1
	middle Holocene					?x	Giebultov	Młynarski, 1961
0.01								
	Holsteinian	x					Brașov	Bolkay, 1913; Estes, 19
	late Biharian	x			x		Kozi Grzbiet	Młynarski, 1977
	Biharian	x					Zalesiaki 1-fauna A	Młynarski, 1977
1.8								
	early Biharian	x			x		Betfia	Venczel, unpublished
	Villányian MN 17	x			x		Vcelare 6/1	Hodrova, 1985; Roček,
	early Villányian MN 16	x		x			Rebielice Królevskie IA	Sanchizs Młynarski, 19 Młynarskis Szyndlar, 1
	Csarnotian MN 15	x	x		x	x	Ivanovce	Hodrova, 1984
5.5								
	upper Miocene MN 8—9			x			Suchomasty 3	Hodrova, 1987
	upper Miocene MN 7		x				La Grive-Saint-Alban	Estess Hofstetter, 1976
24								
		T. cristatus	*T. marmoratus*	*T. montandoni*	*T. vulgaris*	*T. alpestris*		

Table 2

Main secondary sexual characters in males of the *Triturus vulgaris* species-group

Species/subspecies	Dorsal crest	Height of dorsal crest	Tail tip	Dorsolateral fold	Toe flap
T. v. vulgaris	denticulated	high (3—5 mm)	rounded	absent	poor develop
T. v. ampelensis	smooth edged	low (1—3 mm)	pointed	well developed	well develop
T. v. borealis	smooth edged	low (2,7 mm)	pointed	present	poor develop
T. v. dalmaticus	smooth edged	low	filament	present	poor develop
T. v. graecus	smooth edged	low	filament	well developed	well develop
T. v. kosswigi	smooth edged	low	filament	well developed	well develop
T. v. lantzi	denticulated	high (2—4 mm)	filament	poor developed	well develop
T. v. meridionalis	smooth edged	low	pointed	well developed	poor develop
T. v. schmidtlerorum	denticulated	medium (2 mm)	pointed	absent	poor develop
T. helveticus	absent	—	filament	well developed	strong webbi
T. montandoni	absent	—	filament	well developed	absent

and it can be assumed that they caused fragmentation of the range of *T. vulgaris* into a number of regions that should roughly correspond to the present ranges of the subspecies (26). Bolkay (7) states that *T. v. graecus* resembles most closely an ancestral species that migrated Southward during Pleistocene glaciations period. After glaciations it spread to the North and eventually gave rise to new subspecies (like *vulgaris* and *meridionalis*). Fuhn (14) also considers *T. v. vulgaris* a phylogenetically younger subspecies.

During glaciations some populations of *T. vulgaris* spread to the South and Southeast crossing the now submerged continental bridges. Anatolia was colonized mostly along the Black Sea and Mediterranean Sea Coast, that had a warmer climate. The last glaciation (Würm) left isolated populations in the Caspian refuge that eventually differentiated in the *lantzi* subspecies.

An electrophoretic survey of the genetic variability in various populations of *T. vulgaris* from Yugoslavia showed that the populations of *T. v. vulgaris* appear to be genetically the most variable with an average heterozygosity of 9.8% (25). The populations of other subspecies occupying much smaller areas show substantially lower levels of genetic variability. Average heterozygosities are 6.5% for *T. v. meridionalis*, 4.6% for *T. v. graecus* and 3.5% for *T. v. dalmaticus* (25). A morphological and electrophoretical study of *T. v. schreiberi* denied its separate taxonomic position (27), (42).

T. v. ampelensis differs from the other subspecies in that it is distributed in an area relatively central with regard to the distribution of the species and is surrounded by *T. v. vulgaris*. For this situation Bănărescu (3, p. 94) suggests the gradual, recent formation by appearance of mutant individuals that eliminate through intraspecific and even intrapopulational competition the individuals belonging to the ancestral type. This hypothesis seems quite improbable since *T. v. vulgaris* is by far the most competitive subspecies. We believe that in Pliocene *T. vulgaris* had a relatively uniform distribution that was broken up during glaciations. Some populations survived in the Apuseni Mountains, together with other relict species and gave rise to *T. v. ampelensis*. Preliminary electrophoretical data indicate that the differences between *ampelensis* and the nominal form are high ($D_H = 0.17$) (Rafinski and Cogălniceanu, unpubl.).

All this data suggest that geographic isolation played the most important part in subspeciation among *T. vulgaris*.

Bănărescu (3) states that primitive species survive at the periphery of the distributional range while the most evolved and competitive in the central part. This is the case for *T. montandoni* which is restricted to an area in the Eastern part of the *Triturus* range. In Romania, *T. montandoni* and *T. vulgaris* have essentially a parapatric distribution, allowing for a narrow contact zone (10). It is probably a zone of postglacial secondary contact (1). Most of the places inhabited by the two species are above 500 m, where *T. vulgaris* reaches the limit of its ecological optimum and is less abundant and competitive (15). Since the ethological barriers for reproductive isolation are not very effective, this can sustain the hypothesis of *T. vulgaris* extending its range recently. The speed of this postglacial invasion must have been high. For example, the British

Isles were separated from the continent about 10—12,000 years ago. England is inhabited by both *T. vulgaris* and *T. helveticus*, but only *T. v. vulgaris* is found in Ireland. Ireland was separated from the continent first, so that only the Amphibians that were able to cross before the sea-level rose can be found there (4). This means that *T. helveticus* reached the west coast of England after the separation of Ireland. This lateness might be due to lower colonizing abilities compared to *T. v. vulgaris*. The great morphological uniformity of *T. v. vulgaris* over its entire range suggests a recent postglacial invasion that probably started from Central Europe.

Lascu (29) suggests that by the end of the Miocene most of present Romania was covered by sea leaving only two islands, that roughly correspond to the present distribution of *T. montandoni* and *T. v. ampelensis*. If this hypothesis proves to be correct it will support further the role of geographic isolation in speciation and subspeciation in the genus *Triturus*.

4. BEHAVIOURAL DATA

Two main directions regarding the evolution of reproductive behavi our can be observed among the species of the genus *Triturus*. The larg species (*T. cristatus* superspecies complex, *T. marmoratus* and *T. vitta tus*) have evolved towards a pronounced sexual dimorphism and dichroism, visual cues being most important during courtship. Some forms of territorial behaviour (46) and even intraspecific aggression (39) have been observed, suggesting a lek breeding system. In the smaller species, sexual dimorphism is less pronounced, olfaction is more involved (11) and males have developed forms of sexual interference (45). Belvedere et al. (5) observed that the effectiveness of courtship odorants in promoting a positive chemotaxis and sniffing display seems to be inversely related to the degree of sexual dimorphism of the species, increasing from *T. cristatus* (with high sexual dimorphism and less olfaction) to *T. alpestris* and finally *T. italicus* (less sexual dimorphism and high olfaction).

The secondary sexual characters in male newts are important in the prereproductive ethological barriers for sexual isolation. A correct understanding of a biological system requires consideration of both the form and function of that system. Unfortunately, the biological significance of most of the secondary sexual characters is not yet clear (9), (18), (21). *T. v. vulgaris* does not fit in this classification because although a small species it has a pronounced sexual dimorphism. Like males of the large species, males of *T. v. vulgaris* have a well developed dorsal crest.

The dorsal crest is present only during the breeding season and is represented by a vascularized tegumentary extension. It may serve as an auxiliary respiratory organ during courtship when air breathing is suppressed (20). It is not clear, however, if this is a primary or a secondary function. Cutaneous crests in breeding males evolved several times in the evolution of the genus and perhaps their primary adaptive value was connected with some aspects of sexual selection (34). The presence of the dorsal crests in the larger species of newts may be explained by a low body surface/body volume ratio. Taking into account the importance

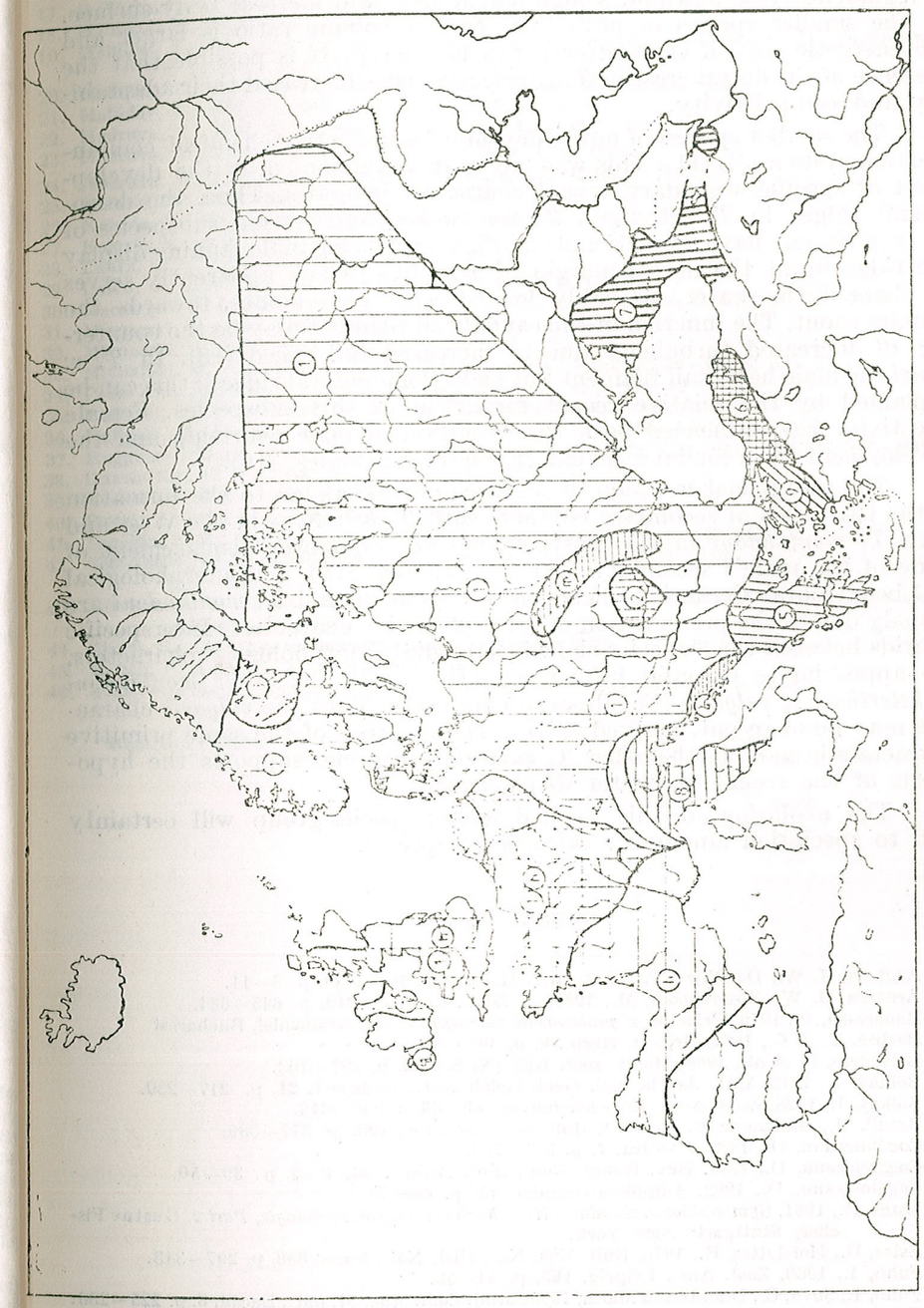

Fig. 1. — Distribution map for *Triturus vulgaris*, *T. helveticus* and *T. montandoni*, after (30) and (44). 1 — *T. v. vulgaris*; 2 — *T. v. ampelensis* 3 — *T. v. boreal is*; 4 — *T. v. dalmaticus* 5 — *T. v. graecus*; 6 — *T. v. kosswigi*; 7 — *T. v. lanzi*; 8 — *T. v. meridionalis*; 9 — *T. a.*; *schmidtlerorum*; h — *T. helveticus*; m — *T. montandoni*.

of tegumentary respiration, a high dorsal crest will increase body surface. In the smaller species of newts the surface/volume ratio is larger and the energetic cost of crest growth can be spared. It is possible that the presence of the dorsal crest in *T. v. vulgaris* males increased their adaptability and competitivity.

The smaller species of newts developed an effective chemical communication system (5), (11). This was probably accompanied by the development of specific secondary sexual characters in males. Thus, the dorso-lateral ridges in *T. helveticus*, *T. montandoni* and several subspecies of *T. vulgaris* may have a functional significance. During male fanning display the ridge forms the dorsal margin of a groove, which apparently serves to channel the water, probably loaded with pheromones, towards the females snout. The function of the caudal tail filament may be the countering of increased turbulence due to increased tail speed (18). In *T. v. lantzi* the male has a tail filament but lacks dorso-lateral ridges ; this can be explained by the relative recent formation of this subspecies. Female selectivity has influenced both the evolution of male courtship and the development of secondary sexual characters in males (19).

The postglacial invasion of *T. v. vulgaris* that led to the formation of the two zones of secondary contact (with *T. helveticus* in the West and with *T. montandoni* in the East) caused the character displacement of some of the male's secondary sexual characters (18). The morphological and behavioural similarities between *T. helveticus* and *T. montandoni* are probaly due to the preservation of some primitive characters. Interspecific hybrids between the 3 species show intermediate morphological characters, the approximate parental influence increasing in the order *montandoni* < *helveticus* < *vulgaris* (Cogălniceanu, unpubl.). Thus the *vulgaris* characters may be of recent, derived origin. The presence of the same primitive characters in most of the other *T. vulgaris* subspecies supports the hypothesis of the recent character displacement.

The evolution of this very dynamic species-group will certainly lead to speciation among *T. vulgaris* subspecies.

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Faculty of Biology
Bucharest, Splaiul Independenţei 91—95
and
Bihor District Museum
Oradea

ECOLOGICAL AND ZOOGEOGRAPHICAL RESEARCHES OF ORIBATID FAUNA *(ACARI – ORIBATIDA)* IN ROMANIAN FORESTRY ECOSYSTEMS

VIORICA HONCIUC

The paper presents the results of ecological and zoogeographical researches of the oribatid populations belonging to 140 species signaled out in some beech forests in Romania.

Previous researches concerning some aspects of oribatid fauna in ecosystems with beech in Romania proved that vegetation, altitude, the type of soil and the quality of organic sphere represent determining factors for the density and for the biomass production respectively at the level of oribatid communities.

In the present work the use of synthetic indices of dominance individualizes (Q da ; Q dr) the bioindicatory character of some oribatid species and the qualitative and quantitative (Q_j; Q_R) similarity indices render evident the participation of oribatid populations to the division into zones of the studied beech forests. The zoogeographical analysis is meant to underline the ubiquist character and the ecological valence of these microarthropods.

MATERIAL AND METHOD

The faunistic material resulted from thirteen surfaces (Table 1) that belong to the different forestry ecosystems previously described (4, 8, 9, 10, 11) has been vernally and aestivally collected between 1979—1985.

The investigations have been made in the organic sphere with the boundary underspheres, fermentations and soil. The samples collected by the Mac Fadyen soil core-borer have been selected according to the Berlese-Tullgren method ; the oribatid fauna registered 140 species with 6910 individuals (adult forms).

The structure of the oribatid communities was the basis of calculating the following indices :

a) the dominance index (7)* of the species calculated according to the formula :

— absolute dominance (Q da) : Σ p DCS

— relative dominance (Q dr) : $\Sigma \frac{\text{p DCS}}{\text{n}}$

where n is the number of dominating taxons (D + C + S), the framing was achieved on the basis of Table 2.

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Table 1

Some characteristics of the studied surfaces and of the oribatid populations

Surface	Relief Altitude (in m) Exposition	Soil type and its characteristics	The decomposition Coefficient Jenny	pH to 0—10 cm	Forest type and vegetal association	Oribatids number of indiv.	Abbreviations used
Cororăştii — Misli	hill 320 NE	luvic reddish-brown soil pseudogleyed, clayey-loamy clayey, medium content in mull type humus	0.42	6.03	Beech with hornbeam and *Carex pilosa*	233	C
Bocşa Montană	hill 350 NV	typic luvic brown soil, loamy—sandy, small content in mull type humus	0.42	6.9	Beech with *Asperula-Asarum-Stellaria*	568	B
Govora	hill 350—400 NE	acid brown soil cryptospodic clay-loamy, moderate mull type humus content	0.41	4.4	Beech with *Luzula luzuloides*	534	Gv. L
Govora	hill 350 NE	typic acid brown soil, loamy—sandy, mull type humus content	0.49	5.8	Beech with mull flora and *Asperula-Asarum*	612	Gv. M
Galbenul	mountainous premountainous 745 NE	typic podzolic brown soil, moderate mull — moder type humus content	0.40	4.1	Beech with *Luzula luzuloides*	503	Gl. L
Galbenul	mountainous premountainous 740 E—SE	cryptospodic acid brown soil, loamy — sandy, middle mull type humus content	0.37	4.0	Beech with mull flora and *Oxalis-Dentaria-Asperula*	245	Gl. M
Galbenul	mountainous premountainous 740 SV	typic argilic brown soil, middle mull type humus content	0.37	5.0	Beech with *Festuca drymeia*	359	GlF.
Lespezi	mountainous premountainous 790—840 E—SE	typic acid brown soil, sandy-loamy, moderate mull type content	0.47	6.0	Beech with mull flora and *Asperula-Asarum*	321	L
Piscul Cîinelui	mountainous premountainous 1330—1380 SV	typic acid brown soil, middle edaphic, mull type humus content	0.49	5.3	Beech with *Oxalis-Dentaria-Asperula*	243	P. C.
Furnicoşi	hill 580 SE	typic acid brown soil, loamy-sandy, mull-moder type humus content	—	5.6	Beech with mull flora and *Oxalis-Dentaria-Asperula*	133	F
Butin	mountainous premountainous 670 S	andic cryptospodic acid brown soil, highly edaphic, sandy-loamy, medium mull type humus content	0.28	5.4	Beech with *Oxalis-Dentaria-Asperula*	326	Bu
Huedin	mountainous premountainous 900 V	typic podsolic brown soil, sandy-loamy to loamy-sandy, medium mull type humus content	0.43	5.3	Beech with *Festuca drymeia*	1292	H
Gutin	mountainous premountainous 720—880 SV	cryptospodic acid brown soil, sandy to sandy-loamy very high mull type humus content	0.34	4.6	Beech with *Vaccinium*	1541	Gu

Table 2

	p > = x + 3s	p > = x + 2s	p> = x + s	p > = x
p. max. > = x+3s	D+C	S	I	A
p. max. > = x + 2s	—	D + C	S	I
p. max. > = x + s	+	—	D + C	S
p. max. > = x	—	—	—	D + C

p = the relative abundance x = the mean ; s = the standard deviation ;
D = dominant (p = max) C = codominant (p < > max)S = underdominant ;
I = accompanier ; A = accidental.

b) the index of qualitative similarity Jaccard :

$Q_j = \frac{C}{a + b - c}$; a, b — the number of species present in the A or B ecosystems.

c — number of species common to both ecosystems.

c) the index of quantitative similarity Renkonen

$Q_R = \Sigma$ min. {p(i)} ; p(i) = proportion of individuals of the species in a sample.

The zoogeographical framing of oribatid fauna has been achieved by the use of reference material (1, 2, 12).

RESULTS AND DISCUSSIONS

a) Comparing the percentage values of the dominance indices (Table 3) we draw the conclusion that from the whole studied oribatid fauna, only six species registered significant values.

The obviously dominant species for most of beech forests is *Oppia ornata*, the codominant species are *Oppia minus*, *Oppia neerlandica* and the underdominant : *Oppia getica*, *Quadroppia quadricarinata*, *Oppia paradecipiens*. It is only in beech forests Galbenul with *Luzula luzuloides* and Galbenul with mull flora, that the values registered by *Oppia ornata* place it as an underdominant species. The ecological significance of the species nucleus may be also reflected by the differences of percentage values mirrored by the two indices.

Thus, the absolute dominance (Q da) that underlines the quantitative participation of these species registered a minimum value of 0.2454 in the beech forest of Galbenul with *Festuca drymeia* and a maximum value of 0.4348 in the beech of Gutin. The relative dominance (Q dr) that reflects the qualitative participation of the species nucleus, registered the minimum value of 0.1339 in the beech forest of Galbenul with mull flora and the maximum value of 0.4149 was registered in Piscul Cîinelui. In seven of the studied surfaces the oribatid fauna registered a value parallelism of the two indices. By its dominant and bioindicatory character, the group of the six species with then relative abundances may fulfill a great part from the decomposition processes exercised by the whole oribatid population, at the level of each ecosystem, under the specific conditions of each beech forest studied.

Table 3

The representative oribatid species in the researched ecosystems, identified by using the dominance indices (Q da) and (Q dr).

Species	C		B		F.		P. C.		H		Bu.		Gu.		L		Gv. L		Gv. M		Gl. L		Gl. M		Gl. F	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
1 *Oppia ornata*	0.3120		0.2588				0.3315										0.3315		0.2512		0.1710		0.1014		0.1270	0.2454
2 *Oppia neerlandica*																										
3 *Oppia minus*																					0.1392				0.1107	
4 *Oppia getica*																			0.1248							
5 *Oppia paradecipiens*																										
6 *Quadroppia quadricarinata*																										
7 *Suctobelba trigona*																							0.1639			
Q da	0.3120		0.2588				0.3315										0.3315		0.3760		0.4116		0.4016		0.2454	
Q dr	0.3120		0.2588				0.3315										0.3315		0.1880		0.1372		0.1339		0.2454	
L	P. C.		F.		H		Bu.		Gu.																	
D	S		D	S	D	S	D	S	D	S																
1 0.2430	0.4149		0.3869		0.2487		0.40		0.1603																	
2									0.1838																	
3																										
4																										
5																										
6	0.1246																									
7																										
0.3676	0.4149		0.3869		0.2487		0.40		0.1603								0.40		0.4325		0.1838		0.0980		0.0785	0.0980
0.1838	0.4149		0.3869														0.40		0.2162						0.0980	
																									0.4348	
																									0.1087	

D — dominant; C — codominant; S — underdominant;

The maximum values of the indices (Q da; Q dr) render evident the oribatid fauna on the two surfaces and lends it a structural and dynamically dominant character.

b) Analyzing the diagram of the qualitative similarity (Q_j) we notice that a division of zones into two large groups ($Q_j = 0.1984$) of oribatid fauna in the studied beech forests is achieved.

The most striking similarity (Figure 1) joins the beech forests of

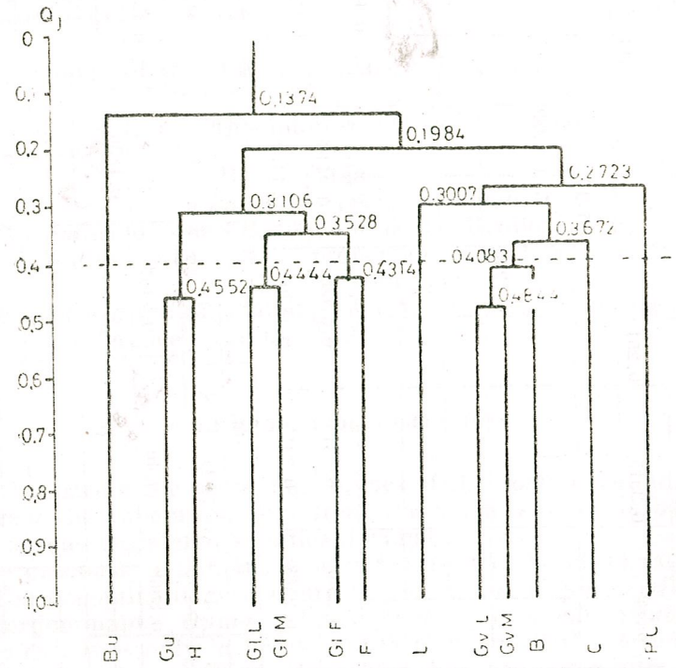

Fig. 1. — Jaccard similarity index (Q_j).

Govora to *Luzula luzuloides*, Govora with mull flora and *Asperula-Asarum* at an index of 0.4844 and frames them into the first grouping. It is here that beech forests of Boeşa Montană with *Asperula-Asarum-Stelaria*, Cocorăştii Misli with *Carex pilosa*, Lespezi with mull flora and *Asperula-Asarum*, Piscul Ciinelui with *Oxalis-Dentaria-Asperula* can be included.

The second grouping presents very close values of similarity and is formed by the beech forests of Galbenul with *Luzula luzuloides*, Galbenul with mull flora and *Oxalis-Dentaria-Asperula*, Huedin with *Festuca drymeia*, Gutin with *Vaccinium*.

The common and constant species signaled out in the beech forests of the first group are: *Oppia ornata*, *Oppia obsoleta*, *Oppia neerlandica*, *Suctobelba trigona*, *Quadroppia quadricarinata* (Table 4).

Within the second grouping and in addition to the species previously signaled out there still appear: *Oppia minus*, *Chamobates cuspidatus*,

otope	Biogeographical element
	Eur.
	hol.
	C.—Eur.
	hol.
	s. bip.—hol.—sbt. cosmp.
	Eurosibir.
	C.—N.—Eur.; S.—Am.
	Eur.; As.
	Eur.
rests	hol.
	R.
hyll lawns	pal.; h.—Eur.
sts	h.—Eur.
	h.—Eur.
	C.—N.—SE.—Eur.
	C.—N.—SE.—Eur.
	C.—N.S.—Eur.; N.—Am
	eur.
hyll lawns	Eur.; maghreb.
ns	Eur.; W.—Sibir.; pal.; S.—As.
synusia	Eur.; W.—Sibir.; maghreb; S.—As.
	Eur.; W.—Sibir.
st forests	C.—S.—Eur.
	Eur.; Cauc.
	Eurosibir.; S.—Eur.
	C.—N.—SE.—Eur.; Sibir. N.—Am.
	C.—SE.—Eur.
	C.—SE.—Eur.
	hol.
	Eur.; pal.
	hol.
	pal.
	Eur.
	pal.
ests, lawns	Eurosibir.
	s.bip.—hol.—sbt.
	Eur.
	—

Eur. = East-European; SE.—Eur. = South—East-Euro-
East—Asiatic; sbt. = subtropical; s. bip. = subbipolar;
nop. = cosmopolitan; h.—Eur. = holo—European; h.—

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Tectocepheus velatus, *Suctobelbella baloghi*, *Metabelba pulverulenta*, *Oppia getica*, *Oppia subpectinata*, *Oppia insculpta*, etc. The larger number of common and constant species in this grouping also determined a closeness of the similarity index values (Q_j).

The grouping of the beech forests is influenced by the values of the index as well as by the presence of the common and constant species signaled out in the two large groupings.

c) Using the Renkonen (Q_R) quantitative similarity index and following the steps also suggested by other authors (6) and replacing the term stand table (relevé) by the sample, the divisioning of the beech forests into zones, on quantitative criteria, was achieved.

From the construction of the cluster diagram (Figure 2) we have concluded that the whole faunal material is distributed into two groups, divided into subgroups, thus determining the division of the beech forests and suggesting the next considerations; except for the beech forests of Piscul Cînelui, Butin, Furnicoși with *Oxalis-Dentaria-Asperula*, included

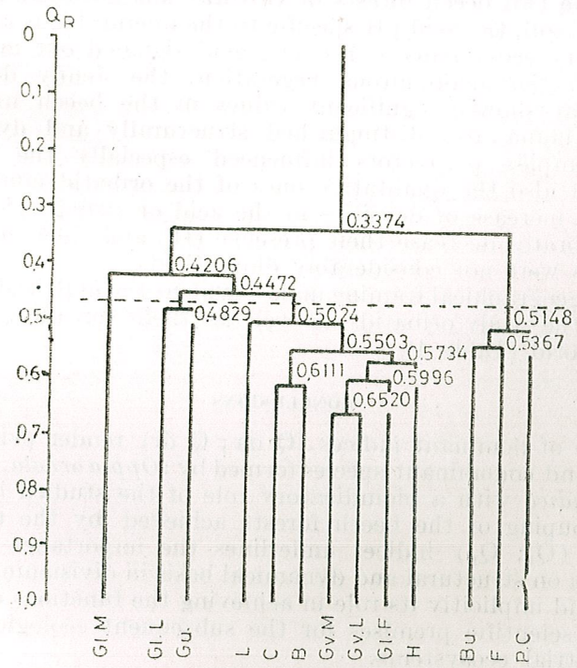

Fig. 2. — Renkonen similarity index (Q_R).

in the second group, the other beech forests represent a part of the first group; the most striking similarity generated by larger densities of the oribatid fauna is contained by the beech forests of Govora with *Luzula luzuloides*, Govora with mull flora and *Asperula-Asarum*, very close values have also been registered in Cocorăștii Misli with hornbeam and *Carex pilosa*, Bocșa Montană with *Asperula-Asarum-Stelaria* and Galbenul with *Festuca drymeia*.

The values of this index have grouped more densely the studied beech forests rendering evident the importance of the species with high density. In this way there have been marked the appearance of a group of species of the order of alliance formed by the three species belonging to the dominant codominant group to which *Oppia obsoleta* was added.

The use of the values of these indices leads to a clear structural and dynamical separation of a group of species with a bioindicatory character, of the studied beech forests as well as to a separation of the oribatid fauna from Govora with *Luzula luzuloides* and Govora with *Asperula-Asarum* as being the most significant and marking this character to the respective beech forests too.

In divisioning the beech forests, in addition to the index use, a most important part was played by the bio-edaphic factors as such; the altitude position as a richer distribution of the oribatid fauna into common and constant species, in the mountainous and premountainous beech forests, compared to the oribatid fauna in the hill beech forests (except for the two beech forests of Govora) was noticed; the structure and texture of soil, the acid pH specific to the mountainous and premountainous forestry ecosystems and strong acid signaled out in the Govora beech forests, the acidophitous vegetation, the Jenny decomposition coefficient that showed significant values in the beech forests, where the oribatid fauna also distinguished structurally and dynamically.

This complex of factors influenced especially the quantitative variations but also the qualitative ones of the oribatid fauna and registered a slight increase of densities in the acid or strong acid soils where other invertebrates decrease their presence (10) and thus the decomposition processes were not considerably diminished.

The zoogeographical framing makes evident the multitude of biotopes preferred by the study oribatids, as well as their spreading in Romania and in the world (Table 4).

CONCLUSIONS

The use of dominant indices (Q da; Q dr) render evident a group of dominant and codominant species formed by: *Oppia ornata*, *Oppia minus*, *Oppia neerlandica* with a bioindicatory role of the studied beech forests.

The grouping of the beech forests achieved by the use of values of similarity (Q_j; Q_R) indices underlines the importance of using the oribatid fauna on structural and dynamical basis in divisioning the forestry ecosystems and implicitly its role in achieving the functions of the ecosystem offering scientific premises for the subsequent ecological researches in the terrestrial ecosystems.

The zoogeographical analysis rendered evident the frequency of the European and palearctic species existing in the oribatid fauna in the beech forests of Romania.

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