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Nouveaux Lacertidae (Reptilia, Squamata) de l'Eocène inférieur européen

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RÉSUMÉ. Les localités de Dormaal et de Prémontré, de l'Eocène inférieur européen, ont fourni de nombreux fossiles de lézards. Parmi ceux-ci nous décrivons un nouveau genre de Lacertidé, *Dormaalisaurus*, représenté par deux espèces, *D. girardoti* et *D. rossmanni*. Une analyse cladistique menée sur les principales familles actuelles de Lacertilia et comprenant le nouveau genre fossile *Dormaalisaurus* fait de celui-ci le groupe frère des Lacertidae actuels.

MOTS CLÉS: Lacertidae, Eocène inférieur, Europe, Systématique.

New Lacertid lizards (Reptilia, Squamata) from the lower european Eocene.

ABSTRACT. The lower European Eocene localities Dormaal and Prémontré have yielded a rather diverse assemblage of lizards. Among them we recognize a new genus of lacertid lizard, *Dormaalisaurus*, represented by two species, *D. girardoti* and *D. rossmanni*. We undertook a cladistic analysis of the major extant taxa in the Lacertilia and the fossil genus *Dormaalisaurus*. Our results find *Dormaalisaurus* to be the sister-group of the extant Lacertidae.

KEY WORDS: Lacertidae, Lower Eocene, Europe, Systematics.

INTRODUCTION

Deux espèces de Lacertidae sont connues avec certitude de l'Eocène européen. *Plesiolacerta lydekkeri* Hoffstetter, 1942, était un lézard de grande taille (au moins 60 cm), enregistré depuis l'Eocène moyen (MP16) jusqu'à l'Oligocène inférieur (MP22) (HOFFSTETTER, 1942; RAGE & AUGÉ, 1993). BÖHME & WEITSCHAT (1998) ainsi que BORSUK-BIALYNICKA et al. (1999) ont redécrit un petit lézard conservé dans l'ambre de la Baltique (Eocène moyen) que KLEBS (1910) assimilait à l'espèce actuelle *Nucras tessellata* Smith, 1838. Les auteurs précédents ont montré que l'on avait affaire à un genre nouveau appartenant aux Lacertidae, *Succinilacerta succinea*. Un autre lézard conservé dans l'ambre de la Baltique, cette fois de grande taille, est assigné aux Lacertidae par KOSMOWSKA-CERANOWICZ et al. (1997), mais ce fossile reste à décrire. Dans un bref commentaire, BÖHME & WEITSCHAT (1998) excluent une appartenance de ce Lacertilia à l'espèce *Succinilacerta succinea*.

Lorsqu'il décrit *Eolacerta robusta* Nöth, 1940, d'après du matériel de l'Eocène moyen du Geiseltal (MP12), NÖTH (1940) range cette espèce dans les Lacertidae, position reprise notamment par ESTES (1983). Des doutes récurrents ont surgi quant à l'identité familiale du genre *Eolacerta*, émis d'abord par RIEPPEL (1980) puis par MÜLLER (1998). Ce dernier auteur pense qu'*Eolacerta robusta* est un scincomorphe mais qu'il ne peut être rangé dans les Lacertidae.

Les fossiles de Lacertilia de l'Eocène inférieur recueillis par l'un d'entre nous (R. Smith) à Dormaal (Belgique, MP7) et par les membres de la Société Laonnoise et Axonaise de Paléontologie à Prémontré (France, MP10) comprennent des restes de petits Lacertidae que nous décrivons ici. La datation des gisements fait référence aux niveaux standard établis d'après

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les faunes de mammifères (SCHMIDT-KITTLER, 1987; BIOCHROM'97). La stratigraphie précise du gisement de Dormaal a été établie par SMITH & SMITH (1996).

Une analyse phylogénétique des familles de Lacertilia, fondée en grande partie sur les caractères définis par ESTES et al. (1988) et incluant nos taxons fossiles, permet de préciser leur position systématique.

ETUDE SYSTEMATIQUE

Squamata Oppel, 1811
Scincomorpha Camp, 1923
Lacertidae Bonaparte, 1831

Dormaalisaurus n. gen.

Espèce-type: *Dormaalisaurus girardoti* n. sp..

Derivatio nominis: d'après la localité de Dormaal, Belgique.

Deux espèces dans le genre: *D. girardoti* et *D. rossmanni*.

Diagnose

Lacertidé de petite taille, qui semble toutefois plus grand que le genre *Succinilacerta*. Le bord ventral du dentaire et la lame horizontale sont courbes, mais moins que chez la plupart des autres membres de la famille. Sur le dentaire, la lame horizontale forme un bord mésial épais et vertical antérieurement, elle s'arrondit et s'amincit vers l'arrière. Le sulcus Meckeli devient très étroit à partir de la dixième position dentaire (comptée à partir de l'avant) et le splénial devait se terminer à ce niveau. La lame bordant le bord dorso-postérieur du sulcus Meckeli est fortement redressée. L'apex des dents porte une légère striation. L'arc neural des vertèbres dorsales est fortement échancré antérieurement.

Dormaalisaurus girardoti n. sp.

Holotype: un dentaire droit légèrement incomplet, coll. IRSNB, N° R205, Fig. 1.

Localité type: Dormaal, niveau DIII (SMITH & SMITH, 1996), Belgique.

Age: Eocène inférieur, niveau standard de Dormaal, MP7.

Répartition stratigraphique et géographique: Eocène inférieur, Dormaal (MP7), Belgique et Prémontré (MP10), Est du Bassin de Paris.

Derivatio nominis: espèce dédiée au paléontologue belge Michel Girardot.

Matériel: Dormaal (MP7, niveau DIII), un dentaire droit holotype (R205), quatre dentaires droits incomplets, trois dentaires gauches incomplets. Prémontré (MP10), un dentaire presque complet (PMT17, fig. 3), quelques dentaires et maxillaires incomplets.

Diagnose

Lacertidé de petite taille dont le dentaire se distingue par un sulcus Meckeli étroit et orienté ventro-linguale-ment à l'avant. A la différence de *Dormaalisaurus rossmanni*, la dentition est nettement hétérodonte, avec des dents bicuspides à apex pointu.

Description

Dentaire, Figs. 1 et 3 (R205 & PMT17).

Une petite partie des extrémités postérieure et antérieure du dentaire holotype manquent. Un dentaire de Prémontré montre cependant ces deux extrémités. Le fossile a une forme générale grêle et allongée. Sept foramens labiaux percent la face labiale qui est régulièrement convexe. Il existe une sorte de replat en position dorso-postérieure qui s'étend jusqu'à la deuxième position dentaire (comptée à partir de l'arrière) et correspond certainement au contact avec l'apophyse dentaire du coronoïde. La marge ventrale du fossile est légèrement arquée, mince à l'avant, elle présente un léger repli mésial à l'arrière. En vue linguale, le sulcus Meckeli se rétrécit de l'arrière vers l'avant où il est nettement étranglé à partir de la dixième position dentaire (comptée à partir de l'avant). C'est à ce niveau que devait se terminer le splénial dont on distingue la trace de contact sur la partie ventrale de la lame horizontale. A l'arrière, le sulcus Meckeli s'ouvre lingualement. Plus antérieurement, l'ouverture devient linguo-ventrale. La lame horizontale est arquée vers le bas, elle forme un bord mésial assez haut (épais) et vertical à l'avant, elle devient plus mince et arrondie postérieurement et se termine après la dernière dent. Sous la frange postérieure de la lame horizontale, on remarque une petite lame, souvent appelée coin supérieur du sulcus Meckeli, qui devait recevoir le processus antéro-lingual

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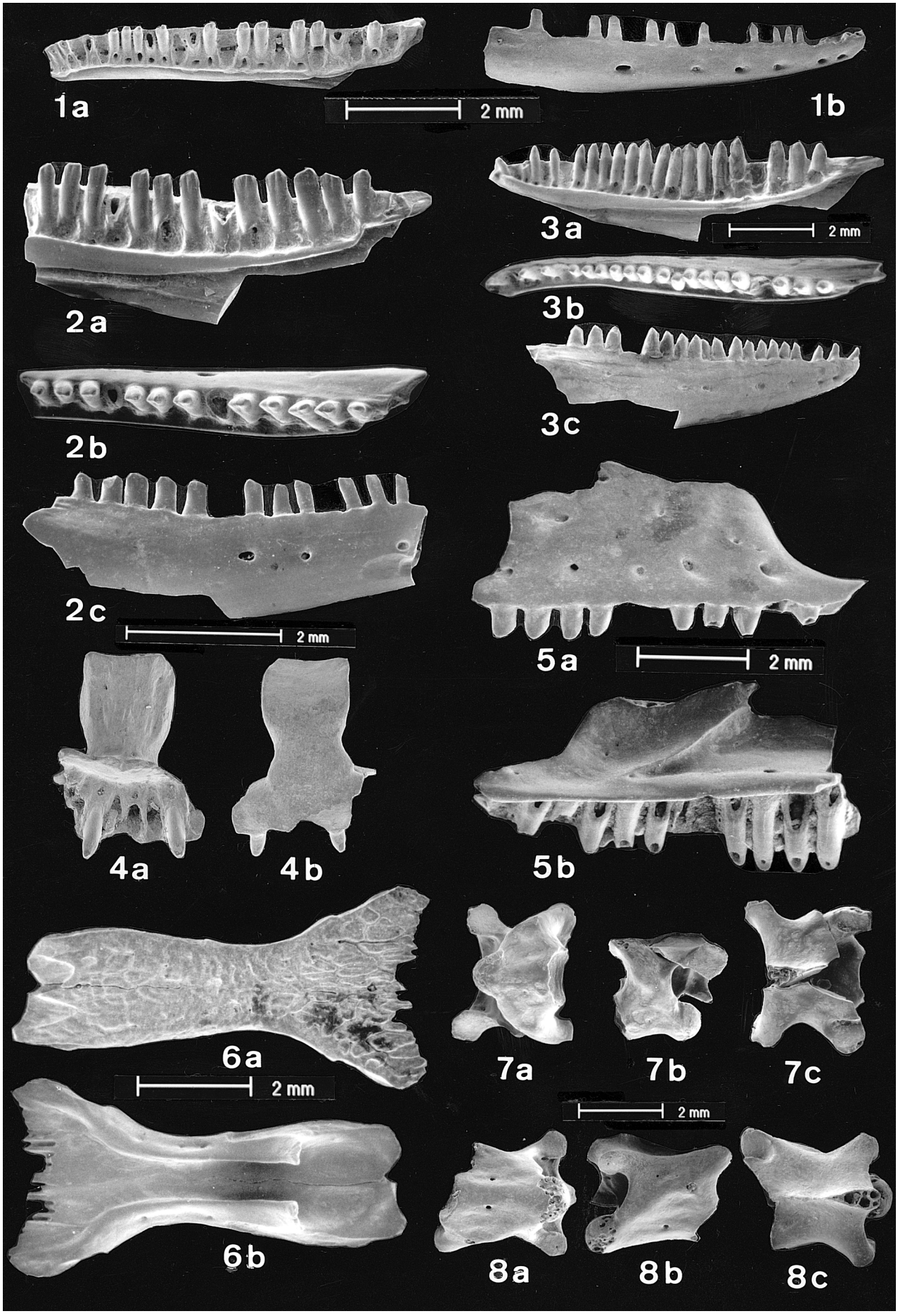
Figs 1-8. – *Dormaalisaurus girardoti* n. sp., dentaire holotype, IRSNB, N°R205 Dormaal, niveau DIII; 1a: face linguale, 1b: face labiale. 2. – *Dormaalisaurus rossmanni* n. sp., dentaire holotype, IRSNB, N° R206, Dormaal, niveau DIII; 2a: face linguale, 2b: face dorsale, 2c: face labiale. 3. – *Dormaalisaurus girardoti* n. sp., dentaire, MNHN, N° PMT17, Prémontré; 3a: face linguale, 3b: face dorsale, 3c: face labiale. 4. – *Dormaalisaurus sp*., prémaxillaire, IRSNB, N° R207, Dormaal, niveau DIII; 4a: face postérieure (linguale), 4b: face antérieure. 5. – *Dormaalisaurus sp*., maxillaire (partie antérieure), MNHN, N° PMT13 Prémontré; 5a: face labiale, 5b: face linguale. 6. – *Dormaalisaurus sp.*: frontal, MNHN, N° PMT14, Prémontré; 6a: face dorsale, 6b: face ventrale. 7. – *Dormaalisaurus sp.*: vertèbre dorsale, MNHN, N° PMT15, Prémontré; 7a: face ventrale, 7b: face latérale, 7c: face dorsale. 8. – *Dormaalisaurus sp.*: vertèbre dorsale, MNHN, N° PMT16, Prémontré; 8a: face ventrale, 8b: face latérale, 8c: face dorsale.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du coronoïde. Le plateau dentaire, assez large, est séparé de la marge dorsale de la lame horizontale par une angulation marquée, avec un sulcus dentalis important, longeant les bases dentaires.

La dentition, pleurodonte et modérément hétérodonte, compte vingt-trois emplacements dentaires. La taille des dents s'accroît régulièrement de l'avant vers l'arrière, les premières ne portent pas de cuspide, une petite cuspide antérieure apparaît après la dixième position dentaire alors que la cuspide principale est beaucoup plus forte et pointue. Les dents sont verticales ou inclinées vers l'arrière, leur apex présente une nette inflexion mésiale et une courbure plus discrète vers l'arrière. L'apex porte une striation. Les dents ont une forme générale cylindrique, avec quelquefois un léger renflement à mi-hauteur du fût dentaire. Les bases dentaires ne s'élargissent pas et il n'y a guère de cément autour. On observe, sur quelques dents, une cavité de résorption en position médiane.

Dormaalisaurus rossmanni n. sp.

Holotype: un dentaire droit incomplet, IRSNB, N° R206, Fig. 2.

Localité type: Dormaal, niveau III (voir Smith & Smith, 1996), Belgique.

Age: début de l'Eocène inférieur, niveau standard de Dormaal, MP7.

Répartition géographique et stratigraphique: espèce connue dans le gisement de Dormaal (MP7) et celui de Prémontré (MP10); Belgique et Est du Bassin de Paris.

Derivatio nominis: espèce dédiée au paléontologue allemand T. Rossmann.

Matériel: Dormaal (MP7, niveau III), dentaire holotype (R206), un maxillaire droit incomplet. Prémontré (MP10), un dentaire gauche, partie postérieure d'un maxillaire droit (PMT18).

Diagnose

Dormaalisaurus rossmanni diffère de *D. girardoti* en premier lieu par sa dentition: les dents ne portent pas de véritable cuspide. Leur apex est pincé labio-lingualement et prend une forme plus ou moins arrondie ou même horizontale. A l'avant du dentaire, le sulcus Meckeli paraît presque uniquement ouvert ventralement alors qu'une ouverture partiellement linguale subsiste chez *D. girardoti*.

Description

Dentaire, Fig. 2 (R206).

Sa morphologie, si l'on excepte les dents, rappelle tout à fait celle du dentaire de *Dormaalisaurus girardoti*, aussi nous nous attarderons surtout sur la description de la dentition.

Le sulcus Meckeli devient très mince antérieurement, sous la septième position dentaire et il s'ouvre alors presque uniquement ventralement. La lame horizontale est haute (épaisse) à l'avant, où elle forme une surface d'abord verticale, puis inclinée dorso-mésialement à ventro-lingualement au voisinage de la symphyse.

La dentition est hétérodonte, pleurodonte. Les premières dents ont un apex pointu, un peu recourbé vers l'arrière. A partir de la huitième position dentaire, l'apex se pince légèrement labio-lingualement et devient plus ou moins arrondi ou même horizontal. Il ne porte pas de cuspide. Dans quelques cas on remarque un renflement antérieur qui peut évoquer l'ébauche d'une cuspide. Quelques stries ornent l'apex. Le fût dentaire est presque vertical. Les dents sont assez serrées et on compte vingt-trois à vingt-quatre positions dentaires. La dentition dépasse la crête dentaire sur un tiers de sa hauteur.

Une portion postérieure de maxillaire de la localité de Prémontré (coll. Sabatier, MNHN) peut être attribuée à l'espèce *Dormaalisaurus rossmanni*.

En vue dorsale, un foramen maxillaire assez large s'ouvre sur la face dorsale de la lame horizontale. Il est en position sub-centrale à labiale. Une dépression large et peu profonde prolonge ce foramen jusqu'à l'extrémité postérieure du maxillaire. Le bord lingual de la lame horizontale ne produit pas d'expansion mésiale importante au niveau du foramen maxillaire.

Latéralement, la retombée postérieure du processus dorsal du maxillaire forme un léger décrochement (escalier) juste avant l'extrémité postérieure de l'os mais on ne peut être certain que le fossile soit intact à ce niveau.

Il reste douze positions dentaires, assez serrées. Les dents sont pleurodontes, avec un fût cylindrique et presque droit. La base des dents ne s'élargit pas beaucoup et il n'y a pratiquement pas de cément qui l'entoure. L'apex est pincé latéralement (aplati linguo-labialement) et se termine par un bord dorsal horizontal à faiblement incliné, avec quelquefois l'ébauche d'une cuspide postérieure.

La forme des dents, sans cuspide antérieure, permet d'affirmer que ce maxillaire appartient à l'espèce *Dormaalisaurus rossmanni*.

Dormaalisaurus indéterminé

Matériel: Dormaal (MP7, niveau DIII), trois dentaires incomplets; deux maxillaires incomplets, un prémaxillaire incomplet. Prémontré (MP10), quelques dentaires et maxillaires incomplets, deux frontaux, deux vertèbres dorsales.

Prémaxillaire

Un fossile incomplet, IRSNB, N° R207, Dormaal, niveau DIII (voir Smith & Smith, 1996), Fig. 4.

La base du processus nasal est pincée, puis elle s'élargit nettement. La face interne du processus nasal porte des sillons verticaux et un bourrelet médian peu en relief. La lame horizontale est presque d'un seul tenant, sans épine prémaxillaire ni processus vomériens bien développés (terminologie FEJERVARY-LANGH, 1923). La partie de l'os qui porte les dents est mal conservée, deux dents demeurent néanmoins. Elles sont banales, cylindriques avec un apex rétréci. La face antérieure (ou externe) du prémaxillaire est lisse, sans foramen ni dépression au contact entre processus nasal et partie inférieure de l'os. Le rétrécissement à la base du processus nasal, ainsi que l'absence d'épine prémaxillaire et de véritables processus vomériens plaident pour l'appartenance de ce fossile aux Lacertidae (la morphologie de ce prémaxillaire évoque d'assez près celle d'un Lacertidé actuel comme *Lacerta agilis* Linnaeus (Fig. 1a, p. 99, RAUSCHER, 1992).

Maxillaire

Une partie antérieure de maxillaire gauche, Prémontré, MNHN, N° PMT 13, Fig. 5.

Les processus prémaxillaires sont courts, une échancrure nasale profonde se situe entre leur extrémité postérieure, un petit foramen s'ouvrant à la jonction des deux processus. En vue dorsale, ces derniers apparaissent déviés mésialement.

En vue linguale, la paroi interne du processus nasal (ou processus montant) est fortement concave. Au niveau de la huitième position dentaire, une amorce de crête orientée ventro-antérieurement à dorso-postérieurement (carina maxillaris, MÜLLER, 1996) se dessine à partir du bord supérieur de la lame horizontale. La cassure de la pièce interrompt très tôt cette structure.

Les huit dents conservées sont pleurodontes, avec une base élargie et un apex pointu et légèrement recourbé postérieurement pour les premières. L'apex des suivantes (à partir de la sixième dent) s'aplatit et les dents tendent à devenir cylindriques. Une cavité de résorption, en position médiane, entame la base de la quatrième dent (comptée à partir de l'avant).

La taille de ce maxillaire correspond tout à fait à celle des dentaires de *Dormaalisaurus*, ainsi que la forme des dernières dents. La faible extension des processus prémaxillaires, le fort enfoncement de l'échancrure nasale et la présence d'une arête interne sur le processus dorsal sont caractéristiques des Lacertidae. L'absence de l'intégralité de la dentition empêche une attribution précise à l'une des deux espèces connues.

Frontal

Frontal presque complet de Prémontré, MNHN, N° PMT 14, Fig. 6.

C'est un os impair, avec tout de même des traces de suture sagittale parfaitement distinctes en vue ventrale. Le frontal a une forme générale allongée, étroite antérieurement, rétrécie entre les orbites et évasée postérieurement. Dorsalement et à l'avant, on découvre deux traces symétriques de recouvrement par les os nasaux. La surface dorsale est ornementée par un réseau de sillons, plus dense à l'arrière. Il n'y a pas de traces d'écailles conservées sur cette surface.

Latéralement, il existe une longue face de contact antérieure avec l'os préfrontal (elle couvre presque la moitié de la longueur de l'os) et une face de contact postérieure avec le postfrontal, plus courte.

En vue ventrale, il existe deux processus subolfactifs (processus descendants) qui ne se rejoignent pas. Ils sont bien développés à l'avant et leur hauteur se réduit à partir de la moitié postérieure du frontal. Ces processus s'atténuent fortement à l'arrière mais restent tout de même en léger relief. La marge postérieure du fossile, bien qu'incomplète, porte d'importantes digitations qui devaient s'ajuster avec celles du bord antérieur du pariétal. Toujours à l'extrémité ventro-postérieure du frontal, mais latéralement, il semble exister deux traces de recouvrement du pariétal par le frontal. Ces deux dispositions du frontal seraient des apomorphies des Lacertoidea (la seconde) et des Lacertidae (la première), selon ESTES et al. (1988).

Vertèbre dorsale

Deux petites vertèbres du gisement de Prémontré, MNHN, N° PMT 15-16, Figs. 7 & 8.

Leur forme générale est plutôt courte et large, moyennement élevée. On n'observe pas l'ensemble de la neurépine, en partie brisée; elle se prolongeait jusqu'au bord antérieur par une lame mince. Les pré- et post-zygapophyses ont à peu près la même extension latérale. Une forte échancrure entame la marge antérieure de l'arc neural.

Un large canal neural occupe la face antérieure avec, au-dessus, un faible pseudozygosphène formé par deux replis antérieurs de l'arc neural, situés très près des prézygapophyses. Le cotyle et le condyle ont une forme arrondie. Latéralement, les synapophyses sont peu marquées et plutôt punctiformes. En vue ventrale, la base du centrum porte une carène hèmale large et arrondie qui joint le cotyle au condyle.

La taille de ces vertèbres s'accorde avec celle des autres fossiles de Prémontré attribués au genre *Dormaalisaurus*. D'autre part, la forme arrondie du cotyle et du condyle, ainsi que la synapophyse faiblement allongée se retrouvent surtout chez les Lacertidae parmi les Scincomorpha.

DISCUSSION

Voici les caractères qui autorisent, selon nous, l'attribution du genre *Dormaalisaurus* aux Lacertidae. Le dentaire

a une forme générale arquée, avec un splénial qui devait s'étendre loin vers l'avant, près de la symphyse (voir HOFFSTETTER, 1944). Il existe un replat labial sur la partie postéro-dorsale du dentaire, correspondant à l'insertion de l'apophyse dentaire du coronoïde. Les dents sont bicuspides, au moins sur l'une des espèces décrites. Sur la partie antérieure du maxillaire, la faible extension des processus prémaxillaires, l'enfoncement marqué de l'échancrure nasale entre la base des processus maxillaires et la présence d'une carène maxillaire sur la face linguale du processus dorsal se conforment au type commun rencontré chez les Lacertidae modernes. De même, la partie postérieure du maxillaire porte un foramen maxillaire important et la face dorsale de la lame horizontale est faiblement creusée par une large dépression. Le frontal, rétréci entre les orbites, montre, sur sa marge postérieure, des digitations qui devaient s'ajuster avec celles du pariétal. Le prémaxillaire a un processus nasal pincé à la base, qui ne se rétrécit pas vers le haut.

L'analyse cladistique (voir plus loin) confirme entièrement l'attribution du taxon *Dormaalisaurus* aux Lacertidae. Nous pouvons ajouter que ces petites espèces de Lacertidae de l'Eocène inférieur étaient déjà de type moderne, proches des genres et espèces actuelles (notion de «crown group», voir JEFFERIES, 1979 et BORSUK-BIALYNICKA et al., 1999).

D'après la taille des fossiles disponibles, les espèces de *Dormaalisaurus* ne devaient guère dépasser 8 cm de longueur (museau-cloaque), il se démarque donc très nettement des formes éocènes de grande taille, *Plesiolacerta lydekkeri* et *Eolacerta robusta*, qui ont été attribuées aux Lacertidae, même si c'est de façon douteuse pour *Eolacerta robusta*.

On peut comparer les dimensions des deux espèces de *Dormaalisaurus* à celles du petit Lacertidé éocène conservé dans l'ambre, *Succinilacerta succinea*. Le frontal autorise une comparaison directe, sa longueur est de 6,5 mm, sa largeur entre les orbites de 1,6 mm chez les espèces de *Dormaalisaurus*. Les mêmes dimensions prises chez *Succinilacerta succinea*, d'après la figure 3 de BORSUK-BIALYNICKA et al. (1999), sont de, respectivement 3,8 mm et 1,1 mm, ce qui est nettement plus petit que chez les espèces de *Dormaalisaurus*. Les deux genres paraissent donc pouvoir être distingués d'après leur taille mais la question de la synonymie générique se pose tout de même pour ces deux genres de Lacertidae éocènes, puisque l'anatomie osseuse de *Succinilacerta succinea* ne peut être étudiée.

Les différences entre les espèces de *Dormaalisaurus* et *Lacerta s.l. filholi*, Augé, 1988, une espèce de Lacertidé de taille relativement petite, connue à partir de l'Oligocène inférieur (MP22) du Quercy (AUGÉ, 1988) sont sans ambiguïté: sur le dentaire, l'encoche postéro-dorsale qui reçoit le processus labial du coronoïde est beaucoup mieux exposée chez *Lacerta s.l. filholi*. Le sulcus Meckeli ne devient très mince, chez *Lacerta s.l. filholi* qu'à proximité immédiate de la symphyse mandibulaire et une partie de sa dentition est tricuspide.

Nous dirons les raisons qui nous amenènent à créer deux espèces dans le genre *Dormaalisaurus*. Ces deux espèces ne peuvent être reconnues que sur la dentition, portant deux cuspides chez *Dormaalisaurus girardoti*, n'en portant pas chez *D. rossmanni*. Lorsqu'une portion suffisante de la dentition (autre qu'antérieure) est disponible, on peut toujours distinguer sans problème les deux espèces. Il est vrai que certaines dents présentent une morphologie intermédiaire (ébauche de cuspide par exemple), mais cela ne se vérifie jamais sur l'ensemble de la dentition. Cependant, c'est une raison plus impérative qui nous a conduit à établir ces deux espèces. Les différences morphologiques constatées entre elles, bien que relativement minimes, se retrouvent à l'identique dans les deux gisements étudiés, Dormaal (MP7) et Prémontré (MP10), alors qu'ils sont séparés par au moins trois millions d'années. Des différences morphologiques enregistrées dans le même gisement, à un moment précis peuvent ressortir à une variation intra-spécifique. Il n'en est plus de même lorsque ces différences persistent, inchangées, dans plusieurs gisements séparés par plusieurs millions d'années. On doit alors privilégier l'existence de deux groupes dont les caractères se maintiennent sur de longues périodes, sans fusion ni intermédiaires morphologiques; selon toute vraisemblance les deux groupes ne se reproduisaient pas entre eux ce qui est la définition de l'espèce selon MAYR (1963) et la plupart des auteurs qui ont abordé la question.

ANALYSE CLADISTIQUE

Le propos de cette étude phylogénique est, en premier lieu, de préciser la place du genre *Dormaalisaurus* dans la classification des Lacertilia. Ensuite, nous ferons quelques commentaires sur le cladogramme obtenu. Le genre *Sphenodon*, un taxon basal parmi les Lepidosauria, est choisi comme extra-groupe. Le cladogramme a aussi été enraciné par rapport à un extra-groupe théorique dont tous les caractères montrent l'état primitif.

Caractères utilisés

L'analyse se fonde sur les caractères osseux employés par ESTES et al. (1988). Dans la liste de caractères (Tableau 1, en annexe), nous avons indiqué, pour chacun d'entre eux, le numéro du caractère tel qu'il apparaît dans la liste d'ESTES et al. (1988). Leur polarité n'a pas été indiquée dans notre liste, on se reportera à ESTES et al. (1988). D'autres caractères ont été ajoutés ou modifiés, dans ce cas ils apparaissent en gras dans la liste et les références dont ils s'inspirent éventuellement sont aussi indiquées. La polarité de l'un des caractères employés par ESTES et al. (1988) a été modifiée: dans leur matrice de caractères, ils codent une faible extension postérieure de la table pariétale comme un caractère dérivé, alors que l'importante extension postérieure du pariétal visible chez

beaucoup de scincomorphes est comptée comme un caractère primitif car c'est aussi l'état qui prévaut chez les lépidosauromorphes autres que les squamates. Cependant, dans les commentaires donnés à propos de ce caractère, ESTES et al. (1988) écrivent que la plus simple explication pour la distribution de ce caractère est de considérer un pariétal court comme primitif chez les squamates et comme une apomorphie de certains membres des scincomorphes. Ils ajoutent que l'extension postérieure du pariétal chez les scincomorphes n'est que superficiellement semblable à celle des lépidosauromorphes autres que les squamates: chez les scincomorphes l'extension postérieure du pariétal n'est obtenue que par des dépôts secondaires d'os dermique.

Taxons utilisés

Dans la présente analyse, on suppose que tous les taxons utilisés sont monophylétiques, ce qui n'est pas acquis pour les Iguanidae* et les Agamidae* qui sont souvent considérés comme des métataxons. L'emploi de taxons de rang restreint (genre par exemple) est préconisé comme une solution par GAUTHIER et al. (1988) mais on ne connaît pas l'état des caractères pour bon nombre d'entre eux. Nous avons donc admis quelques métataxons dans notre analyse, comme l'ont fait beaucoup d'auteurs, le but de la cladistique étant d'ailleurs de résoudre ces difficultés.

Matrice de caractère

Les taxons pour lesquels on ne connaît pas l'état d'un caractère, quelle qu'en soit la cause, sont marqués « ? » pour ce caractère (Tableau 2, en annexe). Les caractères qui se présentent sous plusieurs états dans un taxon donné sont notés avec ces états différents, le premier étant le plus fréquemment rencontré. Cependant, lors de la construction des cladogrammes, seul l'état le plus fréquemment rencontré à été retenu, procédé déjà employé par ESTES et al. (1988).

Cladogrammes obtenus

Les états de caractères ont été soumis au programme heuristique dans Hennig 1986, de J.M. FARRIS. Les deux cladogrammes obtenus réunissent dans un taxon monophylétique les Lacertidae et *Dormaalisaurus*. On peut donc inclure ce genre dans les Lacertidae.

Ces deux cladogrammes ont une longueur de 381, un index de consistance de 41, et un index de rétention de 57, l'un d'eux place les Gekkonidae comme le groupe frère des Anguimorpha, alors qu'ils forment le groupe frère des Scincomorpha dans l'autre option (Fig. 9, voir EVANS & CHURE, 1998).

Dans les deux cladogrammes, les Scincidae, Cordylidae, Xantusiidae, Teiidae, Gymnophthalmidae et

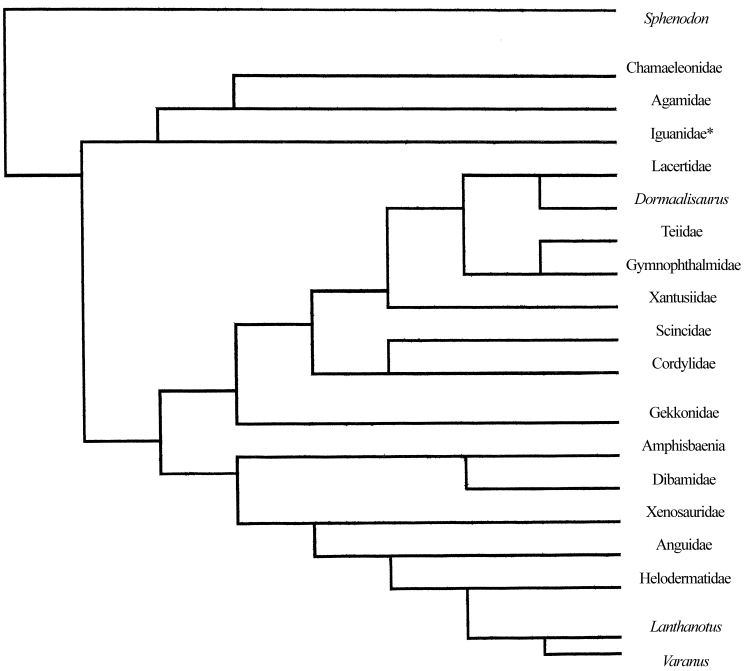

Fig. 9. – Cladogramme montrant les relations de *Dormaalisaurus* à l'intérieur des Lacertilia . *Dormaalisaurus* forme le groupe frère de la famille des Lacertidae dans laquelle nous l'incluons. Un autre cladogramme de même longueur a été obtenu avec les caractères utilisés, *Dormaalisaurus* y occupe la même position (groupe frère des Lacertidae), la seule différence notable entre les deux cladogrammes concerne la position des Gekkonidae qui forment le groupe frère des Scincomorpha et non plus des Anguimorpha + (Amphisbaenia et Dibamidae).

Lacertidae font partie d'un groupe monophylétique, les Scincomorpha. L'arrangement des taxons à l'intérieur des Scincomorpha ne varie pas non plus. On distingue un ensemble réunissant les Xantusiidae, Lacertidae, Teiidae et Gymnophthalmidae (= Lacertoidea Camp, 1923) d'un autre comprenant les Scincidae et Cordylidae (= Scincoidea Oppel, 1811). Ces résultats sont conformes au concensus proposé par ESTES et al. (1988), au moins sur ce point précis (voir aussi CALDWELL, 1999). Par contre, ils sont en désaccord partiel avec ceux d'EVANS & BARBADILLO (1998) et EVANS & CHURE (1998) qui séparent Teiidae et Gymnophthalmidae des Lacertidae et Xantusiidae, réunis avec les Scincoidea. Mais les caractères utilisés par ces auteurs sont plus nombreux que les nôtres puisqu'ils combinent la liste de ESTES et al. (1988) avec celle de GAUTHIER et al. (1988).

CONCLUSION

L'analyse cladistique rejoint les résultats que nous avions obtenus par une méthode comparative plus traditionnelle, limitée à l'examen de quelques caractères jugés pertinents pour décider des attributions d'un taxon. *Dormaalisaurus* est un Lacertidé de l'Eocène inférieur européen, représenté par deux espèces, *D. girardoti* et *D. rossmanni*, à la fois dans les gisements de Dormaal (MP7) et de Prémontré (MP10). La question d'une identité générique avec un petit Lacertidé éocène trouvé dans l'ambre de la Baltique (*Succinilacerta succinea*) peut être posée bien que les espèces de *Dormaalisaurus* paraissent plus grandes que *Succinilacerta succinea*. Les cladogrammes obtenus sont conformes à ceux de ESTES et al. (1988), par contre il existe des différences avec ceux d'EVANS & BARBADILLO (1998) ou EVANS & CHURE (1998), notamment sur la place des Teiidae et, en partie, des Gekkonidae (Gekkota). Comment juger de la validité de ces cladogrammes ? A priori, l'index de consistance permet ce genre de comparaison, il est de 73 pour le cladogramme d'EVANS & BARBADILLO (1998) alors que cet indice n'atteint que 41 dans notre cas et qu'il est proche de 42 chez ESTES et al. (1988). Or cet indice de consistance est très sensible au nombre de caractères et de taxons employés, il tend à diminuer quand le nombre de caractères augmente. Ces données vont dans le même sens et favorisent une acceptation des options d'EVANS & BARBADILLO (1998). Malgré tout, si l'on examine leur matrice de caractères, beaucoup d'entre eux ne présentent aucune variation parmi les taxons actuels de Lacertilia. KITCHING et al. (1998) écrivent que l'inclusion de caractères ne portant pas d'information relative aux groupes étudiés augmente la valeur de l'index de rétention. Ceci revient à dire que les index de rétention considérés ne peuvent pas être comparés dans le cas présent.

Il n'y a donc pas d'accord général quant à la classification des Lacertilia. La validité respective des cladogrammes proposés sera toujours difficilement comparable tant que le nombre et le rang des taxons soumis à l'analyse ne seront pas les mêmes.

ABRÉVIATIONS

IRSNB : Institut royal des Sciences naturelles de Belgique, Bruxelles.

MNHN : Museum national d'Histoire naturelle, Paris.

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TABLEAU 1

Caractères utilisés dans l'analyse. On trouvera l'état des caractères dans Estes et al. (1988), le numéro entre parenthèses indiquant le numéro du caractère tel qu'il apparaît dans la liste de Estes et al. (1988). Les caractères numérotés en gras sont soit inédits, soit modifiés par rapport à Estes et al. (1988), soit repris chez d'autres auteurs.

1 Fusion prémaxillaires (1).
2 Processus nasal sans constriction à la base, base plutôt large, le processus se rétrécit vers le haut (0) ; présence d'une constriction ou processus étroit à la base, le processus ne se rétrécit pas vers le haut (1). Pregill, 1992.
3 Epine prémaxillaire (terminologie Fejervary-Langh, 1923), forme un processus dirigé vers le bas et qui naît de la partie médiane de la face ventrale de la lame horizontale : absent ou peu développé ou séparé en deux parties non soudées (0), bien développé, en une seule partie (1), bien développé et bilobé dans sa partie terminale (2).
4 Extension narine (2).
5 Fusion nasaux (3).
6 Nasal-préfrontal (4).
7 Contact préfrontal avec os derrière orbite (5).
8 Fusion des frontaux (6).
9 Bords latéraux des frontaux (7).
10 Bord antérieur du frontal (8).
11 Processus descendants du frontal (9).
12 Processus descendants du frontal (10).
13 Extension postérieure du frontal (11).
14 Contact frontal-pariétal : sans digitations importantes (0) ; digité (1).
15 Postfrontal présent (12).
16 Forme en fourche du postfrontal (13).
17 Fusion du postfrontal (14).
18 Taille du postfrontal (15).
19 Postorbitaire présent (16).
20 Contribution du postorbitaire au bord de l'orbite (17).
21 Fusion des pariétaux (21).
22 Facettes antérieures du pariétal (22).
23 Processus descendants du pariétal (23).
24 Table pariétale réduite à l'arrière, processus supratemporaux longs (0). Table pariétale étendue à l'arrière, processus supratemporaux courts (1). Réversé d'après Estes et al., 1988 (24).
25 Bords latéraux du pariétal : comprimés (0) ; presque parallèles, sans constriction (1).
26 Foramen pariétal (26).
27 Foramen pariétal dans le pariétal (0) ; décalé vers l'avant, sur suture fronto-pariétale (1) ; entièrement dans le frontal (2) en partie d'après Frost & Etheridge, 1989 (25).
28 Origine musculature adductrice (54).
29 Contact jugal squamosal (18).
30 Fenêtre supratemporale ouverte, arc supratemporal présent (0) ; ouverte arc absent (1) ; fermée, arc présent (2). Caldwell, 1999 (15).
31 Extension postérieure du maxillaire (27).
32 Processus prémaxillaires du maxillaire assez longs (0) ; processus prémaxillaires courts (1).
33 Lacrimal présent (28).
34 Fusion du lacrimal (29).
35 Foramen lacrimal (30).
36 Bord antérieur de l'orbite (31).
37 Bord postérieur de l'orbite (32).
38 Squamosal présent (33).
39 Processus dorsal du squamosal (34).
40 Supratemporal (35).
41 Ossifications palpébrales (36).
42 Vomers (38).

43 Taille des vomers (39).
44 Contact médian des septomaxillaires (40).
45 Expansion dorsale du septomaxillaire : septomaxillaire plat ou concave, organe de Jacobson petit (0) ; septomaxillaire convexe, organe de Jacobson de grande taille (1). (41) voir aussi CALDWELL, 1999.
46 Bord postérieur de l'organe de Jacobson (42).
47 Extensions médianes du palatin (43).
48 Fosse choanale du palatin (44).
49 Contact ectoptérygoïde avec le palatin (45).
50 Taille ectoptérygoïde (46).
51 Epiptérygoïde présent (47).
52 Crête ptérygoïde du carré (37).
53 Processus alaire du prootique (49).
54 Processus supratrigeminal du prootique (50).
55 Fusion opisthotique-exoccipital (51).
56 Veine latérale de la tête entourée par crête prootique (52).
57 Ouverture postérieure du canal vidien (53).
58 Forme générale du dentaire : allongée, droite (0) ; plus ou moins arquée (1).
59 Extension du processus coronoïde du dentaire (60).
60 Extension postérieure du dentaire : extension jusqu'au niveau du coronoïde ou au-delà (0) ; faible extension, près limite antérieure du coronoïde (1).
61 Extension postérieure du dentaire : extension pas au-delà de l'extrémité postérieure du coronoïde (0) ; extension au-delà de l'extrémité postérieure du coronoïde (1). GAUTHIER et al., 1988 (66 in GAUTHIER et al.).
62 Forme extrémité postérieure du dentaire (63).
63 Articulation dentaire-postdentaire (64).
64 Sulcus Meckeli (55).
65 Septum intramandibulaire (56).
66 Ouverture du sulcus Meckeli (57).
67 Plateau dentaire : horizontal (0) ; incliné dorso-labialement à ventro-lingualement (1).
68 Sulcus dentalis à la base des dents : absent ou faiblement développé (0) ; présent, bien développé (1).
69 Lame horizontale (59).
70 Lame horizontale (58).
71 Nombre de dents du dentaire : quinze positions dentaires et plus (0) ; moins de quinze dents (1).
72 Nombre de dents du dentaire : Moins de vingt-sept positions dentaires (0) ; vingt-sept ou plus (1).
73 Vue latérale du supraangulaire isolé (61).
74 Vue mésiale du préarticulaire (62).
75 Crête préarticulaire (73).
76 Extension antérieure du splénial : splénial ne s'étend pas jusque près de la symphyse mandibulaire (0) ; s'étend jusque près de la symphyse (1).
77 Extension antérieure du splénial (65).
78 Extension postérieure du splénial : se termine avant l'extrémité antérieure de la fosse mandibulaire (0) ; splénial se termine au niveau ou après l'extrémité antérieure de la fosse mandibulaire (1).
79 Extension postérieure du splénial (66).
80 Suture splénial-dentaire (67).
81 Processus dentaire du coronoïde : absent (0) ; présent (1). Estes et al., 1988 (68, modifié pour les Cordylidae et les Scincidae).
82 Extension antérieure du coronoïde (69).
83 Extrémité antérieure du coronoïde (70).
84 Angulaire (72).
85 Contact angulaire-splénial : recouvrement avec inter-digitation (0) ; pas de recouvrement articulation proche de la verticale (1) ; face verticale avec projection de l'angulaire sous le splénial (2). 52 in CALDWELL, 1999.
86 Vue dorsale processus rétroarticulaire (74).
87 Direction du processus rétroarticulaire (75).
88 Bord mésial du processus rétroarticulaire (76).
89 Ressaut du processus rétroarticulaire (77).
90 Largeur postérieure du processus rétroarticulaire (78).
91 Torsion du processus rétroarticulaire (79).
92 Fosse mandibulaire (81).
93 Nombre de cuspides : la plupart des dents avec trois cuspides ou sans cuspide (0) ; la plupart des dents avec deux cuspides (1).
94 Apex dentaire : dents avec cuspides ou présence d'un bord coupant (0) ; apex pointu, recourbé, dents caniniformes (1).
95 Implantation des dents (84).
96 Remplacement des dents (85).
97 Base dentaire (86).
98 Largeur base dentaire : non ou peu élargie (0) ; élargie, jusqu'à la marge mésiale du plateau dentaire (1).
99 Marge des dents du maxillaire (87).
100 Dents palatines (82).
101 Dents ptérygoïdes (83).
102 Nombre d'ossicules scléraux (88)
103 Nombre d'ossicules scléraux (89).
104 Deuxième épibranchial présent (90).
105 Deuxième cératobranchial présent (91).
106 Articulation vertèbres dorsales : procoele (0) ; amphicoele (1). ESTES et al., modifié (93).
107 Zygosphène vertèbres dorsales (96).
108 Zygosphène vertèbres (95).
109 Orientation du cotyle vertébral (92).
110 Constriction du centrum vertébral avant le condyle (94).
111 Forme du condyle et du cotyle vertébral : arrondie (0) ; étirée latéralement (1).
112 Relief sous le centrum vertébral (hypapophyses) : existe (0) ; centrum lisse (1).
113 Intercentre sur les vertèbres postérieures du tronc : absent (0) ; présent (1). Estes et al., 1988, modifié (99).
114 Nombre de vertèbres présacrées (104).
115 Nombre de vertèbres présacrées (105).
116 Nombre de vertèbres présacrées (106).
117 Attachement intercentre des vertèbres cervicales (97).
118 Attachement intercentre vertèbres cervicales (98).
119 Nombre de vertèbres cervicales : 7-9 (0) ; 6 ou moins (1). (74 in CALDWELL, 1999).
120 Fracture d'autotomie sur vertèbres caudales (103).
121 Processus transverses des vertèbres caudales : une seule paire (0) ; deux paires convergentes (1) ; deux paires divergentes (2) ; absent (3). ESTES et al., 1988 (100-101) ; EVANS, 1998 (87).
122 Plan de fracture des vertèbres caudales (102).
123 Côtes postxiphisternales (110).
124 Nombre de points d'attachement pour les côtes (109).
125 Emargination (=échancrure) scapulaire (111).
126 Emargination antérieure du coracoïde (112).
127 Emargination postérieure du coracoïde (113).
128 Extension du cartilage épicoracoïde (114).
129 Clavicule présente (115).
130 Angulation de la clavicule (116).
131 Articulation dorsale de la clavicule (117).

132 Interclavicule présente (118).
133 Processus latéral de l'interclavicule (119).
134 Forme de l'interclavicule et taille du processus antérieur (120).
135 Fontanelle sternale (121).
136 Foramen ectépicondyle (122).
137 Entaille de l'épiphyse (123).
138 Pubis en vue ventrale (124).
139 Ostéodermes ventraux (corps) (126).
140 Ostéodermes dorsaux (corps) (127).
141 Ostéodermes céphaliques (128).
142 Rugosités crâniennes absentes (0); étendues sur le frontal et le pariétal (1); étendues au-delà du pariétal et du frontal (2). D'après FROST & ETHERIDGE, 1989, modifié.
143 Rugosités crâniennes présentes: non vermiculées (0); vermiculées (1). (129).
144 Os postcloacal (125).
145 Fusion de l'épiphyse (130).

TABLEAU 2

Matrice de caractères avec les taxons employés dans l'analyse

	1	2	3	4	5	6	7	8	9	10
Agamidae*	1	0	0	0	0	0	0	1	1	1
Anguidae	1	1	2	0	0	0,1	0,1	0,1	0,1	0
Chamaeleontidae	1	?	0	0,1	0,1	?	0,1	1	1	1
Cordylidae	1	0	1	0	0	0,1	0	0,1	0	0
Dibamidae	1	0	2	0	0	1	0	0	0	0
Gekkonidae	0,1	0,1	2	0	0,1	0,1	0	1,0	0	0
Gymnophthalmidae	1	1	0	0	0	0,1	0	1	0,1	0
Helodermatidae	1	1	2	0	0	0	1	0	0	0
Iguanidae*	1	0,1	1,2	0	0	0	0	1	1,0	1
Lacertidae	1	1	0	0	0	1	0	0,1	1,0	0
Lanthanotus	1	1	2	1	1	0	1	0	0	0
Scincidae	0,1	0	0	0	0,1	1,0	0,1	0,1	0	0
Teiidae	1	1	0	0	0	0,1	0	0,1	0	0
Varanus	1	1	0	1	1	?	0	0	0	0
Xantusiidae	1	1	1	0	0	1,0	0	0,1	0	0
Xenosauridae	1	0	2	0	0	0	0	0,1	1	0
Amphisbaenia	1	0	0	0	0	1	1	0,1	0	0
Dormaalisaurus	1	1	0	?	?	?	?	0,1	1	0
Sphenodon	0	0	0	0	0	0	0	0	0	0

	11	12	13	14	15	16	17	18	19	20
Agamidae*	0	0	0	0	1	?	0	?	0	0
Anguidae	1	0,1	0	0	0	1	0,1	0	0	1
Chamaeleontidae	0	0	0,1	0	1	?	0	?	0	0
Cordylidae	1	0	0	0	0	1	0	0	0	1
Dibamidae	1	0	0	0	0,1	1	0	0	1	?
Gekkonidae	1	1	0	0	0	1	0	0	1	1
Gymnophthalmidae	1	1,0	1	0	0	1	0,1	0	0	1
Helodermatidae	1	1	0	0	0	1	0	0	1	1
Iguanidae*	0,1	0	0	0	0,1	0	0	1	0	0
Lacertidae	1	0	0	1	1,0	1	0,1	0	0	1
Lanthanotus	1	0	0	0	0	1	0	0	1	1
Scincidae	1	0	0	0	0	1	0,1	0	0,1	1
Teiidae	0	0	0	0	0	1,0	0,1	0	0	1
Varanus	1	1	0	0	0	1	1	0	0	1
Xantusiidae	1	0,1	0	0	1	1	0	?	0	1
Xenosauridae	1	0	0	0	0	1	0,1	0	0	1
Amphisbaenia	1	1	0	0	1	1	0	0	0,1	?
Dormaalisaurus	?	0	0	1	?	?	?	?	?	?
Sphenodon	0	0	0	0	0	1	0	0	0	0

	21	22	23	24	25	26	27	28	29	30
Agamidae*	1	0,1	0	0	0	0	1	0,1	1	0
Anguidae	1	0	0	0	0	0	0	0,1	0	0
Chamaeleontidae	1	0,1	0	1,0	0	0,1	2	0,1	1	0
Cordylidae	1	0,1	1	1,0	1	0,1	0	1	0	2
Dibamidae	1	1	?	0	1	1	?	0	?	1
Gekkonidae	0,1	0	0	0	0,1	1	?	1	?	1
Gymnophthalmidae	1	1	1	0	1	1	?	0,1	0	0
Helodermatidae	1	0	0	0	0	1	?	1	?	1
Iguanidae*	1	0,1	0	0	0	0,1	0,1,2	0,1	1	0
Lacertidae	1	1	0	1	1	0,1	0	1	0	2
Lanthanotus	1	0	0	0	0	1	?	0	?	1
Scincidae	1	0,1	0,1	0	1	0,1	0	1	1	2
Teiidae	1	1	1	0	0	0,1	0,1	0	1	0
Varanus	1	0	0	0	0	0	0	0	0	0
Xantusiidae	0,1	1	1	1	1	0,1	0	1	0	2
Xenosauridae	1	0	0	1,0	0	0	0	0,1	1	0
Amphisbaenia	1	?	0	0	1,0	0,1	0	0	?	1
Dormaalisaurus	?	?	?	?	?	?	?	?	?	?
Sphenodon	0	0	0	0	0	0	0	0	0	0

	31	32	33	34	35	36	37	38	39	40
Agamidae*	0	0	0,1	0	0	0,1	0	0	0	0,1
Anguidae	0,1	0	0	0	0	1	0,1	0,1	1	0,1
Chamaeleontidae	0	0	0,1	0	0	0,1	0	0	0	0
Cordylidae	0	0	0,1	0	0	0,1	0	0	1	0
Dibamidae	1	1	1	?	?	?	1	0,1	1	1
Gekkonidae	0	0	1	?	?	0,1	1	0,1	1	0,1
Gymnophthalmidae	0	0	0,1	0,1	0	1	0	0	1	0
Helodermatidae	1	0	0	0	0	1	0	0	1	0
Iguanidae*	0	0	0,1	0	0	1	0	0	0	0
Lacertidae	0	1	0	0	0	0,1	0	0	1	0
Lanthanotus	1	0	0	0	1	1	0	0	1	0
Scincidae	0	0	0,1	0	0	0,1	0,1	0	1	0
Teiidae	0	1	0	0	0	1	0	0	0,1	0
Varanus	1	0	0	0	1	1	1	0	1	0
Xantusiidae	1	1	?	?	0	0	0	0	1	0
Xenosauridae	0	0	0	0	0	1	0	0	0,1	0
Amphisbaenia	0	1	0,1	0,1	0	1	0,1	0	1	1
Dormaalisaurus	?	1	?	?	?	?	?	?	?	?
Sphenodon	0	0	1	?	0	0	0	0	0	1

	41	42	43	44	45	46	47	48	49	50
Agamidae*	0	0	0	0	0	0	0	0	0	0
Anguidae	1	0	1	1	1	0,1	0	1	0,1	0
Chamaeleontidae	0	1	0	?	?	0	0	0	0	0
Cordylidae	0,1	0	1	1	1	0	0	1	0	0
Dibamidae	0	0	1	1	1	1	1	1	1	0
Gekkonidae	0	0,1	1	1	1	0	0	1	0,1	0
Gymnophthalmidae	0	0,1	1	1	1	0,1	0	1	0	0
Helodermatidae	0	0	1	1	1	0	0	1	1	0
Iguanidae*	0	0	0	0	0	0	0	0	0	0
Lacertidae	1	0	1	1	1	0	0	1	0	0
Lanthanotus	0	0	1	1	1	1	0	1	1	0
Scincidae	1,0	0,1	1	1	1	0,1	1	1	0	0
Teiidae	0	0	1	1	1	0	0	1	1	1
Varanus	1	0	1	1	1	1	0	1	1	0
Xantusiidae	0	1	1	1	1	0,1	0	1	1	1
Xenosauridae	1	0	1,0	1	1	0	0	1	0	0
Amphisbaenia	0	0	1	1	1	1	0	1	0,1	0
Dormaalisaurus	?	?	?	?	?	?	?	?	?	?
Sphenodon	0	0	0	0	0	0	0	0	0	0

	51	52	53	54	55	56	57	58	59	60
Agamidae*	0	1	0	1,0	1	0	0	0	0	0
Anguidae	0	1	1	0	1	0	1	1	0,1	0
Chamaeleontidae	1	1	0	0	1	0	0	0	0	0
Cordylidae	0	1	1	0	1	0	0,1	0	1	0
Dibamidae	0,1	1	0	0	0	0	2	0	1	0
Gekkonidae	0	1	1	0	1	1,0	0	0	0	0
Gymnophthalmidae	0	0	1	0	1	0,1	0	0	0	0
Helodermatidae	0	0	1	0	1	0	1	1	0	0,1
Iguanidae*	0,1	0,1	0,1	1,0	1	0	0	0,1	0,1	0
Lacertidae	0	0	1	0	1	0	1	1	0	0
Lanthanotus	0	1	1	0	1	0	1	1	0	1
Scincidae	0	1	1	0	1	0	0,1	0	1,0	0
Teiidae	0	0	1	0	1	0	0	1	0	0,1
Varanus	0	1	1	0	1	0	0	0,1	0	0,1
Xantusiidae	0	1	1	0	1	1	0,1	0	1	0
Xenosauridae	0	1	1	0	1	0	0,1	1	0,1	1
Amphisbaenia	1,0	1	1	0	1	0	?	1	1,0	0
Dormaalisaurus	?	?	?	?	?	?	?	1	0	?
Sphenodon	0	0	0	0	0	0	0	0	0	0

	61	62	63	64	65	66	67	68	69	70
Agamidae*	1	0	0	0	0	0	?	0	0,1	0
Anguidae	0	1	0	0	1	1	1	0	0	0
Chamaeleontidae	1	0	0	0	0,1	0,1	?	0	0,1	0
Cordylidae	0,1	0	0	0,1	0	0	0	1	0	1
Dibamidae	1	0	0	2	?	?	0,1	0	0	1
Gekkonidae	1	0	0	2	0	?	0	1	0	1
Gymnophthalmidae	0,1	0	0	0,2	0	0	0	?	0	0,1
Helodermatidae	0	1	1	0	1	1	1	0	1	0
Iguanidae*	0,1	0	0	0,1	0	0	0	0	0	0
Lacertidae	0	0	0	0	0	0	0	1	0	1
Lanthanotus	0	2	1	0	1	1	1	0	1	0
Scincidae	0	0	0	0,1	?	0	0	1	0	1
Teiidae	0	0	0	0,1	0	0	0	1	0	1
Varanus	0	2	1	0	1	1	1	0	1	0
Xantusiidae	1	0	0	2	0	?	0	?	0	1
Xenosauridae	0	1	0	0	1	1	1	0	0	0
Amphisbaenia	1	0	0	0,1	1,0	0,1	0,1	0	0,1	0,1
Dormaalisaurus	?	0	0	0	0	0	0	1	0	1
Sphenodon	1	0	0	0	?	0	0	0	1	0

	71	72	73	74	75	76	77	78	79	80
Agamidae*	0	0	0	0	0	0	1,2	0	1	0
Anguidae	0,1	0	0	0	0	0	0,1	0	0	0
Chamaeleontidae	0	0	0	0	0	0	2	0	?	?
Cordylidae	0	0	0	0	0	0	0	0	0	0
Dibamidae	1	0	0	0	0	0	2	?	?	?
Gekkonidae	0	1,0	0	0	0	0	1,2	0,1	0	0
Gymnophthalmidae	0	0	0	0	2	0	0,1	0	0,1	0
Helodermatidae	1	0	1	0	0	0	1	0	1	1
Iguanidae*	0	0	0	0	0	0	0,1,2	0,1	0,1	0
Lacertidae	0	0	0	0	1	1	0	0	0	0
Lanthanotus	1	0	2	1	0	0	0	0	2	1
Scincidae	1,0	0	0	0	0	0	0,1	0	0	0
Teiidae	0	0	0	0	2	1	0	0	0	0
Varanus	1,0	0	2	1	0	0	0	0	1	1
Xantusiidae	0	0	0	0	1	0	1	0	1	?
Xenosauridae	0	0	0	0	0	0	0	0	0	0
Amphisbaenia	1	0	0	?	0	0	1,2	0	1	0
Dormaalisaurus	0	0	?	?	?	1	0	?	?	0
Sphenodon	0	0	0	1	0	0	2	?	?	?

	81	82	83	84	85	86	87	88	89	90
Agamidae*	0	0	0	0	0	0	0	0	0	0
Anguidae	1	0	0	0	0	1	1	0	0	1
Chamaeleontidae	0	0	0	0	0	?	0	0	0	0
Cordylidae	0,1	0	0	0	0	0,1	1	1	0	1,0
Dibamidae	0	0	?	1	0	1	0	0	0	1
Gekkonidae	1	0	0	0,1	0	1	1	0	1	1
Gymnophthalmidae	1	0	0	0	0	0,1	0	0	0	0
Helodermatidae	1	1	0	0	0	1	1	0	0	0
Iguanidae*	0,1	0	0,1	0,1	0	0,1	0	0	0	0
Lacertidae	1	0	0	0	0	0	0	0	0	0
Lanthanotus	0	1	1	0	2	1	1	0	0	0
Scincidae	0,1	0	0	0,1	0	1	1	1	0	1
Teiidae	1	0	0	0	0	0	0	0	0	0
Varanus	0,1	1	0	0	0	1	1	0	0	0
Xantusiidae	0	0	?	1	0	0	0	0	0	0
Xenosauridae	1	0	0	0	0	1	0	0	0	0
Amphisbaenia	0,1	0	0,1	0,1	0	1	0	0	0	0
Dormaalisaurus	1	?	?	?	?	?	?	?	?	?
Sphenodon	?	1	0	0	0	?	?	?	?	?

	91	92	93	94	95	96	97	98	99	100
Agamidae*	0	0	0	0	1	?	0	0	0	1
Anguidae	1	0	0	1,0	0	1	0,1	1,0	0	0,1
Chamaeleontidae	0	0	0	0	1	?	0	0	0	1
Cordylidae	1	0	0	0	0	0	0	0	0	1
Dibamidae	1	0	0	1,0	0	1	0	0	0	1
Gekkonidae	1	0	0	0	0	0	0	0	0	1
Gymnophthalmidae	0	1	0	0	0	0,1	0	0	1	1
Helodermatidae	1	0	0	1	0	2	1	1	0	0,1
Iguanidae*	0	0	0	0	0	0	0	0	0,1	0,1
Lacertidae	0	1	1	0	0	0	0	0	1	1
Lanthanotus	1	0	0	1	0	2	1	1	0	0
Scincidae	1	0	0	0	0	0	0	0	0	1
Teiidae	0	1	0	0	0	0,1	0	0,1	1	1
Varanus	1	0	0	1	0	2	1	1	0	1
Xantusiidae	0	0	0	0	0	0	0	0	0	1
Xenosauridae	1	0	0	0,1	0	1	0	0,1	0	1
Amphisbaenia	1	0	0	0,1	0,1	1,2	0	0,1	0	1
Dormaalisaurus	?	?	1,0	0	0	0	0	0	0	?
Sphenodon	?	0	0	0	1	?	0	0	0	0

	101	102	103	104	105	106	107	108	109	110
Agamidae*	1	1	1	0,1	0,1	0	1	0	0	0,1
Anguidae	0,1	1	0,1	0,1	1	0	1	0	0	0
Chamaeleontidae	1	1	1	1	1	0	1	0	0	0
Cordylidae	0,1	1	0,1	0	0,1	0	0,1	0,1	0	0
Dibamidae	1	1	1	1	1	0	1	0	0	0
Gekkonidae	1	0,1	0,1	0,1	0,1	1,0	1	0	0	0
Gymnophthalmidae	0,1	1	0,1	1	0,1	0	0	1,0	0	0
Helodermatidae	0	1	1	0	1	0	1	0	1	0
Iguanidae*	0,1	1	0,1	0,1	0,1	0	0,1	0,1	0	0
Lacertidae	0,1	1	0,1	0	0	0	0	1	0	0
Lanthanotus	0	1	1	1	1	0	1	0	1	1
Scincidae	0,1	1	0,1	0,1	0,1	0	0	0	0	0
Teiidae	0,1	1	0	0	0,1	0	0	1	0	0,1
Varanus	1	0	0	1	1	0	1	0	1	1
Xantusiidae	1	1	0	0	0	0	1	0	0	0
Xenosauridae	0,1	1	0	1	1	0	1	0	0	0
Amphisbaenia	1	1	1	1	0,1	0	1	0	0	0
Dormaalisaurus	?	?	0	?	?	0	1	0	0	0
Sphenodon	1	0	0	0	0	1	0	0	0	?

	111	112	113	114	115	116	117	118	119	120
Agamidae*	0	0	0	0,1	0	0	0	1,2	0	1
Anguidae	1	0,1	0	0	1	1	2	0	0,1	0,1
Chamaeleontidae	0	0	0	1,0	0	0	0	0	1	1
Cordylidae	1	0	0	0	0,1	0,1	0,1	0,1,2	0	0
Dibamidae	0	1	0	0	1	1	2	0	1	0
Gekkonidae	0	0	1	0	1,0	0,1	0	0	0	0,1
Gymnophthalmidae	0,1	0	0	0	0,1	0,1	0	1,2	0	0
Helodermatidae	0,1	1,0	0	0	1	1	1,2	0	0	1
Iguanidae*	0	0	0	0,1	0,1	0	0,1	0	0	0,1
Lacertidae	0	0	0	0	0,1	0,1	0,1	0,1	0	0
Lanthanotus	1	0,1	0	0	1	1	1,2	0	0	1
Scincidae	1	0	0	0	1	1	1,2	0	0,1	0,1
Teiidae	1	0	0	0	0,1	0,1	0	1	0	0
Varanus	1	0,1	0	0	1	1	1,2	0	0	1
Xantusiidae	0,1	0	0	0	1	0	0	0,1	0	0
Xenosauridae	0,1	0	0	0	1	0,1	2	0	0	0,1
Amphisbaenia	0	1	0	0	1	1	2	0	?	0,1
Dormaalisaurus	0	0	0	?	?	?	?	?	?	?
Sphenodon	?	?	1	0	0	0	0	0	0	0

	121	122	123	124	125	126	127	128	129	130
Agamidae*	0	?	0	0,1,2	0	1	0,1	0,1	0	0
Anguidae	0,1,2	0	0	1,2	0,1	1	0	0	0	1
Chamaeleontidae	0	?	1	3	1	0	0	1	1	?
Cordylidae	0,1,2	0	0,1	0,1	0	1	0	0	0	1,0
Dibamidae	2	0	1	3	?	?	?	?	1	?
Gekkonidae	0	1	0,1	1,2	1	1	0,1	0,1	0	1
Gymnophthalmidae	0	0	0,1	1,3	0	1	1	0	0	1
Helodermatidae	0	?	0	1	0	0	0	1	0	1
Iguanidae*	0,1,2	0,1	0,1	0,1,2	0,1	1	0,1	0,1	0	0
Lacertidae	0,1	0	0,1	1,0	0	1	0	1	0	1
Lanthanotus	0	?	0	3	0	1	0,1	1	0	1
Scincidae	0,1,2	0	0,1	1,3	0,1	1	0	0	0	1
Teiidae	0	0	0	1	0,1	1	1	0	0	1
Varanus	0	?	0	2	0	1	1	1	0	1,0
Xantusiidae	0	0	0	1,2	0	1	0	0	0	1
Xenosauridae	0	0	0	1	0	1	0	0	0	1
Amphisbaenia	2	0	0	3	0	0	0	1	0,1	?
Dormaalisaurus	?	?	?	?	?	?	?	?	?	?
Sphenodon	0	0	0	1,2	0	0	0	0	0	0

	131	132	133	134	135	136	137	138	139	140
Agamidae*	0,1	0	0	0,1	0,1	0	0	0	0	0
Anguidae	1	0,1	0	1	0	0	1	2	1	1
Chamaeleontidae	?	1	?	?	0,1	1	0	0	0	0
Cordylidae	1	0	0	1	0	0	1	2	1	1
Dibamidae	?	1	?	?	0	?	?	?	0	0
Gekkonidae	1,0	0	0,1	1	0,1	0	1	1	0	0,1
Gymnophthalmidae	1	0	0,1	1	1	1	1	2	0	0
Helodermatidae	1	0	1	?	0	0	1	1	0	1
Iguanidae*	1,0	0	0	0	0,1	0	0	0	0	0
Lacertidae	1	0	0	1	1,0	0	1	2	0	0
Lanthanotus	1	0	0	1	0	0	1	1	0	1
Scincidae	1	0,1	0	1	0,1	0	1	2	1	1
Teiidae	1	0	0	1	1	1	1	1	0	0
Varanus	1	0	0	0,1	0,1	0	1	0	0	0,1
Xantusiidae	1	0	0	1	0,1	0	1	2	0	0
Xenosauridae	1	0	0	0,1	0	0	1	1	0	1
Amphisbaenia	1	1	?	?	0	1	?	?	0	0
Dormaalisaurus	?	?	?	?	?	?	?	?	?	?
Sphenodon	0	0	0	0	0	0	0	0	0	0

	141	142	143	144	145
Agamidae*	0	0,1	0	0	0
Anguidae	1	2,1	0,1	0	1
Chamaeleontidae	0	1,2,0	0	0	0
Cordylidae	1	2	1	0	1
Dibamidae	0	0	0	0	?
Gekkonidae	0	0	0	1,0	1
Gymnophthalmidae	0	1	1	0	1
Helodermatidae	1	2	0	0	1
Iguanidae*	0,1	0,1,2	0	0	0
Lacertidae	1	2	1	0	1
Lanthanotus	1	0	0	0	1
Scincidae	1	1	1	0	1
Teiidae	0	1	1	0	1
Varanus	0,1	0	0	0	1
Xantusiidae	0	2	1	0	1
Xenosauridae	1	2	1	0	1
Amphisbaenia	0	0	0	0	1
Dormaalisaurus	1	1	1	?	?
Sphenodon	0	0	0	0	0

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On the myology of the cephalic region and pectoral girdle of three ariid species, *Arius heudeloti*, *Genidens genidens* and *Bagre marinus*, and comparison with other catfishes (Teleostei: Siluriformes)

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ABSTRACT. The muscles of the cephalic region and pectoral girdle of *Arius heudeloti*, *Genidens genidens* and *Bagre marinus* are described and compared with those of non-ariid siluriforms. Our observations and comparisons revealed that, although the configuration of the cephalic and pectoral girdle musculature of these ariid species is basically similar to that of other catfishes, these species present two myological peculiarities that could eventually represent autapomorphic characters of the family Ariidae, namely: 1) the muscle adductor arcus palatini inserts not only on the mesial margin of the suspensorium, but also on a significant part of the lateral surface of this complex structure; 2) the adductor mandibulae Aw is obliquely oriented, with its posterodorsal fibres being significantly dorsal to the upper edge of the coronoid process.

KEY WORDS: Ariidae, *Arius heudeloti*, autapomorphies, *Bagre marinus*, catfish, cephalic region, comparative morphology, *Genidens genidens*, myology, pectoral girdle, Siluriformes.

INTRODUCTION

The Siluriformes are "one of the economically important groups of fresh and brackish water fishes in the world: in many countries, they form a significant part of inland fisheries; several species have been introduced in fish culture; numerous species are of interest to the aquarium industry where they represent a substantial portion of the world trade" (TEUGELS, 1996).

Among the 35 siluriform families (FERRARIS & DE PINNA, 1999), the family Ariidae, with approximately 121 species in 12 genera (TEUGELS, 1996), is surely one of the most studied (see, e.g., REGAN, 1911; STARKS, 1926; LYNN & MELLAND, 1939; MERRIMAN, 1940; BAMFORD, 1948; SRINIVASACHAR, 1958; HUBBS & MILLER, 1960; ALEXANDER, 1965; TILAK, 1965; ROSSEL, 1968; LUNDBERG, 1975, 1993; GOSLINE, 1977; DAN, 1980; TAVOLGA, 1962; SRINIVASA & LAKSHMI, 1984; GAYET, 1987, 1995; VAN NEER & GAYET, 1988; LAKSHMI & SRINIVASA, 1989; GAUDANT, 1993; MO, 1991; ARRATIA, 1995; ARRATIA & GAYET, 1995; CIONE et al., 1996; LADICH & BASS, 1998; etc.). The Ariidae are found worldwide in tropical and subtropical regions. They form an important part of commercial catches in some areas, particularly in the Far East, being also used in aquaculture (TEUGELS, 1996). According to MO (1991), the Ariidae (excluding the Madagascar genus *Ancharius* transferred to Mochokidae) are defined by two uniquely derived features: 1) a greatly enlarged utricular otolith occupying a space formed by the prootic, pterotic and exoccipital bones and 2) an extensive superficial ossification on the ventral side of the complex vertebral centrum.

Despite the numerous studies dedicated to the ariids (see above), the myology of these fishes was never described in detail. This complicates not only the study of the functional morphology of these fishes, but also the study of the phylogenetic relationships between the Ariidae and the other catfish families.

The aim of this work is thus to describe in detail the muscles of the cephalic region (branchial apparatus

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excluded) and pectoral girdle of three ariid species, *Arius heudeloti* (Valenciennes, 1840), *Genidens genidens* (Valenciennes, 1840) and *Bagre marinus* (Mitchil, 1815), and to compare these muscles with those of other catfishes, either studied by us or described in the literature, in order to pave the way for further anatomical, functional and phylogenetical studies on ariids, as well as on catfishes in general.

MATERIAL AND METHODS

The fishes studied are from the collection of our laboratory, trypsine-cleared and alizarine-stained (t&a) or alcohol fixed (alc): 4 alc. *Arius heudeloti*, 1 alc. and 1 t&a *Bagre marinus,* 2 alc. *Genidens genidens*. Dissections and morphological drawings were made using a Wild M5 dissecting microscope equipped with a camera lucida.

The nomenclature of the cephalic muscles is mainly based on WINTERBOTTOM (1974). However, for the different adductor mandibulae sections, we follow DIOGO & CHARDON (2000a), since recent works have pointed out that, with respect to these sections, WINTERBOTTOM's nomenclature (1974) presents serious limitations (see GOSLINE 1989; DIOGO & CHARDON, 2000a). In relation to the muscles associated with the mandibular barbels – which were not studied by WINTERBOTTOM (1974) – we follow DIOGO & CHARDON (2000b). With respect to nomenclature of the pectoral girdle muscles, we follow DIOGO et al. (2001).

RESULTS

In this section, we will describe the myology of the cephalic region and pectoral girdle of *Arius heudeloti*, *Genidens genidens* and *Bagre marinus.* It should be noticed that the abbreviations used in these figures refer mainly to the myological structures being described: for a detailed description of the osteological components of the cephalic region and pectoral girdle of ariid catfishes, see REGAN (1911), STARKS (1926), MERRIMAN (1940), BAMFORD (1948), SRINIVASACHAR (1958), HUBBS & MILLER (1960), ALEXANDER (1965), TILAK (1965), CHARDON (1968), ROSSEL (1968), LUNDBERG (1975, 1993), GOSLINE (1977), TAVOLGA (1962), SRINIVASA & LAKSHMI (1984), GAYET (1987, 1995), VAN NEER & GAYET (1988), LAKSHMI & SRINIVASA (1989), GAUDANT (1993), MO (1991), ARRATIA (1995), ARRATIA & GAYET (1995), CIONE et al., (1996), etc.

Arius heudeloti

Musculus adductor mandibulae (ad.mnd). This muscle is differentiated in several sections. The external section, adductor mandibulae A1-ost (ad.mnd.1-ost), originates on the preopercular and quadrate and inserts on the postero-lateral surface of the angulo-articular (Fig. 1A). The adductor mandibulae A2 (ad.mnd.2), which lies dorso-mesially to the adductor mandibulae A1-ost, attaches caudally to the preopercular, pterotic and sphenotic (Fig. 1A). Rostrally it attaches to the mesial surface of the angulo-articular, laterally to the adductor mandibulae A3'' (Fig. 2B). The adductor mandibulae A3'-d is differentiated into two bundles, adductor mandibulae A3'-d-1 (ad.mnd.3'-d-1) and adductor mandibulae A3'-d-2 (ad.mnd.3'-d-2). The adductor mandibulae A3'-d-1 originates on the hyomandibular and preopercular, mesially to the adductor mandibulae A2 (Fig. 1B), and inserts on the postero-

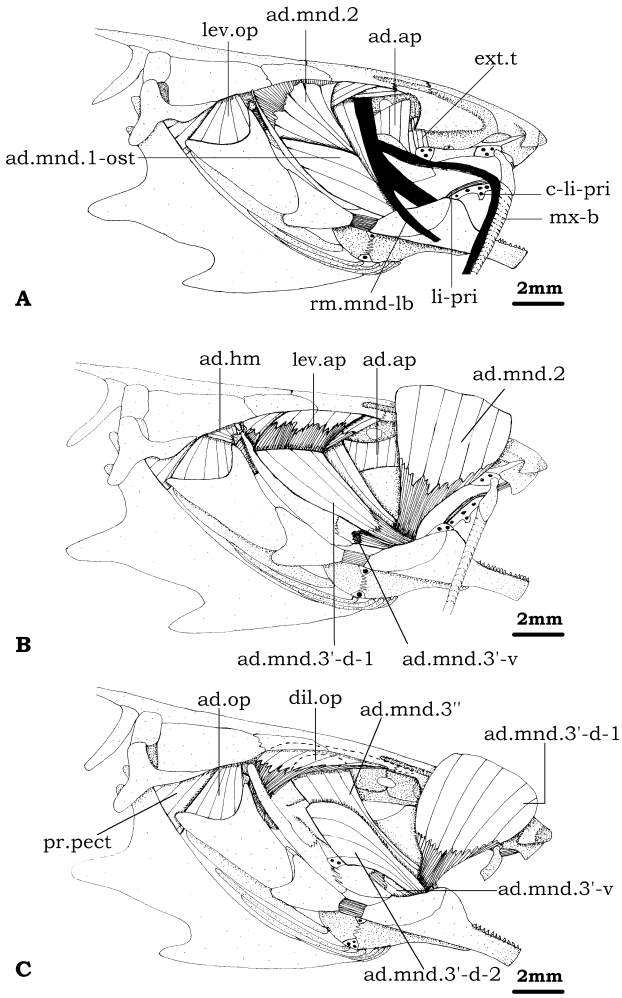

Fig. 1. – Lateral view of the cephalic musculature of *Arius heudeloti*. (A) All the muscles are exposed. (B) Levator operculi, adductor mandibulae A1-ost, ramus mandibularis and ramus maxillaris removed and adductor mandibulae A2 folded back. (C) Adductor mandibulae A2, adductor arcus palatini, levator arcus palatini, primordial ligament and adductor hyomandibularis removed and adductor mandibulae A-3'-d-1 folded back. *ad.ap*, adductor arcus palatini; *ad.hm*, adductor hyomandibularis; *ad.op*, adductor operculi; *ad.mnd.1-ost*, *ad.mnd.2*, *ad.mnd.3'-d-1*, *ad.mnd3'-d-2*, *ad.mnd.3'-v*, *ad.mnd.3''*, sections of adductor mandibulae; *c-li-pri*, cartilage associated with primordial ligament; *dil.op*, dilatator operculi; *ext.t*, extensor tentaculi; *lev.ap*, levator arcus palatini; *lev.op*, levator operculi; *li-pri*, primordial ligament; *mx-b*, maxillary barbel; *pr.pect*, protractor pectoralis; *rm.mnd-lb*, lateral branch of ramus mandibularis.

dorso-mesial edge of the coronomeckelian bone (Fig. 2B). The adductor mandibulae A3'-d-2 (ad.mnd.3'-d-2), which lies antero-mesially to the adductor mandibulae A3'-d-1 (Fig. 1C), originates on a prominent, long lateral crest formed by both the quadrate and the hyomandibular and inserts on the postero-dorso-lateral edge of the coronomeckelian bone (Fig. 2C). The adductor mandibulae A3'-v (ad.mnd.3'-v) runs from the quadrate (Fig. 1B) to the mesial surface of the angulo-articular (Fig. 1C). The deeper bundle of the adductor mandibulae, adductor mandibulae A3'' (ad.mnd.3''), attaches anteriorly to the antero-lateral margin of the hyomandibular (Fig. 1C) and posteriorly to the medial surface of the angulo-articular (Fig. 2A). The smallest section of the muscle adductor mandibulae, the adductor mandibulae Aω (ad.mnd.ω) is well-developed and obliquely oriented, running from the mesial side of the mandible to the tendons of both the adductor mandibulae A3'' and the adductor mandibulae A2 (Fig. 2A).

Musculus levator arcus palatini (lev.ap). It originates on the dorso-lateral surfaces of both the sphenotic and the frontal and inserts on the lateral face of the hyomandibula (Fig. 1B).

Musculus adductor arcus palatini (ad.ap). The adductor arcus palatini originates on the parasphenoid, orbitosphenoid and pterosphenoid. It inserts on the mesial margin of the hyomandibular, as well as on both the mesial and antero-dorsal surfaces of the metapterygoid (Fig. 1A, B).

Musculus dilatator operculi (dil.op). Thick muscle situated medially to the levator arcus palatini (Fig. 1C). It runs from the sphenotic, pterosphenoid, frontal and lateral ethmoid to the antero-dorsal edge of the opercular (medial to the preopercular but lateral to the articulatory facet of the opercular for the hyomandibula) (Fig. 1C).

Musculus levator operculi (lev.op). It originates on the ventro-lateral surface of the pterotic and inserts on the dorsal edge of the opercular (Fig. 1A).

Musculus adductor hyomandibularis (ad.hm). Small muscle situated mesially to the levator operculi. It originates on the ventral surface of the pterotic and inserts on a well-developed postero-dorsal process of the hyomandibula (Fig. 1B).

Musculus adductor operculi (ad.op). Situated mesially to the adductor hyomandibularis. It runs from the ventro-medial surface of the pterotic to the dorso-medial surface of the opercular (Fig. 1C).

Musculus extensor tentaculi (ext.t). It originates on the antero-medial surface of both the lateral ethmoid and the orbitoshenoid (Fig. 1A). It inserts on the mesial and ventral surfaces of the entopterygoid, as well as on the back of the autopalatine (Fig. 1A).

Musculus protractor hyoidei (pr.h). This muscle is differentiated into three parts. The pars ventralis (pr.h-v), in which are lodged the moving parts of the cartilages associated with the mandibular barbels, originates on both the anterior and posterior ceratohyals and inserts on the dentary, meeting its counterpart in a well-developed median aponeurosis (Fig. 3A). The pars lateralis (pr.h-l) originates on the posterior ceratohyal, inserting on the ventro-medial face of the dentary (Fig. 3A). The pars dorsalis (pr.h-d) runs from the anterior ceratohyal to the dentary (Fig. 3A).

Intermandibularis (intm). Well-developed muscle joining the two mandibles (Fig. 3A).

Musculus retractor externi mandibularis tentaculi (r.ex.mnd.t). It runs from the moving part of the cartilage associated with the outer mandibular barbel to the dentary (Fig. 3A).

Musculus retractor interni mandibularis tentaculi (r.in.mnd.t). It originates on the moving part of the cartilage associated with the internal mandibular barbel and inserts on dentary (Fig. 3A).

Musculus protractor externi mandibularis tentaculi (pr.ex.mnd.t). It runs from the posterior ceratohyal to the moving part of the cartilage associated with the outer mandibular barbel (Fig. 3A).

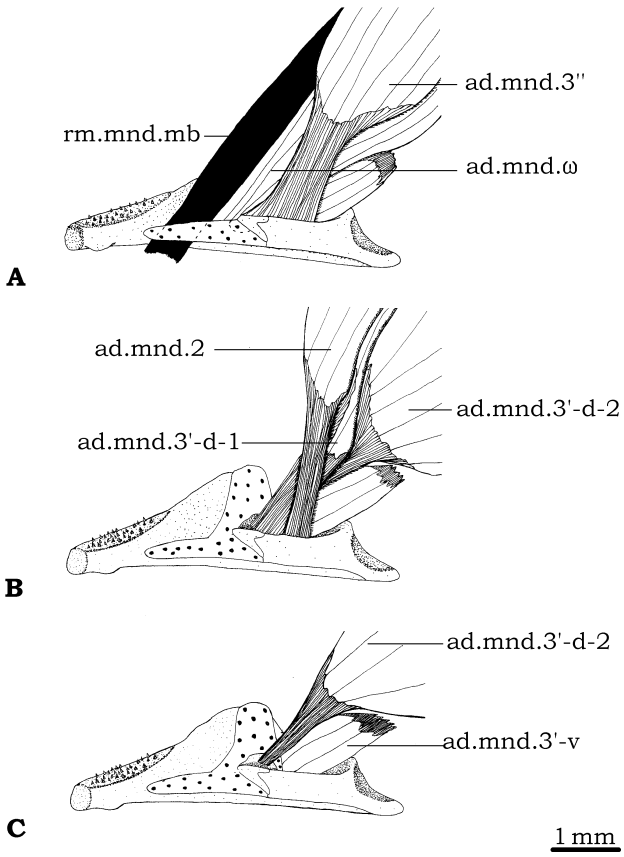

Fig. 2. – Mesial view of the mandible and adductor mandibulae muscle of *Arius heudeloti.* (A) Adductor mandibulae complex exposed. (B) Adductor mandibulae A3'', adductor mandibulae Aω and ramus mandibularis removed. (C) Adductor mandibulae A2 and adductor mandibulae A-3'-d-1 removed. *ad.mnd.2*, *ad.mnd.3'-d-1*, *ad.mnd.3'-d-2*, *ad.mnd.3'-v*, *ad.mnd.3''*, *ad.mnd.ω*, sections of adductor mandibulae; *rm.mnd-mb*, mesial branch of ramus mandibularis.

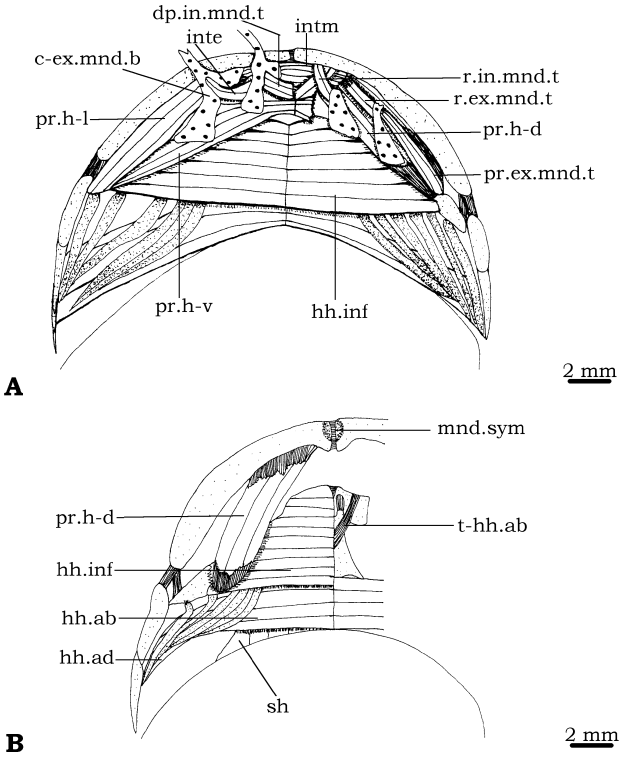

Fig. 3. – Ventral view of the cephalic musculature of *Arius heudeloti.* (A) On the left side all muscles are exposed, on the right side lateral and ventral sections of protractor hyoideus and intertentacularis removed and cartilages associated with mandibular barbels were folded back. (B) On the left side only the hyohyoideus abductor, dorsal section of protractor hyoideus, hyohyoideus adductor, hyohyoideus inferioris and sternohyoideus are represented, on the right side only the hyohyoideus abductor is represented. *c-ex.mnd.b*, cartilage associated with the external mandibular barbel; *dp.in.mnd.t*, depressor interni mandibularis tentaculi; *hh.ab*, hyohyoideus abductor; *hh.ad*, hyohyoideus adductor; *hh.inf*, hyohyoideus inferioris; *inte*, intertentacularis; *intm*, intermandibularis; *mnd.sym*, mandibular symphysis; *pr.ex.mnd.t*, protractor exteni mandibularis tentaculi ; *pr.h-d*, *pr.h-l*, *pr.h-v*, dorsal, lateral and ventral sections of protractor hyoideus; *r.ex.mnd.t*, retractor externi mandibularis tentaculi; *r.in.mnd.t*, retractor interni mandibularis tentaculi; *sh*, sternohyoideus; *t-hh.ab*, tendon of hyohyoideus abductor.

Musculus depressor interni mandibularis tentaculi (dp.in.mnd.t). Small muscle extending from a mesial aponeurosis to the mesial surface of the cartilage associated with the internal mandibular barbel (Fig. 3A).
Intertentacularis (inte). Small muscle running from the mesial face of the cartilage associated with the external mandibular barbel to the lateral face of that associated with the internal one (Fig. 3A).

Musculus hyohyoideus inferior (hh.inf). Thick muscle attaches laterally on the ventral surface of the ceratohyals and medially on a median aponeurosis (Fig. 3A, B).

Musculus hyohyoideus abductor (hh.ab). It runs from the first (medial) branchiostegal ray to a median aponeurosis, which is associated with two long, strong tendons, attached, respectively, to the two ventral hypohyals (Fig. 3B).

Musculus hyohyoideus adductor (hh.ad). Medially it attaches to the first (medial) branchiostegal ray and laterally it attaches to the opercular (Fig. 3B).

Musculus sternohyoideus (sh). Well-developed muscle running from the parurohyal to both the anterior and the antero-dorsal surfaces of the cleithrum (Fig. 4A).

Musculus arrector dorsalis (arr.d). This muscle is differentiated into two well-developed divisions. The dorsal division (arr.d-dd), situated on the dorsal surface of the pectoral girdle, originates on the dorso-mesial edge of the scapulo-coracoid (Fig. 4A) and inserts on the anterior edge of the dorsal condyle of the pectoral spine. The ventral division (arr.d-vd), situated on the ventral surface of the pectoral girdle, originates on the ventral margin of the cleithrum and inserts on the antero-lateral edge of the pectoral spine (Fig. 4B).

Arrector ventralis (arr.v). It runs from the antero-ventral surface of the cleithrum to the ventral condyle of the pectoral spine (Fig. 4B).

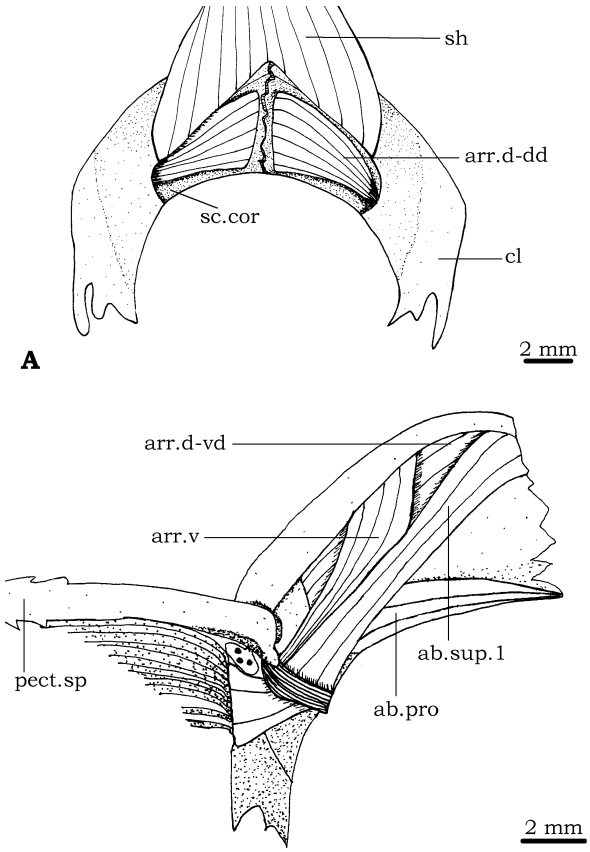

Fig. 4. – Pectoral girdle musculature of *Arius heudeloti.* (A) Dorsal view. (B) Ventral view. *ab.pro*, abductor profundus; *ab.sup.1*, section 1 of abductor superficialis; *arr.d-dd*, *arr.d-vd*, dorsal and ventral divisions of arrector dorsalis; *arr.v*, arrector ventralis; *cl*, cleithrum; *pec-sp*, pectoral spine; *sc.cor*, scapulo-coracoide; *sh*, sternohyoideus.

Abductor profundus (ab.pro). It originates on the postero-mesial edge of the coracoid (Fig. 4B) and inserts on the mesial surface of the dorsal condyle of the pectoral spine.

Abductor superficialis (ab.sup). Paired. This muscle is differentiated in two sections. The larger section (Fig. 4B : ab.sup.1) attaches medially to the ventral face of both the cleithrum and the scapulo-coracoid and laterally to the antero-ventral margin of the ventral part of the pectoral fin rays. The smaller section runs from the postero-lateral edge of the scapulo-coracoid to the antero-dorsal margin of the ventral part of the pectoral fin rays.

Adductor superficialis. This muscle is also differentiated into two sections. The larger one originates on the posterior surfaces of both the cleithrum and the scapulo-coracoid and inserts on the antero-dorsal margin of the dorsal part of the pectoral fin rays. The smaller section runs from the ventro-lateral edge of the mesocoracoid arch and the dorsal surface of the proximal radials to the antero-ventral margin of the dorsal part of the pectoral fin rays.

Genidens genidens

In a general way, the configuration of the muscles of the cephalic region and pectoral girdle of this species resembles that of *Arius heudeloti*. The most significant differences between these species are: I) in *G. genidens* (Fig. 5) the adductor mandibulae A1-ost (ad.mnd.1-ost) contacts a significant part of the lateral surface of the angulo-articular, inserting on a prominent lateral crest of this bone (Fig. 5A, compare with Fig. 1A); II) in *G. genidens* the levator opeculi (lev.op) and the adductor hyomandibularis (ad.hm) are not only originated on the pterotic, but also on the posttemporo-supracleithrum (Fig. 5A, B, compare with Fig. 1A, B).

Bagre marinus

With the exception of a few differences, the configuration of the cephalic and pectoral girdle musculature of *Bagre marinus* also resembles that of *Arius heudeloti.* Most of these differences are related to the fact that *B. marinus* only presents one, and not two, mandibular barbels on each side of the head. Therefore, contrarily to *A. heudeloti* (Fig. 3A), which presents five little muscles on each side of the head exclusively related with the movement of the mandibular barbels (retractor externi mandibularis tentaculi, retractor interni mandibularis tentaculi, protractor externi mandibularis tentaculi, depressor interni mandibularis tentaculi and intertentacularis), in *B. marinus* there is only one muscle associated with the single mandibular barbel in each side of the head. Although this muscle seems to be a retractor of this barbel, it is difficult to specify whether it corresponds to the retractor externi mandibularis tentaculi or to the retractor interni mandibularis tentaculi, due to the incertitude concerning the identity of the mandibular barbels of *B. marinus* (that

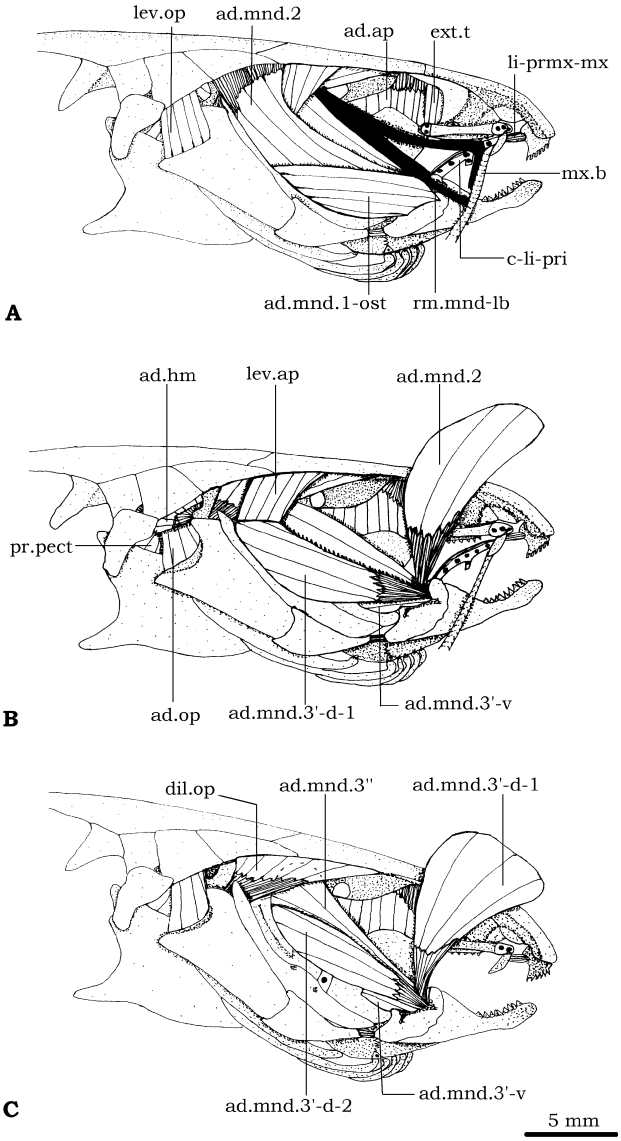

Fig. 5. – Lateral view of the cephalic musculature of *Genidens genidens*. (A) All the muscles are exposed. (B) Levator operculi, adductor mandibulae A1-ost, ramus mandibularis and ramus maxillaris removed and adductor mandibulae A2 folded back. (C) Adductor mandibulae A2, levator arcus palatini, primordial ligament and adductor hyomandibularis removed and adductor mandibulae A-3'-d-1 folded back. *ad.ap*, adductor arcus palatini; *ad.hm*, adductor hyomandibularis; *ad.op*, adductor operculi; *ad.mnd.1-ost*, *ad.mnd.2*, *ad.mnd.3'-d-1*, *ad.mnd3'-d-2*, *ad.mnd.3'-v*, *ad.mnd.3''*, sections of adductor mandibulae; *c-li-pri*, cartilage associated with primordial ligament; *dil.op*, dilatator operculi; *ext.t*, extensor tentaculi; *lev.ap*, levator arcus palatini; *lev.op*, levator operculi; *li-prmx-mx*, ligament between premaxillary and maxillary; *mx-b*, maxillary barbel; *pr.pect*, protractor pectoralis; *rm.mnd-lb*, lateral branch of ramus mandibularis.

is, whether these barbels correspond to the external or to the internal mandibular barbels of other catfishes: see DIOGO & CHARDON, 2000b).

In addition to the differences related to the mandibular barbels, there is another significant difference between *B. marinus* and *A. heudeloti*: in *B. marinus*, as it is the case

in *G. genidens*, the adductor mandibulae A1-ost contacts a significant part of the lateral surface of the angulo-articular, inserting on a lateral crest of this bone (which, however, is not as developed as in *G. genidens*).

DISCUSSION

In a general way, the muscles of the cephalic region and pectoral girdle of *Bagre marinus, Arius heudeloti* and *Genidens genidens* present a quite similar configuration. The most remarkable difference between the cephalic and pectoral girdle musculature of these three species is surely the configuration of the muscles associated with the mandibular barbels. In fact, *A. heudeloti* and *G. genidens*, which present on each side of the head several (five in this specific case) little muscles associated with the movements of the two mandibular barbels, exhibit a quite similar configuration to that found in many other catfishes, such as claroteids, amphiliins, doumeins, bagrids, clariids, plotosids or malapterurids (see DIOGO & CHARDON, 2000b). However, in *B. marinus*, contrarily to these two species, there is only one mandibular barbel and one little muscle associated to it in each side of the head. This muscle seems to be a retractor of the mandibular barbel, since it attaches anteriorly to the mandible and posteriorly to the antero-dorsal surface of the moving part of the cartilage associated with this barbel (see DIOGO & CHARDON, 2000b). However, the present study did not enable us to determine which of the two pairs of mandibular barbels present in most other catfishes (externals mandibular barbels and internal mandibular barbels) corresponds to the single pair found in *B. marinus*. Therefore, it could not be specified if this muscle is a retractor externi mandibularis tentaculi or a retractor interni mandibularis tentaculi. Another significant difference between the cephalic and pectoral girdle muscles of the three ariid species studied is that in both *B. marinus* and *G. genidens*, but not in *A. heudeloti*, the adductor mandibulae A1-ost contacts a great part of the lateral surface of the angulo-articular. A remarkable difference between these three species is also that in *G. genidens* the levator operculi and adductor hyomandibularis are associated with both the pterotic and the posttemporo-supracleithrum, and not only with the pterotic, as it is the case in *A. heu*deloti and *B. marinus*.

As referred to in the Introduction, one of the principal aims of this work is to compare the configuration of the cephalic and pectoral girdle musculature of the three ariid species studied and that of other siluriforms (either studied by us or described in the literature). This comparison revealed that, in a general way, the muscles of the cephalic region and pectoral girdle of *A. heudeloti*, *B. marinus* and *G. genidens* do not differ much from those of most other catfishes in which these muscles have been studied in detail. However, there are three morphological features, which are present in all the three ariid species studied, that, by their rarity, deserve particular attention, being discussed below.

As pointed out by DIOGO & VANDEWALLE (in press), the plesiomorphic condition for catfishes is that in which the adductor arcus palatini inserts on the mesial margin of the suspensorium. However, in the three ariid species studied, *A. heudeloti* (Fig. 1A), *G. genidens* (Fig. 5A) and *B. marinus*, this muscle not only inserts on the mesial margin of both the hyomandibular and the metapterygoid, but also on a significant part of the lateral surface of the metapterygoid. Since such a configuration of the adductor arcus palatini muscle is found in all the three ariid species studied, and in no other catfish studied by us or described in the literature, this morphological feature could represent an ariid autapomorphy. However, it should be noted that much more data on the configuration of this muscle in other ariid species, as well as in many other catfishes, are needed to eventually confirm this hypothesis.

Plesiomorphically in catfishes the adductor mandibulae Aω is a small, anteroposteriorly-oriented bundle lodged in the mesial surface of the mandible (see DIOGO & CHARDON, 2000a). However, in the three ariid species studied, *A. heudeloti* (Fig. 2A), *G. genidens* and *B. marinus*, the adductor mandibulae Aω is a well-developed, obliquely-oriented bundle, with its postero-dorsal fibers being significantly dorsal to the upper edge of the coronoid process. The presence of such a configuration of the Aω in all the three ariid species studied, together with its absence in all other catfish studied by us and/or described in the literature, indicates that this configuration could probably represent an ariid autapomorphy. However, as mentioned above, much more data on the configuration of this muscle in other ariid species, as well as in many other catfishes, are needed to eventually confirm this hypothesis.

In catfishes the adductor operculi connects the neurocranium, the mesial surface of the opercular and, often (e.g., in amphiliins, plotosids, bagrids, diplomystids, clariids, schilbeids), the mesial surface of the hyomandibular (DIOGO & VANDEWALLE, in press). However, in the three ariid species studied, as well as in the auchenoglanidin and clarotein species examined in this work, in the region normally occupied by the adductor operculi, in addition to this muscle there is a small, completely separate muscle (here called adductor hyomandibularis) running from the neurocranium to the postero-dorsal surface of the hyomandibular (see, e.g., Figs 1B, 5B). As the adductor hyomandibularis is found not only in the ariid, but also in the auchenoglanidin and the clarotein species examined, its presence could not constitute an ariid autapomorphy, that is, a derived feature exclusively present in the ariids. Instead, the presence of the adductor hyomandibularis in these three groups seems to support DIOGO et al.'s (in press) study, according to which the auchenoglanidins, ariids and claroteins are probably closely related.

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Révision de *Luxilites striolatus*, poisson marin (Teleostei, Tselfatiiformes) du Crétacé supérieur du Kansas (Etats-Unis)

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RÉSUMÉ. Le crâne de *Luxilites striolatus*, une espèce d'un genre monospécifique téléostéen fossile du Crétacé supérieur marin du Kansas, est étudié. L'ostéologie montre que *Luxilites* est un genre valable de l'ordre des Tselfatiiformes, proche de *Bananogmius*, et qu'il appartient à un sous-groupe de Plethodidae qui, outre *Bananogmius*, comprend aussi *Syntegmodus* et *Niobrara*.

MOTS CLEFS : *Luxilites striolatus*, Teleostei, Tselfatiiformes, Crétacé supérieur marin, Kansas, ostéologie.

Revision of *Luxilites striolatus*, marine fish (Teleostei, Tselfatiiformes) from the Upper Cretaceous of Kansas (United States)

ABSTRACT. The skull of *Luxilites striolatus*, a species of a monospecific genus of fossil teleosts from the marine Upper Cretaceous of Kansas, is studied. The osteology shows that *Luxilites* is a valid genus of the order Tselfatiiformes, close to *Bananogmius*, and that it belongs to a subgroup of Plethodidae comprising, besides *Bananogmius*, also *Syntegmodus* and *Niobrara*.

KEY WORDS : *Luxilites striolatus*, Teleostei, Tselfatiiformes, marine Upper Cretaceous, Kansas, osteology.

INTRODUCTION

JORDAN (1924) a décrit une série de téléostéens marins provenant de la Niobrara Formation (Coniacien à Sénonien) du Kansas et, parmi ceux-ci, cinq nouveaux genres monospécifiques, *Zanclites xenurus*, *Niobrara encarsia*, *Kansanus martini*, *Luxilites striolatus* et *Ferrifrons rugosus*, qu'il a rapportés à une nouvelle famille, les Niobraridae. Cette famille a été mise depuis en synonymie avec celle des Plethodidae, au sein de l'ordre des Tselfatiiformes (=Bananogmiiformes) (PATTERSON, 1993 : 627 ; NELSON, 1994 : 90).

Zanclites xenurus et *Niobrara encarsia* ont fait l'objet d'une récente révision (TAVERNE, 1999, 2001a) qui a prouvé leur appartenance aux Tselfatiiformes ainsi que leur validité générique et spécifique. SCHULTZE et al. (1982 : 35) ont indiqué que *Kansanus martini* ne se rapportait pas aux Plethodidae mais aux Pachyrhizodontidae, ce que mes propres observations ont confirmé. Quant à *Ferrifrons rugosus*, il a été réétudié par ARRATIA & CHORN, 1998 et s'est révélé être un acanthomorphe archaïque. Seul *Luxilites striolatus* n'a pas encore fait l'objet d'un réexamen depuis sa description originale.

Luxilites striolatus n'est connu que par sa tête et le début de sa région abdominale. Le reste du corps est perdu. JORDAN (1924 : 226-227) a donné une description très succincte, incomplète et partiellement erronée de l'ostéologie de ce poisson qui ne permet guère d'émettre un avis quant à sa position systématique au sein des téléostéens. Cet auteur était d'ailleurs conscient de la chose

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puisqu'il écrit (ibid. : 227) que « the relations of this genus are obscure ». Il faut encore ajouter que certaines parties de la tête (la portion antérieure de la mandibule, la partie antérieure de l'entoptérygoïde droit) de *L. striolatus* ont échappé à l'investigation de ce chercheur car ils n'étaient pas rangés avec le reste du matériel provenant de ce poisson. Précisons encore que d'après les dimensions de sa tête, on peut estimer que *L. striolatus* atteignait un peu moins d'un mètre de longueur totale.

Le but du présent travail est donc d'approfondir les connaissances ostéologiques relatives à *L. striolatus*, de prouver ou de réfuter son appartenance aux Tselfatiiformes et, enfin, de préciser éventuellement sa position phylogénétique au sein de cet ordre. Le présent article s'inscrit également dans la série de travaux que je consacre à la révision générale des Tselfatiiformes (TAVERNE, 1975, 1983, 1999, 2000a-d, 2001a, b, sous presse a, b).

Rappelons une fois encore que les Tselfatiiformes ou Bananogmiiformes sont un ordre de grands téléostéens marins appartenant au groupe des Clupeocephala dont ils forment l'un des clades les plus primitifs. Ils ont vécu durant le Crétacé dans la Mésogée eurafricaine et ses dépendances, le Paléoatlantique et la mer intérieure nord-américaine. Ils n'ont plus de descendants dans les mers et les océans d'aujourd'hui.

La morphologie des Tselfatiiformes rappelle celle des Scombridae et des Coryphaenidae, ce qui indique des poissons à nage rapide qui menaient probablement, dans les mers du Crétacé, le genre de vie mené par ces deux familles dans les océans actuels. Le crâne est presque toujours médio-pariétal. La mâchoire supérieure est bordée par le prémaxillaire et le maxillaire, ce dernier surmonté d'un seul supramaxillaire. Les dents des mâchoires, du palais et de la langue sont minuscules et groupées en plages. Les os dentés sont perforés de canalicules très étroits qui, lorsque les dents sont perdues par l'usure ou la fossilisation, donnent à la surface de ces os un aspect ponctué caractéristique. Les nageoires dorsale et anale sont longues et élevées, la dorsale couvrant toute la longueur du dos. Les nageoires pectorales sont insérées haut sur les flancs. La ceinture et les nageoires pelviennes occupent une position abdominale et sont souvent atrophiées. Le complexe urophore comporte une large plaque hypurale faite des quatre premiers hypuraux soudés et elle-même fusionnée à une très petite vertèbre urale I et II. Le parhypural et souvent aussi l'arc hémal préural 1ont disparu. La nageoire caudale est grande, bilobée et compte 19 rayons principaux. L'hypurostégie est importante. Les écailles sont grandes et cycloïdes.

Pour davantage d'informations concernant les Tselfatiiformes ainsi que la discussion de leur position systématique au sein des téléostéens, voir TAVERNE (2000a).

MATERIEL ET METHODES

L'holotype et unique exemplaire de *Luxilites striolatus* est conservé dans les collections paléontologiques de l'Université du Kansas à Lawrence (Kansas, U.S.A.) sous le N° KUVP 295. Il a été récolté par E.H. TAYLOR en 1910 dans le canyon de la ligne de chemin de fer Gove-Trego, dans le comté de Gove, au Kansas. Il provient du Smoky Hill Chalk Member de la Niobrara Formation et est d'âge coniacien (Crétacé supérieur) (SCHULTZE et al., 1982 : 35).

Le matériel a été étudié à l'aide d'un stéréomicroscope Wild M5. L'observation de certains détails crâniens et des sutures entre les os a été facilitée par une immersion dans l'éthanol. Les dessins ont été réalisés par l'auteur au moyen d'une chambre claire (camera lucida).

LISTE DES ABREVIATIONS DES FIGURES

An :	angulaire (= angulo-splénial)
Art :	articulaire
Bo :	fragment du basioccipital
Brstg :	rayons branchiostèges
Cbr 1 :	cératobranchial du premier arc branchial
Chy a. :	cératohyal antérieur
Clt :	cleithrum
Cor :	coracoïde (= hypocoracoïde)
Dbbr 1-3 :	dermobasibranchial des trois premiers arcs branchiaux
Dbhy :	dermobasihyal (= dermentoglosse)
Dn :	dentaire (= dento-splénial, dentalo-splénial)
Dsph :	dermosphénotique
Ec :	écaille
Ecpt :	ectoptérygoïde
Enpt :	entoptérygoïde (= endoptérygoïde, mésoptérygoïde)
Epi :	épiotique (= épioccipital)
Exo :	fragment de l'exoccipital
Fr :	frontal
Hbr 1 :	fragment de l'hypobranchial du premier arc
Hclt :	hypercleithrum (= supracleithrum)
Hhy d. :	hypohyal dorsal
Hhy v. :	hypohyal ventral
Hyom :	hyomandibulaire
Iorb 2, 3, 4, 5 :	infraorbitaires 2, 3, 4, 5
Meth :	mésethmoïde
Mpt :	métaptérygoïde
Na :	nasal
Op :	operculaire
Osph :	orbitosphénoïde
Pa :	pariétal
Pop :	préoperculaire
Ps :	parasphénoïde
Psph :	pleurosphénoïde (= ptérosphénoïde)
Pt :	posttemporal
Pte :	ptérotique
Qu :	carré (= quadratique)
Rad :	ptérygophores pectoraux
Rart :	rétroarticulaire
Sca :	scapula (= hypercoracoïde)
Soc :	supraoccipital

Sop :	sous-operculaire	d. :	droit
Sph :	sphénotique (= autosphénotique)	d. fr. pa. :	dépression médiane fronto-pariétale
St :	supratemporal (= extrascapulaire)	f. t. :	fosse temporale (= posttemporale)
Sy :	symplectique	g. :	gauche
Uhy :	urohyal (= parahyoïde)	lig. os. :	ligaments ossifiés mandibulo-cleithraux
V :	fragment de la première vertèbre	p. op. :	processus opercularis de l'hyomandibulaire

ÉTUDE DU MATERIEL

Le crâne (Fig. 1-4)

Les os dermiques crâniens sont ornementés de fines ridules et parfois de petits tubercules. Cette ornementation est particulièrement prononcée sur les infrorbitaires postérieurs, le supratemporal, les os de la série operculaire et les rayons branchiostèges.

Le museau n'est pas conservé. Rien n'est donc connu du mésethmoïde, des nasaux, des ethmoïdes latéraux et du vomer.

Le toit crânien est large, presque plat et de forme à peu près triangulaire, la largeur étant nettement moins importante au niveau des frontaux qu'à celui des ptérotiques. Les frontaux sont vastes et forment la portion antérieure de ce toit. Les pariétaux sont grands, à peu près quadran-

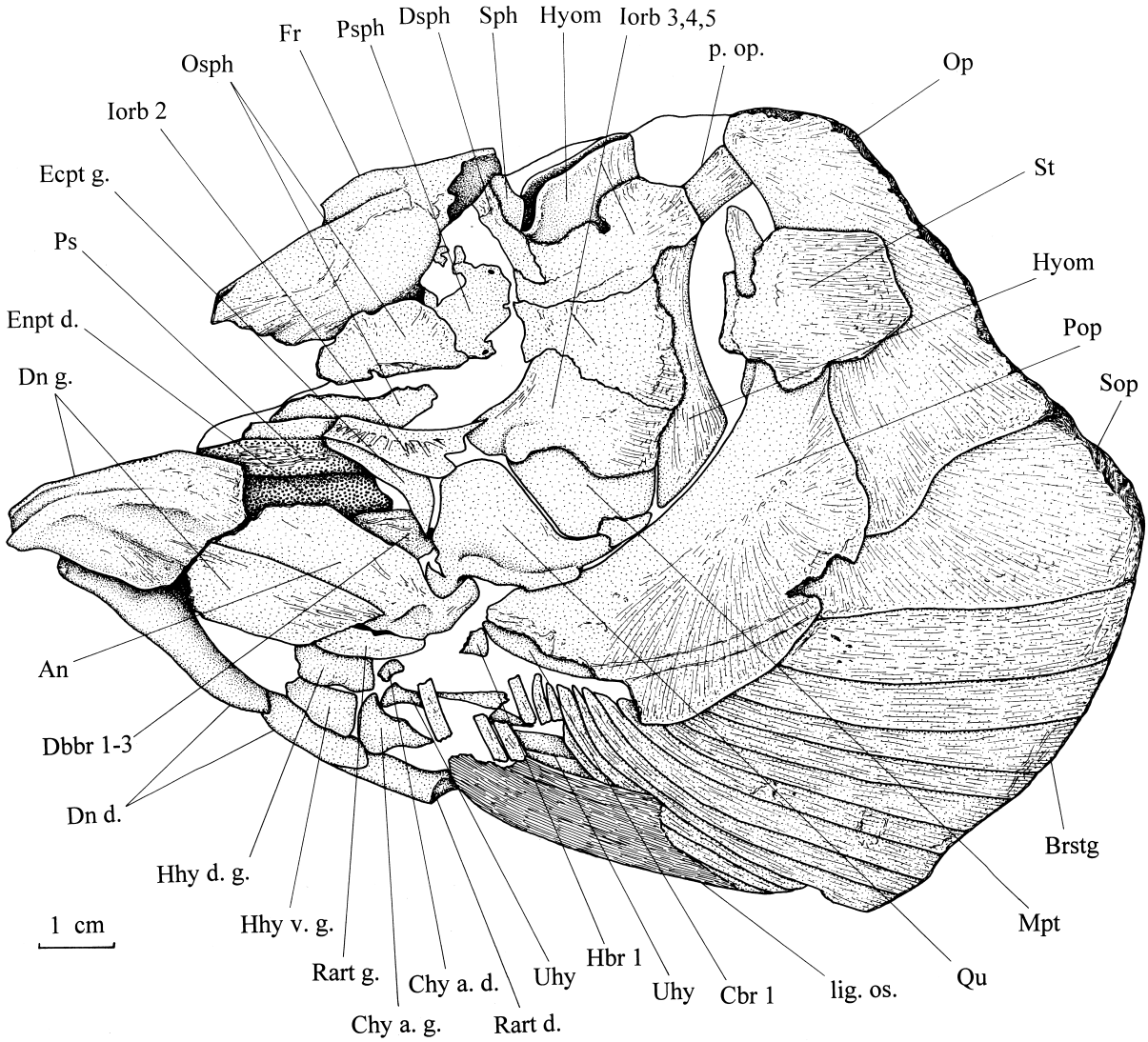

Fig. 1. – *Luxilites striolatus* JORDAN, 1924. Le crâne de l'holotype N° KUVP 295 en vue latérale gauche. Le museau et la mâchoire supérieure manquent. Le substrat est laissé en blanc. Le rétroarticulaire, normalement soudé à l'angulaire, a été brisé et séparé de ce dernier os suite aux aléas de la fossilisation.

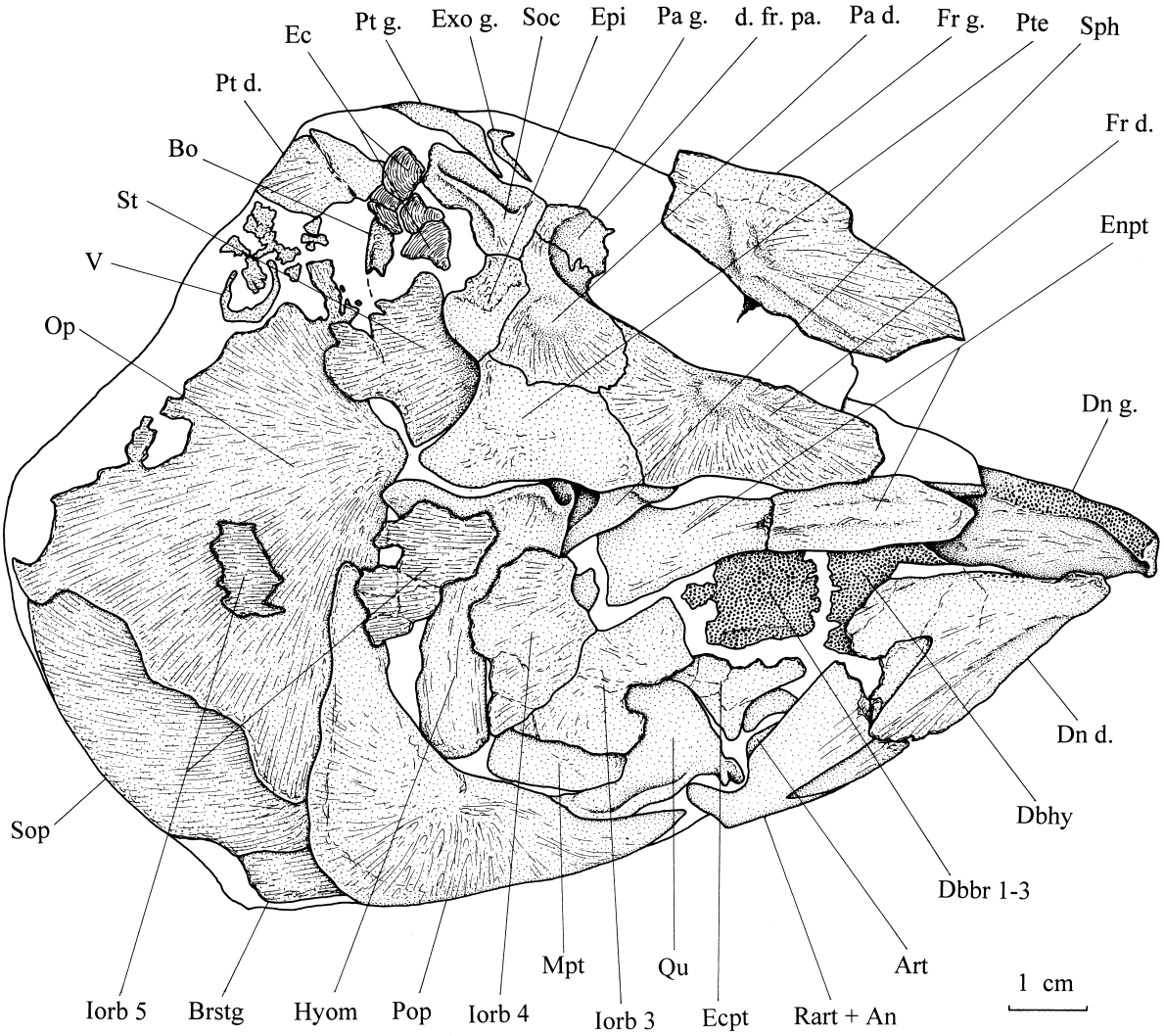

Fig. 2. – *Luxilites striolatus* JORDAN, 1924. Le crâne de l'holotype N° KUVP 295 en vue latérale droite. Le museau et la mâchoire supérieure manquent. Le substrat est laissé en blanc.

gulaires et jointifs, déterminant un crâne de type médio-pariétal. Le frontal et le pariétal portent en leur milieu une petite protubérance d'où irradient les très fines crêtes qui ornementent la surface externe de ces deux os. Le pariétal est bordé antérieurement par le frontal et latéralement par le ptérotique. La dépression fronto-pariétale médiane est allongée, étroite et délimitée par un bord bien marqué. Les ptérotiques sont bien développés et leur aile dorsale (= dermoptérotique) est très vaste. Les sphénotiques développent chacun un large processus postorbitaire qui, en vue dorsale, dépasse le bord latéral du frontal et du ptérotique. Le supraoccipital est assez petit, garni d'une crête médiane peu importante et encadré par les épiotiques.

La fosse temporale est située sur l'arrière du crâne et fermée dorsalement. Le supratemporal droit couvre la fosse correspondante. La fossilisation a, par contre, fait glisser le supratemporal gauche sur l'operculaire et le préoperculaire. JORDAN (1924: 226) a cru voir là le reste d'un morceau de peau garni de tubercules et recouvrant ces deux os et en a fait l'un des caractères distinctifs de son genre *Luxilites.* Les deux supratemporaux possèdent de vastes composants membranodermiques mais ne s'étendent cependant pas jusqu'à la ligne médiane du crâne. La dilatator fossa n'est pas visible car toute entière située sur la face ventrale du neurocrâne. L'extrémité antérieure de la fossette articulaire pour l'hyomandibulaire est visible du côté droit du crâne; elle est creusée dans le sphénotique.

L'orbitosphénoïde est grand et rejoint ventralement la crête médio-dorsale du parasphénoïde, formant ainsi un septum interorbitaire osseux complet auquel participent aussi les pleurosphénoïdes de taille plus réduite. La région trabéculaire du parasphénoïde est longue, large, de forme rectangulaire, presque plate et creusée sur toute sa longueur de puits minuscules, restes de l'implantation de denticules. Sur le fossile, cette région denticulée paraît un peu moins large qu'elle ne l'est en réalité car la partie latérale gauche de l'os est perdue. La région postérieure du parasphénoïde n'est pas visible.

Rien n'est connu des prootiques, de la *pars jugularis*, de l'éventuel basisphénoïde et des intercalaires. Un très petit fragment de l'exoccipital gauche est visible au som-

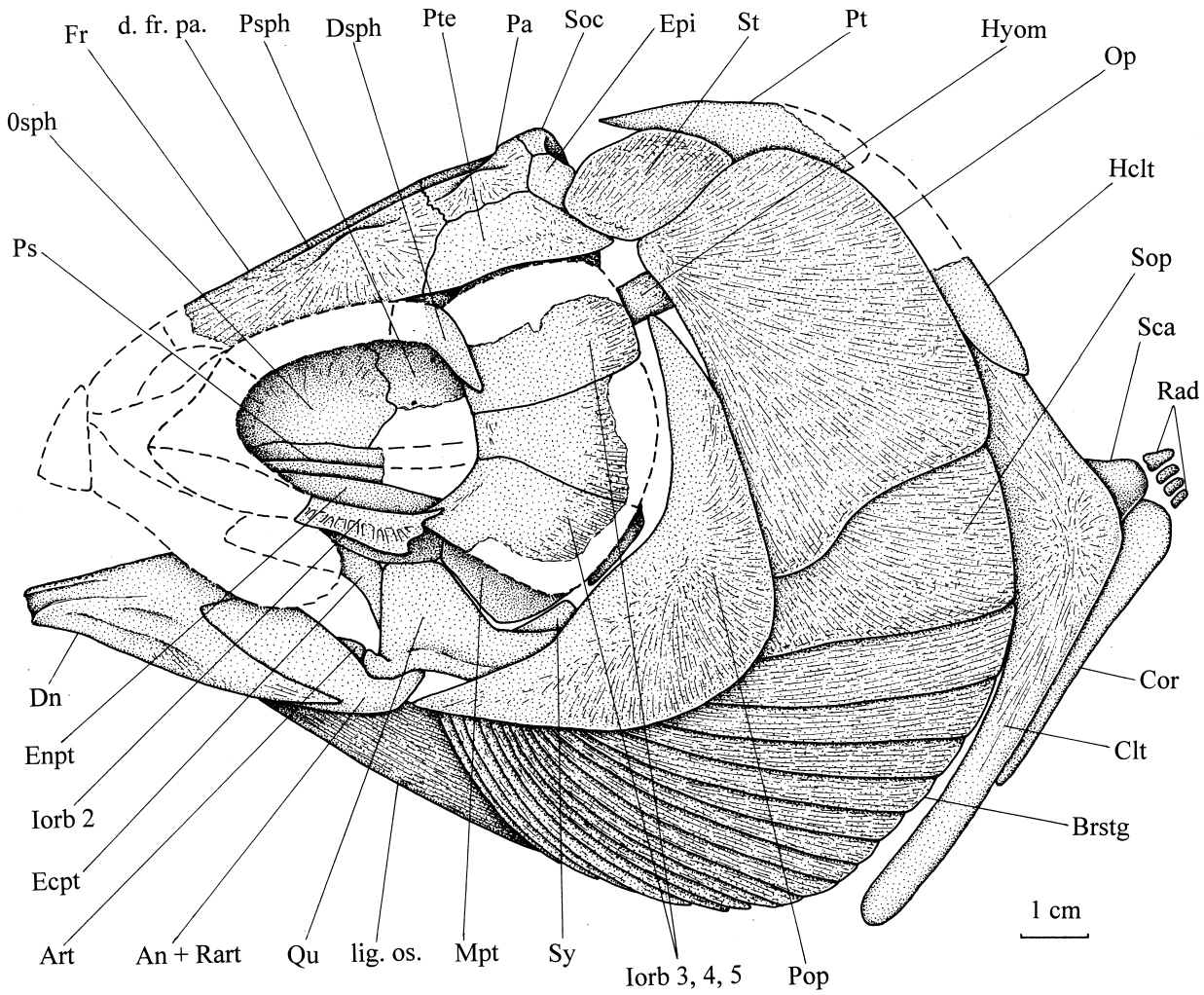

Fig. 3. – *Luxilites striolatus* JORDAN, 1924. Reconstitution du crâne et de la ceinture scapulaire en vue latérale gauche d'après l'holotype N° KUVP 295.

met de la tête, juste à côté du supraoccipital. Un petit morceau de la partie condylaire du basioccipital s'observe du côté droit du crâne, en dessous de quelques restes d'écailles.

Les os circumorbitaires sont incomplètement conservés. L'antorbitaire, le premier infraorbitaire et le supraorbitaire sont perdus. Le deuxième infraorbitaire est conservé du côté gauche de la tête. C'est un os étroit et allongé sur lequel on observe le canal sensoriel infraorbitaire qui émet plusieurs courts canalicules ventraux. Les trois infraorbitaires postérieurs, très vastes, sont présents des deux côtés du crâne. Du côté droit de la tête, le cinquième infraorbitaire est brisé en deux gros fragments dont le postérieur est venu chevaucher l'operculaire. La partie ventrale d'un petit dermosphénotique étroit est présente du côté gauche de la tête.

La mâchoire supérieure manque. Le maxillaire que JORDAN (1924 : 227) décrit est, en fait, l'entoptérygoïde droit. La mandibule est longue, moyennement haute et dépourvue de processus coronoïde clairement marqué. Le bord oral du dentaire porte une large plage denticulée qui ne se voit pratiquement pas en vue externe mais qui déborde largement sur la face interne de l'os. Au niveau de l'hémi-mandibule droite, l'angulaire et le rétroarticulaire sont fusionnés et l'os ainsi composé montre un processus postarticulaire bien développé. Sur l'hémi-mandibule gauche, au contraire, la région du rétroarticulaire a été brisée et séparée de l'angulaire suite aux aléas de la fossilisation, donnant ainsi l'impression que ces deux os sont distincts. Il n'en est rien. L'observation de la pièce montre clairement qu'il s'agit d'une brisure et non pas d'une suture entre deux os. L'articulaire est volumineux et autogène. On ne distingue pas l'ouverture postérieure du canal sensoriel mandibulaire sur la face externe de la mandibule, cette ouverture se situant sur la face interne, comme chez tous les Tselfatiiformes (NELSON, 1973 : fig. 2D, 5D, 6B ; TAVERNE, 2000a : fig. 8).

Le palatin n'est pas conservé. L'entoptérygoïde gauche est long, large et sa face interne est complètement couverte de petits puits, restes de l'implantation des denticu-

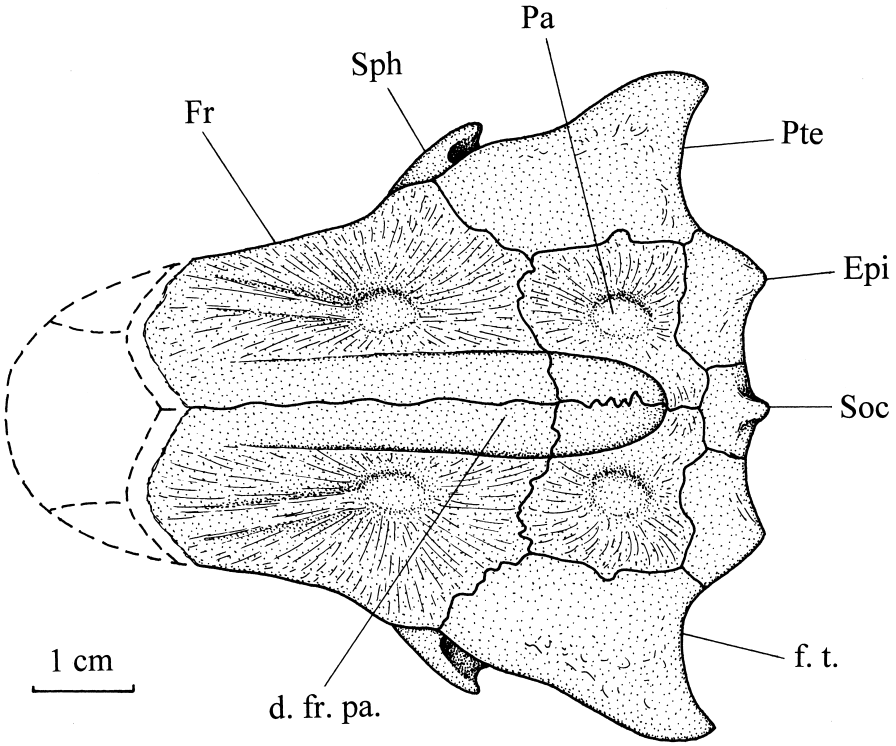

Fig. 4. – *Luxilites striolatus* JORDAN, 1924. Reconstitution du neurocrâne en vue dorsale d'après l'holotype N° KUVP 295.

les perdus suite à la fossilisation. L'ectoptérygoïde est plus étroit et complètement édenté. Seule la partie la plus ventrale du métaptérygoïde est visible ; le sommet de l'os est couvert par le troisième infraorbitaire. Le carré est triangulaire, garni d'un fort condyle articulaire pour la mandibule et son processus quadrato-jugal est situé en arrière du corps de l'os et non pas parallèlement à ce dernier.

La série operculaire est presque complète. Le préoperculaire est vaste, très large dans sa partie basale. Ses deux branches sont longues et de longueur à peu près égale. L'operculaire est grand et de forme vaguement ovale. Le sous-operculaire est long et large. L'interoperculaire n'est pas visible. Les onze derniers rayons branchiostèges sont bien conservés et l'on observe encore des petits débris de cinq rayons antérieurs. Il y avait donc seize rayons branchiostèges au total. Les premiers rayons sont étroits mais ils s'élargissent progressivement et la largeur du dernier de la série vaut presque la moitié de celle du sous-operculaire. Tous ces rayons sont contigus et forment ainsi un volet branchiostège complet.

Le squelette hyoïdeo-branchial (Fig. 1, 2)

L'hyomandibulaire est élargi dorsalement dans sa zone articulaire avec le neurocrâne et se rétrécit ventralement en une forte tige osseuse. Le *processus opercularis* est large et très allongé. Le symplectique est petit. Les hypohyaux dorsal et ventral gauches sont gros. On observe aussi de tout petit débris des cératohyaux antérieurs gauche et droit ainsi que de l'urohyal long et étroit. Les autres pièces de l'arc hyoïdien ne sont pas connues.

Le dermobasihyal et la partie antérieure du dermobasibranchial des trois premiers arcs sont visibles du côté droit du crâne. Ce sont des pièces larges et de forme plus ou moins rectangulaire. La surface supérieure de ces deux os est couverte de petits puits, traces de l'implantation de denticules aujourd'hui perdus. Cette surface est à peu près plate au niveau du dermobasihyal et devient très légèrement convexe sur le dermobasibranchial. On se rappelle que la condition primitive chez les Tselfatiiformes est d'avoir la plaque denticulée linguale composée de trois pièces : le dermobasihyal (= dermentoglosse), le dermobasibranchial des trois premiers arcs et le dermobasibranchial du quatrième arc (HAY, 1903 : fig. 21 ; NELSON, 1973 : fig. 6D ; TAVERNE, 2000b : fig. 10 C, D). Compte tenu des autres traits ostéologiques archaïques conservés par *Luxilites* (voir discussion), il est possible que son squelette branchial comportait également les trois plaques denticulées en question. Du côté gauche du crâne, on remarque encore un petit fragment de l'hypobranchial et presque tout le cératobranchial du premier arc allongé, fin et partiellement couvert par le préoperculaire. Rien d'autre n'est connu du squelette branchial.

Les ceintures (Fig. 3)

La ceinture scapulaire est complète mais il n'y a pas de postcleithrum. Le posttemporal est une vaste plaque osseuse triangulaire. L'hypercleithrum (= supracleithrum)

est long et large. Le cleithrum est bien développé, avec une courte branche dorsale et une branche ventrale longue et orientée obliquement. La surface externe du cleithrum est ornementée. La scapula (= hypercoracoïde) est petite. Le coracoïde (= hypocoracoïde) est long et étroit, moins long cependant que la branche ventrale du cleithrum. Il y a quatre petits ptérygophores pectoraux ossifiés. Seule la base de la nageoire pectorale est conservée. Cette nageoire est insérée haut sur le flanc et comporte 14 rayons et non pas 10 comme le signale JORDAN (1924: 227). En fait, dix rayons sont en effet visibles à l'attache des ptérygophores mais les débris de quatre autres s'observent un peu plus bas. Le premier rayon de la série est plus épais que les suivants. Il n'y a pas de petite épine initiale impaire..

On observe une masse très importante de ligaments ossifiés entre l'arrière de la mandibule et les rayons branchiostèges. FIELITZ & SHIMADA (1999) pensent que ces ligaments, présents chez presque tous les Tselfatiiformes mais inconnus chez les autres téléostéens, relient les barres hyoïdiennes aux cleithra et dérivent de l'ossification des muscles protracteurs hyoïdiens.

La ceinture et les nageoires pelviennes ne sont pas conservées.

Le squelette axial

Quelques restes des premières vertèbres subsistent mais elles sont très mal conservées. Ces vertèbres sont plus hautes que longues, peu resserrées en leur milieu et plutôt petites par rapport à la taille de la tête du poisson

Les huit premières paires de côtes sont présentes. Elles sont longues, fortes et descendent jusqu'au bord ventral du *situs viscerum*.

L'écaillure

Seuls des fragments d'écailles subsistent. Elles sont grandes, cycloïdes et couvertes de fins *circuli* à disposition plus ou moins horizontale. On y observe des anneaux de croissance concentriques mais pas de *radii.*

DISCUSSION

Luxilites striolatus au sein des téléostéens

Le large toit crânien assez plat, les grands pariétaux jointifs, le septum interorbitaire osseux, les dents minuscules et disposées en plages, l'aspect ponctué de la surface des os dentés, la fusion de l'angulaire et du rétroarticulaire, l'articulaire autogène, la forme du supratemporal, la forme du cleithrum, l'insertion haute de la nageoire pectorale, la présence de ligaments ossifiés mandibulo-cleithraux sont autant de caractères qui, associés, attestent sans doute possible que *Luxilites striolatus* est un membre de l'ordre des Tselfatiiformes.

La diagnose amendée de *Luxilites striolatus*

La présente étude permet de définir *Luxilites striolatus* d'une manière nettement plus précise que ne l'avait fait JORDAN (1924: 226-227). La nouvelle diagnose de l'espèce devient donc: tselfatiiforme de taille moyenne; toit crânien triangulaire, large, nettement plus large au niveau des ptérotiques que des frontaux et presque plat; grands pariétaux jointifs, encadrés latéralement par les ptérotiques; dépression fronto-pariétale longue, étroite et cernée par un petit rebord osseux; portion dorsale du ptérotique vaste; supraoccipital petit et garni d'une crête peu marquée; fosse temporale ouverte à l'arrière du neurocrâne et couverte latéralement par le ptérotique; deuxième infraorbitaire étroit et allongé; les trois infraorbitaires postérieurs très développés; dermosphénotique petit; septum interorbitaire osseux complet formé par l'orbitosphénoïde, les pleurosphénoïdes et le parasphénoïde; région denticulée du parasphénoïde large, plate et de forme rectangulaire; supratemporal réduit à sa partie latérale mais conservant un vaste composant membranodermique; entoptérygoïde denticulé sur toute sa surface; ectoptérygïde édenté; carré bien développé avec le processus quadrato-jugal situé en arrière du corps de l'os; préoperculaire à branches bien développées et de longueur presque égale ; mandibule allongée et modérément élevée, dépourvue de processus coronoïde individualisé; dentaire garni d'une plage de denticules orientée vers l'intérieur de la bouche; angulaire et rétroarticulaire fusionnés; articulaire autogène; operculaire et sous-operculaire bien développés; une quinzaine de rayons branchiostèges de grande taille et accolés les uns aux autres; dermobasibranchial et dermobasibranchial des trois premiers arcs denticulés et formant une plaque linguale vaguement rectangulaire; hyomandibulaire large dorsalement, prolongé par une forte tige ventrale et garni d'un fort *processus opercularis*; ligaments ossifiés mandibulo-cleithraux présents; posttemporal grand et de forme triangulaire; hypercleithrum large et allongé; cleithrum à longue branche ventrale; nageoire pectorale insérée haut sur les flancs et comptant 14 rayons; vertèbres plus hautes que larges; grandes écailles cycloïdes couvertes de fins *circuli* à disposition horizontale et d'anneaux de croissance concentriques.

Luxilites étant monospécifique, la nouvelle diagnose de l'espèce *L. striolatus* vaut également pour le genre.

Luxilites au sein des Tselfatiiformes

Luxilites appartient manifestement au groupe majoritaire des Tselfatiiformes à toit crânien plus ou moins plat et non pas à celui minoritaire des formes spécialisées où le toit crânien s'incurve fortement le long de la ligne médiane comme *Tselfatia* ou *Dixonanogmius* (TAVERNE, 1983, 2000a, c).

Dans ce premier groupe, *Luxilites* se montre particulièrement primitif puisqu'il conserve un préoperculaire dont les deux branches sont bien développées et de longueurs

presque égales comme chez *Bananogmius* (WOODWARD, 1923 : fig. A ; FIELITZ & SHIMADA, 1999 : fig. 2 ; TAVERNE, 2001b : fig. 2) et *Niobrara* (TAVERNE, 2001a : fig. 3, 4). Chez les autres Tselfatiiformes, la branche ventrale du préoperculaire est devenue beaucoup plus courte que la branche dorsale (LOOMIS, 1900 : fig. 6 ; ARAMBOURG, 1954 : fig. 66 ; BARDACK, 1965 : fig. 2 ; BARDACK & TELLER-MARSHALL, 1980 : fig. 4 ; TAVERNE, 1983 : fig. 2, 2000b : fig. 1, 9, 2000c : fig. 3, sous presse a : fig. 1). Dans un seul cas, celui de *Zanclites xenurus*, la branche ventrale du préoperculaire s'est, au contraire, considérablement allongée, tandis que la dorsale se raccourcit (TAVERNE, 1999 : fig. 2).

L. striolatus offre un autre trait qui le rapproche de *Bananogmius* et *Niobrara*, l'ectoptérygoïde édenté. C'est là l'une des deux synapomorphies qui lient ces deux derniers genres (TAVERNE, 2001b : fig. 5, 10, 11) et les distinguent des autres Tselfatiiformes qui possèdent un ectoptérygoïde denticulé (LOOMIS, 1900 : pl. XXII, fig. 6, où l'ectoptérygoïde est erronément appelé quatrième cératobranchial ; APPLEGATE, 1970 : fig. 196E , G ; TAVERNE, 1983 : fig. 4, 2000b : fig. 8C, 2000c : fig. 5, sous presse a : fig. 4). Chez *Zanclites xenurus*, l'ectoptérygoïde est également édenté mais c'est suite à sa transformation en une épaisse tige osseuse destinée à soutenir un gros palatin (TAVERNE, 1999 : fig. 2), ce qui est différent des cas de *Luxilites*, *Bananogmius* et *Niobrara*.

Il paraît donc logique de considérer qu'au sein des Tselfatiiformes, *Luxilites* est plus particulièrement apparenté à *Bananogmius* et *Niobrara*, petit groupe de Plethodidae primitifs auquel s'ajoute encore le genre *Syntegmodus* ainsi que TAVERNE (sous presse b) l'a montré.

Dans ce groupe, *Luxilites* semble plus proche de *Bananogmius* que de *Niobrara* ou de *Syntegmodus.* Il ne présente, en effet, aucune des autapomorphies de *Niobrara*, c'est-à-dire l'avancée du frontal le long du bord latéral du pariétal et le rejet concomitant des ptérotiques en arrière des pariétaux, la dépression fronto-pariétale courte, large et triangulaire, le processus aliforme de l'hypercleithrum ainsi que l'excroissance postérieure du cleithrum. Il n'offre pas non plus les autapomorphies de *Syntegmodus*, à savoir le contact réduit du ptérotique et du pariétal, la hauteur très importante du septum interorbitaire et le grand développement de l'aile médio-dorsale du parasphénoïde. En revanche, il ne diffère de *Bananogmius* que par le net élargissement du toit crânien au niveau des ptérotiques et par la forme triangulaire de ce toit qui en résulte. Ce n'est pas une différence énorme mais elle est suffisante pour justifier le statut générique particulier de *Luxilites*.

En fait, par la forme de son toit crânien, *Luxilites* s'intercale dans une série évolutive continue qui va de *Bananogmius* à *Niobrara* en passant par *Syntegmodus* (Fig. 5). Chez *Bananogmius*, le toit crânien est aussi large

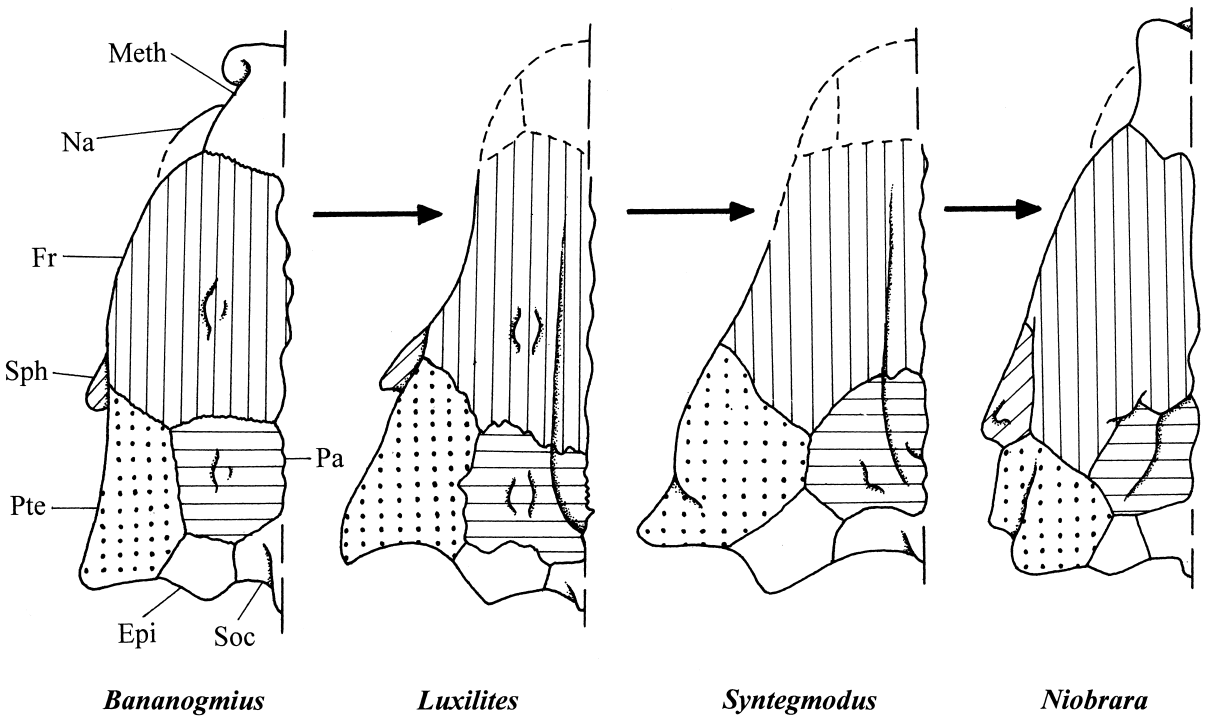

Fig. 5. – Représentation semi-schématique de l'évolution du toit crânien qui va de *Bananogmius aratus* (COPE, 1877) à *Niobrara encarsia* JORDAN, 1924 en passant par *Luxilites striolatus* JORDAN, 1924 et *Syntegmodus altus* LOOMIS, 1900. On y remarque la forme triangulaire acquise par le toit crânien suite à l'élargissement du ptérotique à partir de *L. striolatus*, puis la diminution du contact entre le ptérotique et le pariétal ainsi que la légère avancée postérieure concomitante du frontal chez *S. altus* et enfin le retrait du ptérotique en arrière du pariétal dont le bord latéral est longé par le frontal qui s'avance fortement vers l'arrière chez *N. encarsia*. Frontal hachuré verticalement, pariétal hachuré horizontalement, sphénotique hachuré obliquement et ptérotique pointillé.

au niveau des frontaux que des ptérotiques et le ptérotique borde latéralement le pariétal. Chez *Luxilites*, la partie dorsale (= dermoptérotique) du ptérotique s'élargit considérablement, rendant le toit crânien nettement plus large à ce niveau qu'à celui des frontaux et lui donnant une forme triangulaire. Le ptérotique, dans ce cas, borde toujours latéralement le pariétal. Chez *Syntegmodus*, la forme générale du toit crânien est également triangulaire car l'élargissement des ptérotiques demeure. Mais la ligne de suture entre le ptérotique et le pariétal se réduit et n'intéresse plus que l'arrière du bord latéral du pariétal, tandis qu'une avancée postérieure du frontal vient longer la partie antérieure du bord latéral du frontal. Chez *Niobrara*, enfin, cette avancée du frontal se poursuit le long du bord latéral du pariétal et le ptérotique est presque entièrement rejeté en arrière du pariétal.

L'intégration de *Luxilites* dans ce groupe de quatre genres encadré par *Bananogmius* et *Niobrara* implique probablement que ce poisson montrait aussi, outre la plésiomorphie du préoperculaire à branches longues et subégales et l'apomorphie de l'ectoptérygoïde édenté, les trois autres plésiomorphies et l'autre apomorphie qui caractérisent les deux genres précités quoique la fossilisation n'ait pas permis la conservation des os et des structures concernées chez notre fossile. Ces trois autres plésiomorphies sont la conservation d'une ceinture et de nageoires pelviennes bien développées, d'un complexe hémaxanal de type I (Blot, 1968) et d'un arc hémal préural 1 rudimentaire. Rappelons que, chez les autres Tselfatiiformes où elle est connue, la ceinture pelvienne est atrophiée (Bardack, 1965 : fig. 1 ; Patterson, 1967 : fig. 6 ; Bardack & Teller-Marshall, 1980 : fig. 4, 5 ; Taverne, 1983 : fig. 1, 1999 : fig. 1a, b), que le complexe hémaxanal est de type 3 (Arambourg, 1954 : pl. XIV, fig. 1, 2, pl. XV, fig. 1 ; Patterson, 1967 : fig. 6 ; Bardack & Teller-Marshall, 1980 : fig. 2, 4, 5 ; Taverne, 1983 : fig. 1, 1999 : fig. 1a, b) et que l'arc hémal préural 1 a disparu (Taverne, 1975 : fig. 3, 1983 : fig. 5, 1999 : fig. 5, 2000a : fig. 2). Quant à l'autre apomorphie, il s'agit du coin postéro-ventral de l'antorbitaire qui s'étire en un très long processus pointu. Ce processus manque ou est très peu développé chez la plupart des autres Tselfatiiformes dont les os circumorbitaires ont été figurés (Loomis, 1900 : pl. XXI, fig. 2 ; Stewart, 1900 : pl. LXIV ; Bardack, 1965 : fig. 2 ; Taverne, 1983 : fig. 2, 2000b : fig. 1, 4, 9).

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The reproductive behaviour of the African catfish *Heterobranchus longifilis* (Siluriformes, Clariidae) in an aquarium – Preliminary results

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ABSTRACT. Aggressive and spawning behaviours of *Heterobranchus longifilis* were observed in aquaria under controlled environmental conditions. Reproductive behaviour was induced in 11 groups of two to six fish by means of a single injection of carp pituitary extract . Behavioural data were recorded by direct observation or with a camera system. Sexual behaviour was observed in six groups, but egg release was observed in only one, the responses to hormonal stimulation differing strongly. The complete spawning sequence was observed, including aggressive (lateral display, circling display, biting, and rapid swimming head against head) and spawning behaviours (swimming head against head, male folding his body around the head of the female, spawning and egg release, egg scattering).

KEY WORDS: catfish, reproductive behaviour.

INTRODUCTION

Some species of African catfish of the Clariidae family, such as *Clarias gariepinus* (Burchell, 1822), have been abundantly studied in most aspects of their biology (TEUGELS, 1982). The knowledge gained has been used to develop farming of these species (LEGENDRE & PROTEAU, 1996; MICHA, 1972).

Heterobranchus longifilis Valenciennes, 1840 is a member of the Clariidae family of great interest for aquaculture because it has one of the fastest growth rates among African catfish (LEGENDRE et al., 1992). Multidisciplinary research on this species has recently developed in Belgium. It has focused on morphology (ADRIAENS & VERRAES, 1994; VANDEWALLE et al., 1997), systematics (TEUGELS et al. 1990), genetics and phylogeny (TEUGELS *et al.,* 1992), ecology and behaviour (BARAS et al., 1998; BARAS, 1999). The reproductive cycle of *Heterobranchus longifilis* (FREUND et al., 1995) and the associated histological changes (NUNEZ RODRIGUEZ et al., 1995) have been studied, but the reproductive behaviour of this species has not been described in detail, in contrast to that of *Clarias gariepinus* (BRUTON, 1979; VAN DER WAAL, 1974). Here we focus on the various sexual behaviours of *Heterobranchus* longifilis and their temporal succession during mating in a large aquarium (5 m^3).

MATERIAL AND METHODS

The study was carried out between January and July 1997 in the Laboratory of Fish Ethology of the University of Liège, Belgium (PONCIN & RUWET, 1996). The fish, whose characteristics are listed in Table I, were from the Laboratory of Fish Demography and Aquaculture of the Tihange station where they were born and reared in captivity until reaching sexual maturity (BARAS et al., 1998).

The experiments were carried out in a 5-m^3 aquarium (3.5 m x 1.3 m x 1.2 m) connected to a 750-l biological filter equipped with two circulating pumps (2 x 3m^3/h). The lighting (two 70-W mercury vapour lamps) was maintained constant 12L/12D (7:00 AM – 19:00 PM). During mating, however, the lamps remained on even if mating continued into the night. The temperature of the water was maintained at 26°C. The bottom of the experimental aquarium was covered with artificial plants.

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Outside the experimental period, the fish were kept in a 1-m^3 tank with a separate filter.

Daily, the fish were fed pellets (Trouvit "Tilapia 4.5", 38% protein) and fresh fish ad libitum.

TABLE 1

Characteristics (total length and weight) of the fish of the 11 experimental batches.

Batch n°	Males		Females	
	Length (mm)	Weight (g)	Length (mm)	Weight (g)
1	320	234	350	290
	420	688	440	740
2	420	682	520	1437
	450	762		
3	485	841	510	1196
4	460	784	530	1449
5	530	1187	600	1882
6	480	741	580	1748
7	the same fish as in batch n° 6			
8	340	236	360	340
	390	365	360	343
	340	237	340	303
9	330	233	350	363
	390	373	400	500
	380	360	370	427
10	the same fish as in batch n° 9			
11	390	383	390	484
	410	432	380	455
	330	234	460	825

As we were unable to obtain spontaneous matings, 17 selected specimens received an injection of carp pituitary extract (6 mg/kg for females; 2 mg/kg for males) as described by Woynarovich & Horvath (1981), so as to induce and synchronise spawning. Eleven batches of two to six fish were thus hypophysised (Table 1). Some pituitary extract injections were done in the morning (between 4:00 AM and 9:00 AM) and others at night (between 19:00 and 0:30), as we did not know the response time of the fish.

The behavioural data (sexual and aggressive behaviours) were recorded with a video camera controlled from a room adjacent to the room housing the aquariums (Fig. 1). Females were identified from the males because they exhibited stoutness.

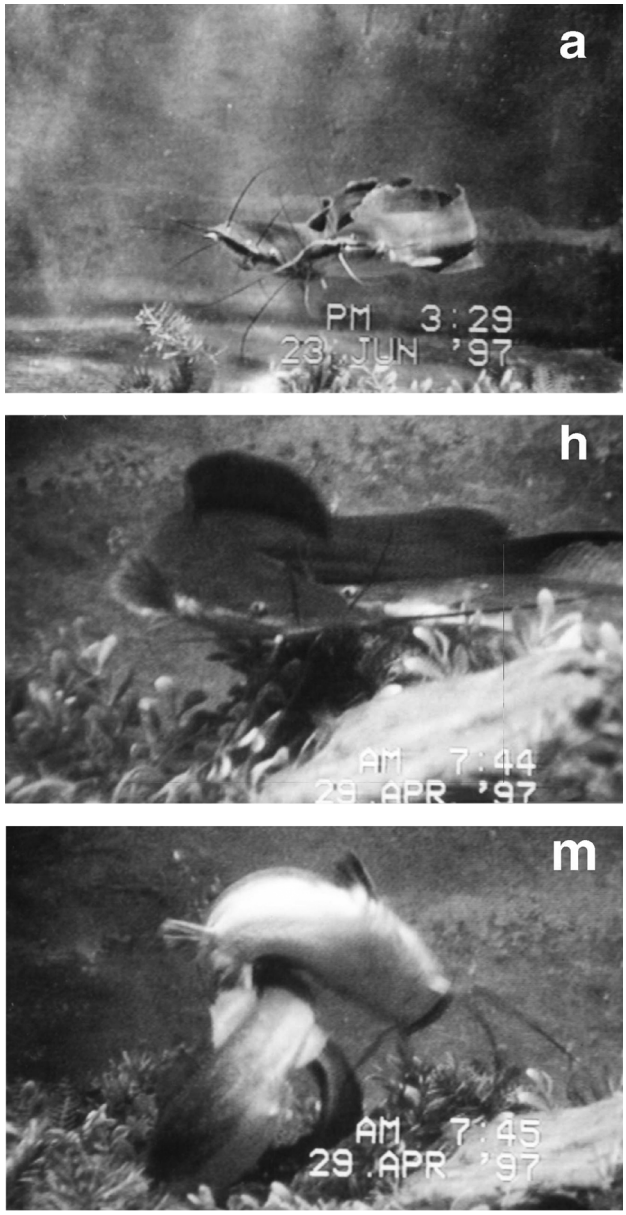

Fig. 1. – Aggressive and spawning behaviour of *H. longifilis* in aquarium. "a", "h" and "m" are related to the same items of Fig. 2. See the text for explanations.

RESULTS

Aggressive behaviours

Various aggressive behaviours were observed in *H. longifilis* with or without hormonal treatment. Both sexes displayed them indifferently. These behaviours were (Fig. 2): lateral display (a), during which both partners advanced slowly side by side with all their fins spread out, executing swimming movements with a seemingly exaggerated amplitude and giving each other powerful tail blows; head-to-tail display (b), a variant of the previous behaviour; carried out by two fish passing each other slowly, it was often followed by a circular motion (c) during which the fish, head to tail, circled in place, effecting a lateral display and biting (d) each other on the tail and side until the dominated individual fled; rapid swimming of the two opponents head against head (e). Neither the aggressive nor the sexual behaviours were associated with any actual colour patterns (the coloration was uniform), but we did observe a darkening of colour in dominant males and during male sexual displays. Dominated individuals were lighter in colour than dominant fish. These changes were rather slight.

Sexual display

The first sexual behaviours began about three hours post-injection (3 hours at 26°C =78 degree-hours). First we observed following (f) (Fig. 2), during which the male accompanied the female, swimming with his head against her sides and abdomen. This following was interspersed with periods of inactivity or solitary swimming. Male and

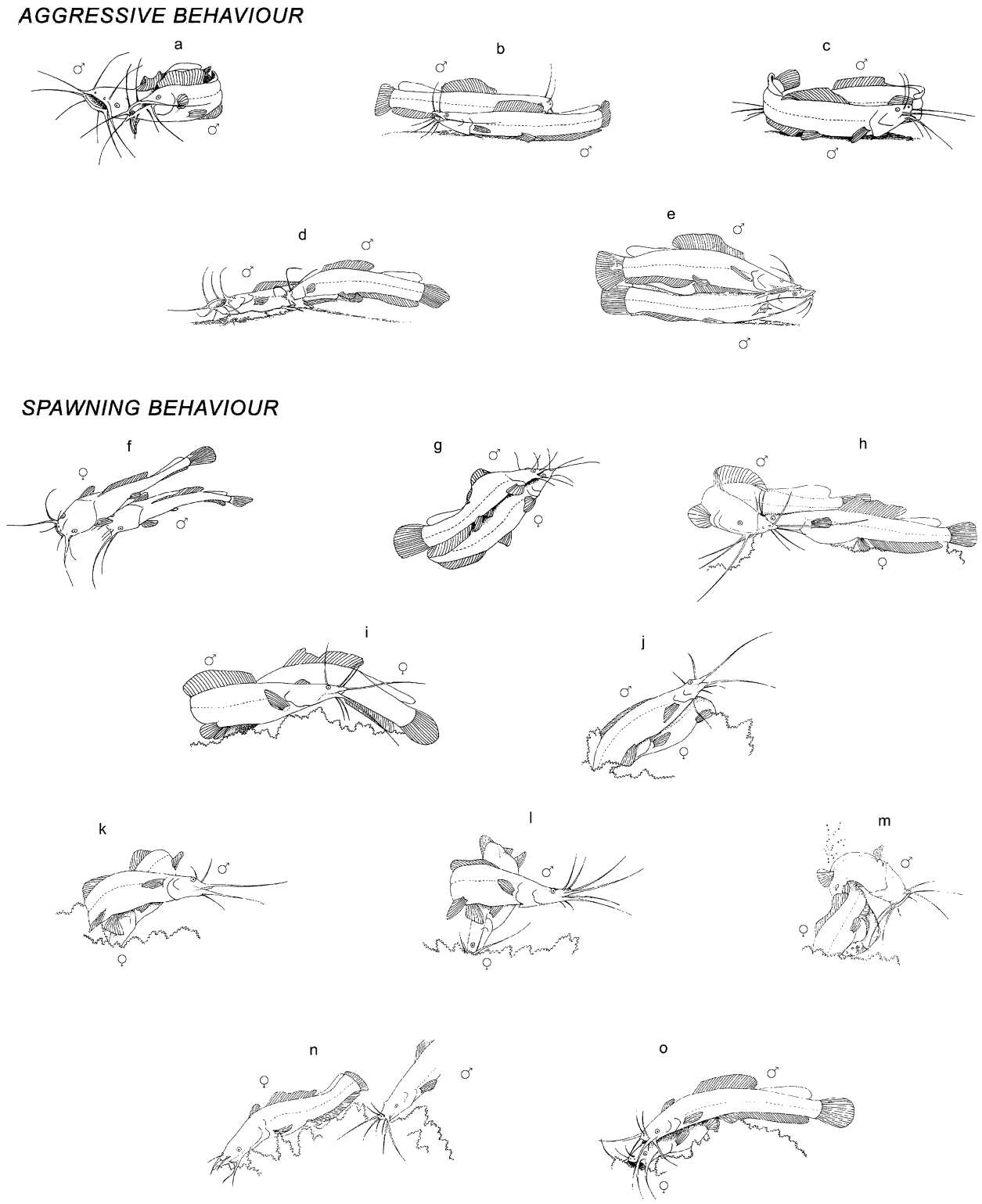

Fig. 2. – Aggressive and spawning behaviours in *Heterobranchus longifilis*: a, b: lateral display; c: circling display; d: biting; e and g: swimming head against head; f: male following female; h and i: male folds his body around the head of female; j, k, l, m: spawning act in which male folds his body around the body of female. Ova, sperm, and bubbles are released; n and o: female pushes her head into the substrate (vegetation) and beats her tail vigorously, mixing sperm and ova and distributing them over the substrate. The behaviours 'a', 'h' & 'm' are also illustrated by the plates with similar references.

female next swam head against head (g) while the male, located above his partner during a slow, calm swim, pressed his head against hers. This behaviour was similar to aggressive behaviour (e), rapid swimming head against head. When the female was ready to spawn, she effected a few burrowing movements in the artificial aquatic plants. The male, his body trembling, then encircled the female's head (Fig. 2: h, i, and j). He tightened his body around his partner's head while exerting pressure on her abdomen until the pair was immobilised by the surrounding vegetation. This amplexus was maintained for about ten seconds. In most batches observed, sexual display did not go beyond this stage. There was only one 'batch' of fish that spawned completely. When mating did continue, the male further tightened his grip and slid along the female's back as she positioned herself almost vertically, her head buried in the substrate (k and l). Then, when the partners' genital papillae were close to each other, she released a batch of eggs often followed by few bubbles of gas escaping from the genital pore or from the female's gills (m); presumably the male released his sperm at this time, but this was not seen. The male then moved away briefly, as the female, jaws anchored to the substrate, beat her tail to disperse the eggs (n and o). The eggs yielded larvae a few days later. Detailed monitoring of the frequency and duration of the main sexual behaviours was carried out on the only pair (in batch n°8) where spawning was observed (Fig. 3). Noteworthy was the high frequency of pursuit behaviour and enfolding behaviour associated with spawning acts.

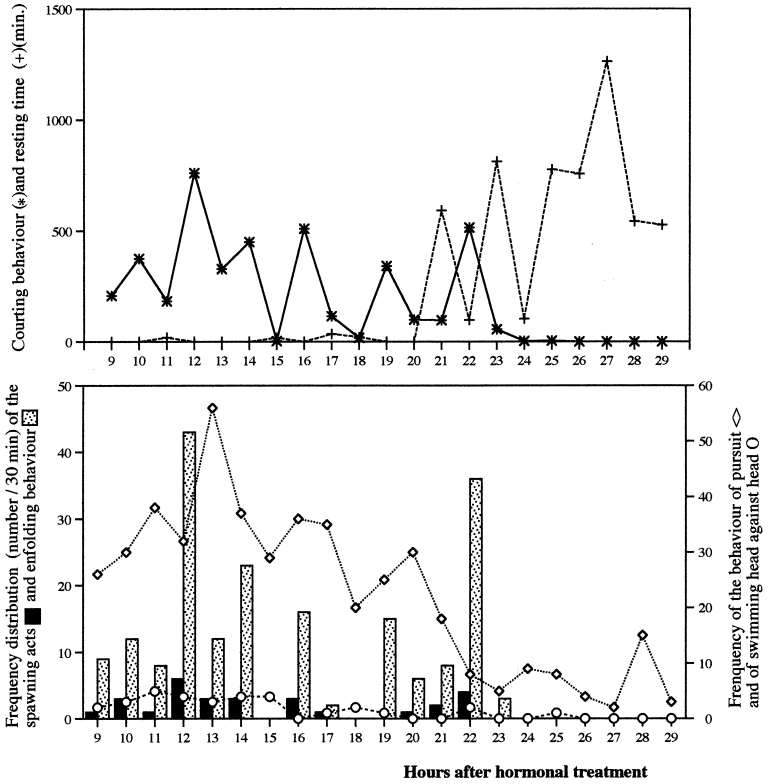

Fig. 3. – Duration of courtship and resting behaviours and frequency distribution of spawning acts, enfolding of the female by the male and pursuit., in a pair of *Heterobranchus longifilis* specimens (batch n° 8, in which egg release was observed).

DISCUSSION

This is the first detailed description of reproductive behaviour in *Heterobranchus longifilis.* This behaviour is quite similar to that of *Clarias gariepinus*, described by BRUTON (1979) and VAN DER WAAL (1974), and *Heteropneustes fossilis* (Heteropneustidae; a family closely related to the Clariidae) (ROY & PAL, 1986). It should be mentioned, however, that swimming head against head was not described by BRUTON (1979) for *Clarias gariepinus* in natural conditions (Lake Sibaya). Despite these differences, given the sympatric distribution of *H. longifilis* and *Cl. gariepinus* in some regions of Africa (MICHA, 1972) and their use of comparable spawning sites (flooded grounds) (GOSSE, 1963), it seems that extremely rare natural hybridisation might occur between these species (TEUGELS, pers. comm.). Moreover, by artificial fertilisation it is possible to obtain hybrids between Clariidae species (NA-NAKORN, 1995), and some hybrids are fertile (LEGENDRE et al., 1992; TEUGELS et al., 1992). However, AGUIGWO (1993) failed to obtain any hybrids of *H. longifilis* and *Clarias albopunctatus*, two genetically and morphologically distant species (AGNÈSE & TEUGELS, 2001). Factors such as morphological features, sound production, and pheromones could be important in bringing male and female catfish together (VAN WEERD, 1990).

Hormonal induction of spawning has been used previously to observe the reproductive behaviour of several fish species when spontaneous mating is hard to obtain in captivity. The similarity between the induced behaviours and those reported in the field (e.g. in *Clarias gariepinus*; BRUTON, 1979; VAN DER WAAL, 1974) indicated that this method does not influence qualitatively the behaviours expressed. To obtain spontaneous spawning (without hormonal stimulation) would require better knowledge of the role of environmental factors (water physico-chemistry, pluviosity, water-level variations,…) in controlling the seasonality of *H. longifilis* reproduction. This has already been investigated in *Clarias gariepinus* (HOGENDOORN, 1979; RICHTER et al., 1987; VAN WEERD, 1990).

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The invasive *Corbicula* species (Bivalvia, Corbiculidae) and the sediment quality in Flanders, Belgium

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ABSTRACT. *Corbicula species*, *C. fluminea* and *C. fluminalis* (Bivalvia, Corbiculidae) started to invade the Belgian section of the river Meuse, and some of the connected canals in the early 1990s. During 1999 and 2000, sediment samples from 33 watercourses in Flanders, Belgium were collected and analysed. The clam was found in six watercourses. *C. fluminea* was present in all of the six watercourses, whereas *C. fluminalis* was found only in three canals. Overall, the density of *Corbicula* species averaged around 200 individuals/m^2, except for four places connected with the river Meuse where the density was higher than 1000 individuals/m^2. In 63% of the invaded sites, the clam was the most abundant taxon. Most of the sites (89%) colonised by *Corbicula* species were (slightly to heavily) polluted by organic contaminants and heavy metals. However, no correlation between the clam density or proportion and the quality of the sediment was observed. Including *Corbicula* species in the Biotic Sediment Index calculation altered biological sediment quality classification in 52% of the cases. The results show that colonisation of *Corbicula* species in Belgium is continuing to expand.

KEY WORDS: *Corbicula*, invasion, abundance, sediment quality.

INTRODUCTION

Corbicula species (Bivalvia, Corbiculidae) are native to Asia, Africa and Australia where they form an important component of the benthic community. In the 1930s, the clam was introduced in the United States and rapidly spread across the country, causing considerable damage to hydro installations and becoming one of the most important molluscan pest species ever introduced (McMahon, 1983). In the early 1980s the presence of *Corbicula* species in some of the European rivers, e.g. Dordogne - France; Tajo - Portugal; Miño - Spain, Portugal (Araujo et al., 1993) was evidence of the invasion of the clam in Europe. In the beginning of the 1990s, successfully established populations of the clam were recorded in Germany and the Netherlands (bij de Vaate & Greijdanus-Klaas, 1990; Haesloop, 1992). In the same period, two species *C. fluminea* (Müller, 1974) and *C. fluminalis* (Müller, 1974) were also found in the Belgian section of the river Meuse and in some of the large connecting canals (Swinnen et al., 1998).

Invasive species of *Corbicula* originally attracted attention as an economically important fouling organism in irrigation and drainage canals, water distribution and industrial water use systems. However, its ecological influence on natural systems has recently become of major concern. In comparison with most freshwater bivalves, the clam is reported to have a high growth rate and a short life span. It also appears to have a relatively high annual production and high filtering rate. Such characteristics have led to speculation that the clam has the capacity to alter the trophic and nutrient dynamics of aquatic systems and to displace native bivalves (Britton & Morton, 1982; Marsh, 1985; Isom, 1986; Ricciardi et al., 1995; Strayer, 1999). On the other hand, some investigators have claimed, on the basis of field studies, that the potential influence of *Corbicula* species on aquatic ecosystems does not appear to be dramatic, and thus believed that the clam would not be a threat to native bivalves (Stites et al., 1995; Miller & Payne, 1994, 1998; Dillon, 2000). Despite different opinions on the ecological effect of *Corbicula* species, the clam is considered as a biological pollutant and the proportion of individuals of *Corbicula* has been recommended as one of the metrics in biological assessment of water quality (Kerans & Karr, 1994; Carlisle & Clements, 1999; US EPA, 1999). The present study aims at contributing to the understanding of the ecological and environmental aspect of *Corbicula* species in Belgian watercourses. More specifically it set out

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to investigate whether or not there is a relationship between the distribution of the clam and the water/sediment quality.

MATERIAL AND METHODS

Sampling

The present study was conducted within the framework of an AMINAL project, which was set up to assess the sediment quality of all watercourses in Flanders, Belgium. The assessment was based on a combination of physico-chemical, ecotoxicological and biological data, i.e. a TRIAD assessment approach (Ministry of Flemish Community, 1998). For this purpose, during the spring of 1999 and 2000, 180 bottom sediment samples were collected from 33 watercourses. The sampling was performed as outlined in the standard protocol for sediment sampling (Ministry of Flemish Community, 1998). A Van Veen grab (surface 250 and 500 cm^2) was used to retrieve the sediment samples from depths varying between 1 and 5m. On arrival in the laboratory, samples were sieved over a pile of metal sieves (1 cm – 1 mm – 0.5 mm). Benthic macroinvertebrates including *Corbicula* species were sorted out and examined under a stereomicroscope.

Physico-chemical classification

Measurements of temperature (°C), dissolved oxygen (%), pH, and conductivity (mS/cm) of water at each sampling site were carried out *in situ*. The physico-chemical parameters measured for assessing the sediment are the following:

1. Clay (%) and organic matter (%)
2. Nonpolar hydrocarbons
3. Extractable organohalogens
4. Sum of the pesticides
5. Sum of 7 PCBs
6. Sum of 6 Borneff PAHs
7. Heavy metals: Cd, Cr, Cu, Ni, Pb, Hg, Zn and As

After being measured, each parameter was compared with its reference value, and the ratio of the measured and the reference value was calculated. All the ratios (indices) obtained were normalised by using logarithmic transformation (0<LOGINDEX<2). Samples were then classified into four classes (Table 1) and the highest class of all physico-chemical parameters was taken as the overall quality class (Ministry of Flemish Community, 1998).

TABLE 1

Sediment quality classification based on physico-chemical parameters.

Log Index	Class	Significance (compared to reference)
0 - < 0.4	**1**	Not deviating
0.4 - < 0.8	**2**	Slightly deviating
0.8 - < 1.2	**3**	Deviating
1.2 - < 2	**4**	Strongly deviating

Biological classification

The sediment quality was also assessed by means of the Biotic Sediment Index (BSI) (De Pauw & Heylen, 2001). The BSI is an adapted version of the Belgian Biotic Index (De Pauw & Vanhooren, 1983; NBN, 1984), which is based on the taxonomic diversity of the benthic macroinvertebrate community and the presence or absence of indicator taxa in a given sediment sample. The BSI score can vary between 10 and 0, corresponding with four sediment quality classes (Table 2). The biological sediment quality was assessed using two approaches: (1) calculating the BSI without considering the presence of *Corbicula* species and (2) calculating the BSI considering *Corbicula* species as a taxon in overall diversity and in the mollusc indicator group.

TABLE 2

Sediment quality classification based on the BSI.

BSI	Class	Significance
7 - 10	**1**	Good biological quality
5 - 6	**2**	Moderate biological quality
3 - 4	**3**	Bad biological quality
0 - 2	**4**	Very bad biological quality

Statistical analysis

Linear regression (Sokal & Rohlf, 2000) was used to examine the correlation between degree of pollutants in the sediments (physico-chemical classification) and (1) the densities (logarithmic transformed of the number of individuals per square meter); (2) the relative abundance (number of the clam in sample/total number of macroinvertebrates in sample) of *Corbicula* species.

RESULTS

Distribution

Corbicula species were found in 27 sampling sites of six different watercourses in Flanders, Belgium (Table 3). Besides the canals Albert and Bocholt-Herentals that were already successfully colonised by the clam (Swinnen et al., 1998), *Corbicula* species was found in the canals Zuid-Willemsvaart, Dessel-Schoten and Willebroek, and in the river Dender. The clam was present in more than 80% of the sampling sites of the canal Herentals-Bocholt, the canal Albert and the canal Zuid-Willemsvaart. In the canal Dessel-Schoten, *Corbicula* specimens were found in three out of ten sampling sites. Ten sites of the river Dender (from Appels to Geraadsbergen) were also sampled, and so far, the clam was found in only one site (Appels).

TABLE 3

Watercourses in Flanders colonised by *Corbicula* species.

Watercourses	N° of sampling sites	N° of sites inhabited by the clam	Sampling date
Canal Bocholt-Herentals	8	7	April, June 1999
Canal Zuid-Willemsvaart	10	8	June 1999
Canal Albert	8	7	May 2000
Canal Dessel-Schoten	10	3	April 1999
Canal Willebroek	5	1	May 2000
River Dender	10	1	March, April, May 2000

Likewise, of the five sampling sites (from Willebroek to Vilvoorde) in the canal Willebroek, only one site (Willebroek) was invaded by species of *Corbicula*.

Two species, i.e. *Corbicula fluminea* (Müller, 1774) and *Corbicula fluminalis* (Müller, 1774), were identified in the samples. The former species was present in all of the 27 invaded sites, while the latter one was found in one site of the canal Albert, two sites of the canal Bocholt-Herentals, and in four sites of the canal Zuid-Willemsvaart.

Density and relative abundance

Sediments of 89% of the sites in which *Corbicula* species was found were strongly deviating (44%), deviating (19%) or slightly deviating (26%) from the chemical-physical standard. Only 11% of the sites had a sediment quality not deviating from the uncontaminated references.

Densities of *Corbicula* species in canal Bocholt-Herentals, canal Albert, canal Zuid-Willemsvaart and canal Dessel-Schoten, are given in Fig. 1. In general, the densities, averaged across all sampling sites, were less than 200 clams/m^2. There were only four sites (A2, B2, B9 and C1) where the clam density reached one thousand or more specimens per square meter. The sites of the canal Willebroek and the river Dender inhabited by *C. fluminea* showed densities of 164 and 60 clams/m^2, respectively. In 63% of the invaded sites, benthic communities were dominated by the clam. Especially in site C7 (canal Albert) no macroinvertebrates were found except *Corbicula* species.

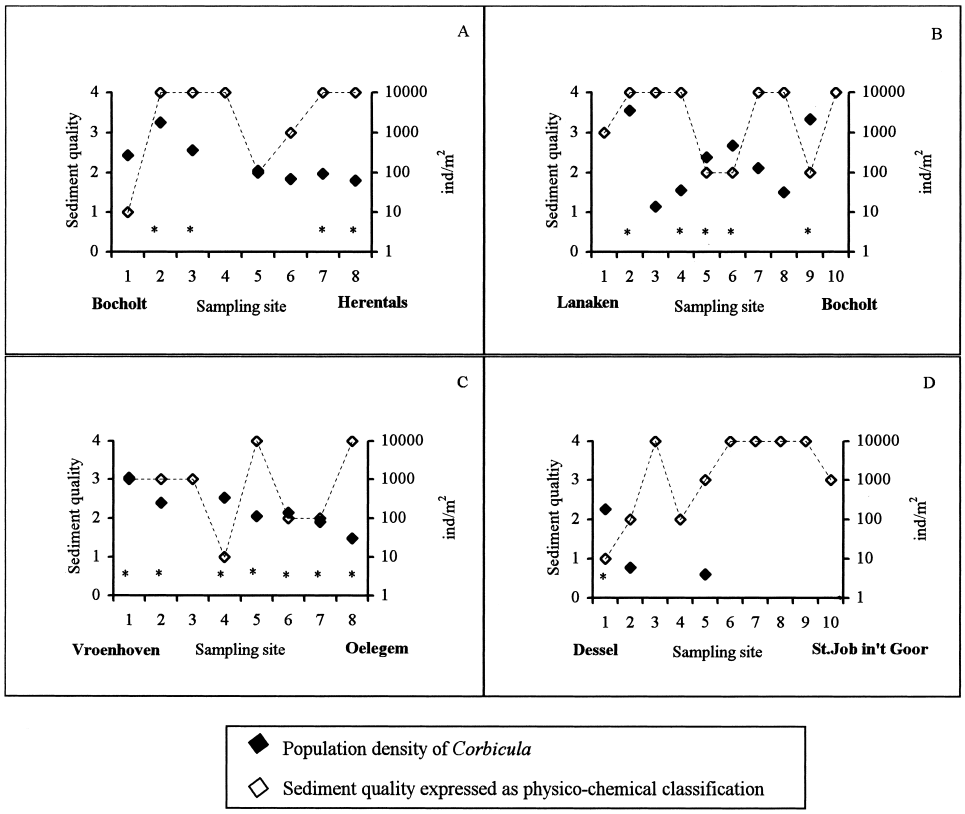

Fig. 1. - Population abundance of *Corbicula* species in (**A**): canal Bocholt-Herentals; (**B**): canal Zuid-Willemsvaart; **C**: canal Albert and **D**: canal Dessel-Schoten. *: Sites where the clam was the most abundant macroinvertebrate taxon.

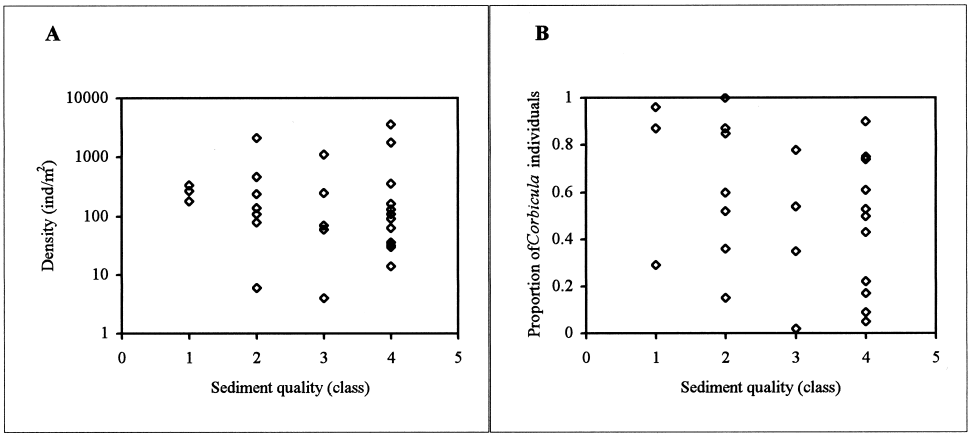

Fig. 2. – Relationship between (**A**) population densities of *Corbicula*; (**B**) proportion of *Corbicula* individuals and the sediment quality expressed as physico-chemical classification in six Flemish waters.

Fig. 2A gives the relationship between the clam density and the physico-chemical sediment quality, and shows no significant correlation ($r = 0.15$, $p>0.05$).

The ratio between the clam abundance and total macroinvertebrate abundance in the canals Bocholt-Herentals, Albert, Dessel-Schoten and Zuid-Willemsvaart ranged from 0.05 to 1 (Fig. 3). The proportion of individuals of *Corbicula* species in relation to the abundance of the whole benthic community in the canal Willebroek and the river Dender was 0.17 and 0.02, respectively. Similarly to the density, the relative abundance of the clam appeared to have no correlation ($r = 0.24$, $p>0.05$) with the physico-chemical quality index (Fig. 2B).

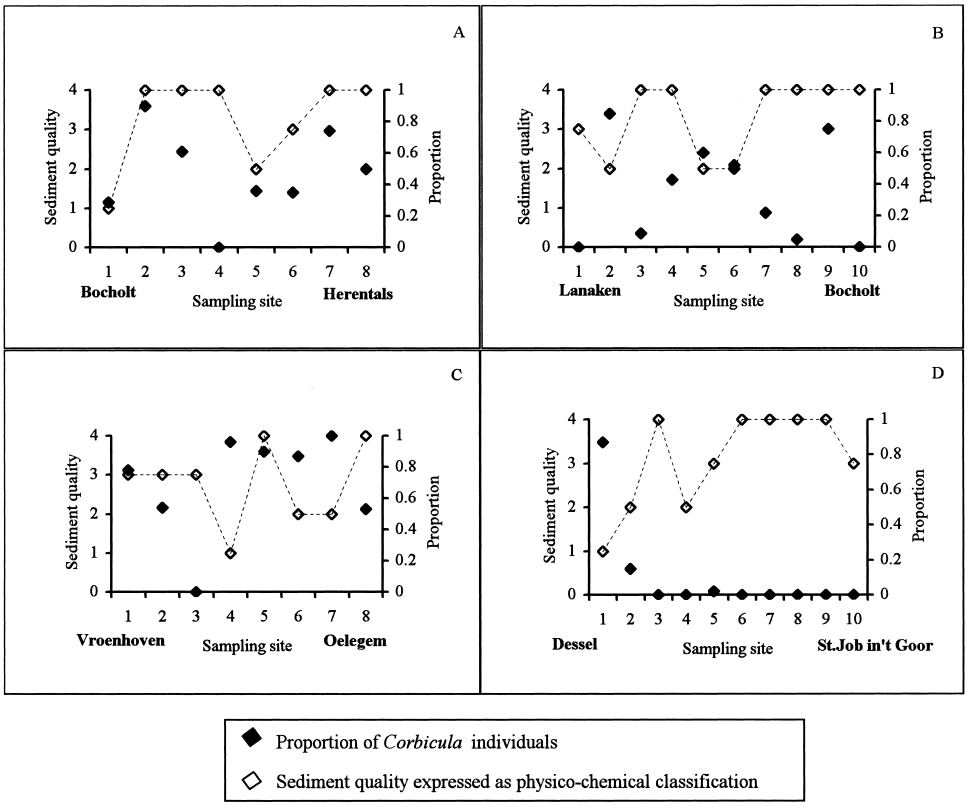

Fig. 3. – Contribution of *Corbicula* species in relation to the abundance of the benthic macroinvertebrate community in (**A**): canal Bocholt-Herentals; (**B**): canal Zuid-Willemsvaart; (**C**): canal Albert and (**D**): canal Dessel-Schoten.

Corbicula species and biological assessment of sediment quality

The sediment quality was also assessed by means of the Biotic Sediment Index (BSI). The distribution of samples over four classes is represented graphically in Fig. 4. The BSI values calculated without considering *Corbicula* species classified the sites as follows: 33.3% in class 1; 29.7% in class 2, 18.5% in class 3, and 18.5% in class 4 (Fig. 4A). However, when *Corbicula* species was included in the calculation of the BSI, the number of sites in class 1 increased to 55.5%, and no sediment was classified as biologically very bad quality (class 4) (Fig. 4B).

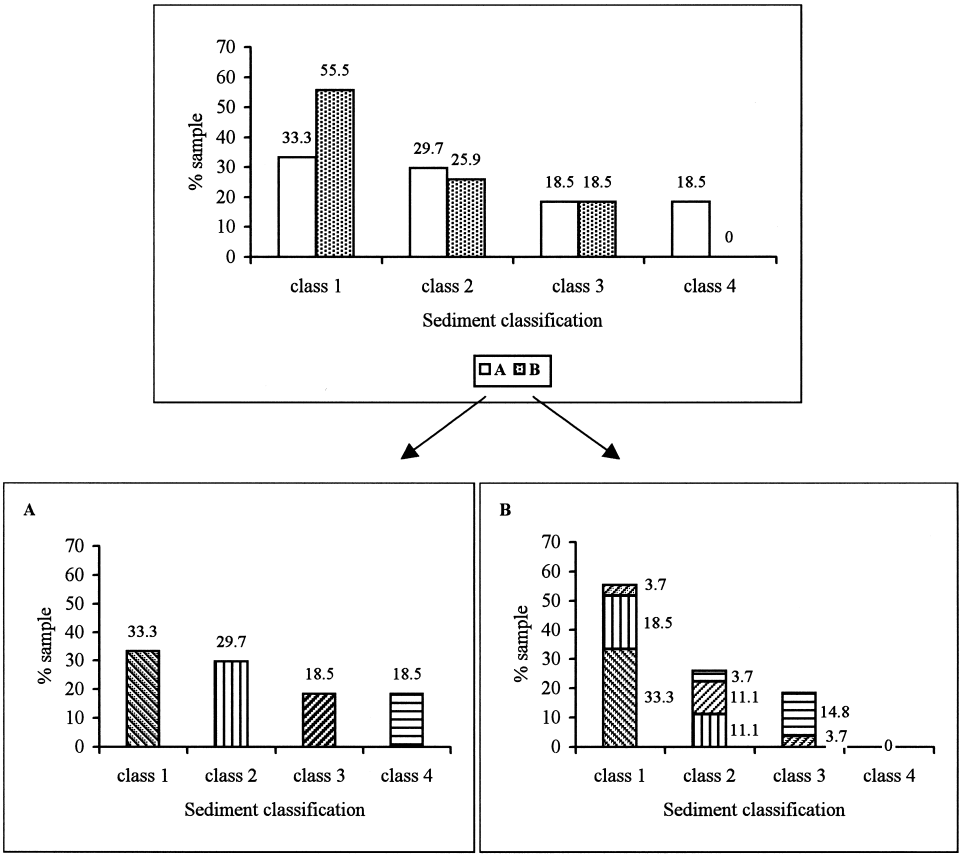

Fig. 4. – Ranking of sediment quality classification based on the Biotic Sediment Index (BSI). (**A**): *Corbicula* species were not considered in the calculation of the Index; (**B**): *Corbicula* species were considered as a (single) taxon in overall diversity and in the mollusc indicator group when calculating the Index. Of the samples shifted in class 1, 18.5% were from class 2 and 3.7% were from class 3. Also, 11.1% and 3.7% of samples from class 3 and 4, respectively, were shifted to class 2. The remaining samples of class 4 (14.8%) were shifted to class 3, leaving no samples in class 4.

DISCUSSION

Of the 33 watercourses investigated, six waterbodies were found to be invaded by *Corbicula* species (Fig. 5). As can be seen, the three watercourses, i.e. canal Albert, canal Bocholt-Herentals, and canal Zuid-Willemsvaart, were completely colonised by the clam. Also, the establishment of *Corbicula* species appeared to shift northward to the canal Dessel-Schoten. Furthermore, the expansion of the clam was moving westward to the canal Willebroek and the river Dender. The presence of *Corbicula* in Belgium was first recorded in 1992 and the genus is represented by two species *C. fluminea* and *C. fluminalis* (SWINNEN et al., 1998). According to the authors, Viersel (canal Bocholt-Herentals) and Tihange (river Meuse) were the farthest west and south locations where the clam had established. The results of the present survey show that the current distribution of *Corbicula* species is broader in comparison with the one reported by SWINNEN et al. (1998), confirming the suggestion of the authors that the distribution of the clam is still continuing to expand.

It has been reported that *C. fluminea* and *C. fluminalis* do not live together in any ecosystem (MORTON, 1986). Yet, mixed populations of the two species have been found in Dutch, German and French parts of the river Rhine. The dissimilarities of the two species in reproductive strategy, spawning periods and different food resources were suggested as the explanations for their successful co-existence (RAJAGOPAL et al., 2000). In the present survey, the co-occurrence of *C. fluminea* and *C.*

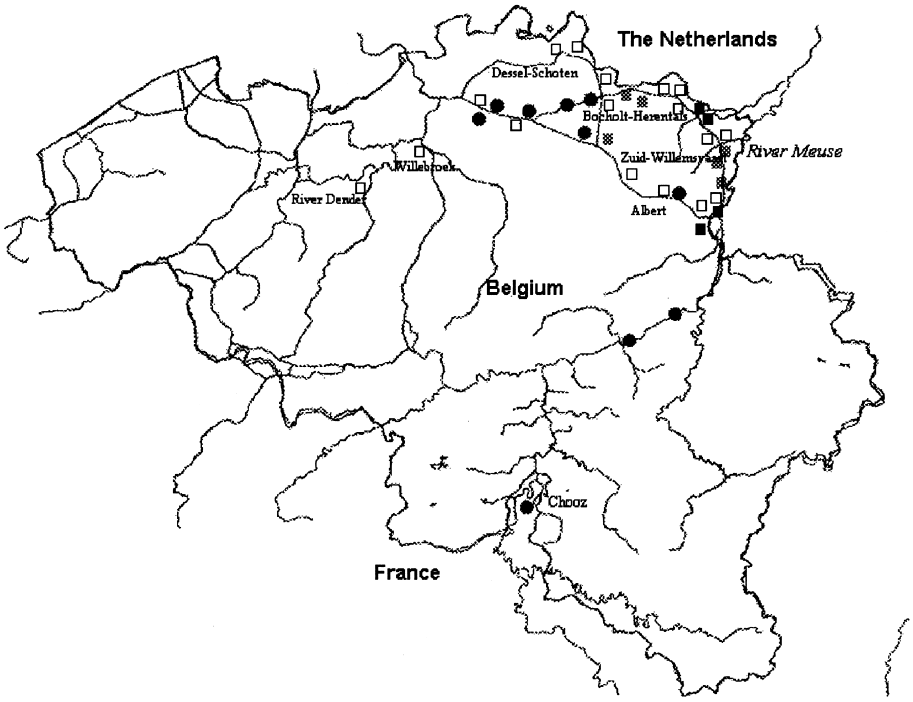

Fig. 5. – Current distribution of *Corbicula* species in Flanders, Belgium. ● : distribution of the clam reported by SWINNEN et al. (1998); □ : *C. fluminea* and *C. fluminalis*; ▩ : *C. fluminea*; ■ : sites where more than one thousand *Corbicula* specimens (*C. fluminea* and *C. fluminalis*) per square meter were found.

fluminalis was also found in several sites of the canals Albert, Bocholt-Herentals, and Zuid-Willemsvaart (Fig. 5). However, the co-existence of the two species was not (longitudinally) perpetual over the length of the above-mentioned canals. This discontinuity in co-inhabiting of *C. fluminea* and *C. fluminalis* in the Flemish waters seems to corroborate the conclusions made by RAJAGOPAL et al. (2000) that beside reproductive strategy, spawning periods and food preferences, other environmental factors are also of importance for their co-existence.

The geographic distribution of *Corbicula* in North America was reported to be limited to areas below latitude 40°N due to intolerance of the clam to severe winter conditions (BRITTON & MORTON, 1982). Temperatures below 2°C were suggested as the major restriction on the distribution of the clam (MCMAHON, 1983). Despite this prediction, *Corbicula* species in America was recently found to continue to move northward (KREISER & MITTON, 1995). Thermal refuges provided by power plants, spring inputs etc., and evolution of the cold tolerance of the clam, were thought to be the two reasons for this phenomenon. Waterbodies receiving heated water (e.g. the cooling-water ditches) were also suggested to be the places for *C. fluminalis* populations to survive during extremely cold winters in Germany (HAESLOOP, 1992). In Flanders, Belgium, survival of *Corbicula* species during winter seems not to be restricted, as in winter water temperatures are usually not lower than 2°C (FLEMISH ENVIRONMENTAL AGENCY, 2000). Moreover, thermal effects of power plants located in the region where the clam was found could also be responsible for its rapid colonisation and stabilisation. As extremely cold winters are rare, and the clam was recently introduced, successful establishment of *Corbicula* species in Belgian watercourses appears to be linked to suitable survival conditions rather than to its adaptation to the coldness.

In general *Corbicula* densities in Flemish watercourses were low in comparison with other populations, which often exceed 1000 individuals/m^2 (DILLON, 2000). It has been documented that in the beginning of the invasion, densities of *C. fluminea* were low (few specimens per square meter). During its establishment, it could increase to more than ten thousand per square meter, and after that the population seemed to stabilise around 2000 individuals/m^2 (GRANEY et al., 1980). The average density of *C. fluminea* in the Potomac River has also been reported to have increased from a few individuals per square meter to more than one thousand specimens per square meter after four to five years of invasion (COHEN et al., 1984). In the present survey, the four sites where the clam densities were greater than a thousand individuals/m^2 were in the waters connected with the river Meuse. After these points the clam densities averaged around 200 individuals per square meter (Fig. 1 and Fig. 5). This observation on the clam densities seems to support the suggestion of SWINNEN et al. (1998) that the colonisation by *Corbicula* species in Belgium originated from the river Meuse, and spread out through the service water system of power plants along the river and some of the connecting canals.

High densities of *Corbicula* species were often found to be concurrent with its dominance in the benthic community (SICKEL, 1986). Yet, in the present study the clam was the most abundant group in 63% of the invaded sites, irrespective of its density (Fig. 1). This finding could probably be explained by the capacity of the clam to re-inhabit following an environmental disturbance (MILLER & PAYNE, 1998). In 89% of the sites where *Corbicula* species established, sediments were slightly or heavily contaminated by organic pollutants and heavy metals. With regard to the sampling time, it has been reported that the population abundance of *Corbicula* varied between seasons with the highest density in spring (PHELPS, 1994; STITES et al., 1995). In the present study, seasonal variation between densities/samples could be rejected as all sampling was performed in the same period, i.e. the spring.

Corbicula species were found in both polluted and unpolluted sediments, and no correlation between physico-chemical sediment quality and the abundance of the clam was observed (Fig. 2A). Similar results were reported by BOLTOVSKOY et al. (1997), who also found no association between environmental stress and *C. fluminea* density. Although the previous study indicated that low dissolved oxygen concentrations were associated with reduced *C. fluminea* densities (BELANGER, 1991), the present survey shows that one of the highest population densities of the clam in the canal Bocholt-Herentals (1870 individuals/m^2 – site 2A, Fig. 1) was in fact found in the location where the dissolved oxygen concentration was the lowest (45% saturation value) of all. The ratio of *Corbicula* abundance and the total macroinvertebrate abundance has been documented to increase with increasing environmental perturbation (KERANS & KARR, 1994). In the present study, the relative proportion of *Corbicula* species at each site was also analysed, and no correlation pattern between it and the sediment quality was observed (Fig. 2B).

The interactions between invasive species and water quality have been investigated by numerous authors. Widely monitored water quality variables such as transparency, concentration of soluble inorganic nitrogen and phosphorus, and dissolved oxygen have been reported to alter due to the invasion of *Corbicula* and *Dreissena* species (COHEN et al., 1984; FOE & KNIGHT, 1985; LAURISTEN, 1986; WAY et al., 1990; PHELPS, 1994; CARACO et al., 1997). These exotic bivalves can also affect the performance of biological indices of water quality causing a change in the index without a corresponding change in water quality (STRAYER, 1999). With regard to the BSI, the presence of the recently introduced *Corbicula* species in benthic macroinvertebrate diversity has been expected to lead to a misreading of sediment quality. Indeed, the results of the present study demonstrated that, with inclusion of the clam as a taxon in the overall diversity and in the mollusc indicator group, the BSI values had changed in 52% of the cases. In all of these altered cases, the biological quality of the sediment shifted from a lower to a higher class (Fig. 4).

In conclusion, the exotic clam species *C. fluminea and C. fluminalis*, which recently invaded several Belgian waters, are continuing to extend their colonisation. Most of the places (89%) where *Corbicula* species were found were (slightly to heavily) polluted by organic contaminants and heavy metals. However, no correlation between the clam density, clam proportion in relation to the abundance of the benthic community, and the quality of the sediment was found. The present study shows a clear influence of *Corbicula* species on the performance of the BSI. It is, therefore, suggested that for the time being the clam should only be considered as a taxon in the overall species richness when calculating the index. Further investigation of the impact of *Corbicula* species on the biological assessment of water and sediment quality is recommended.

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Conservation of the lesser horseshoe bat (*Rhinolophus hipposideros* Bechstein, 1800) (Mammalia: Chiroptera) in Belgium. A case study of feeding habitat requirements

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ABSTRACT. The aim of this study was to determine the habitat use of the last important Belgian colony of *Rhinolophus hipposideros*, Bechstein, 1800, one of the most endangered bat species in Europe. During 71 evenings from April to August 1998, ultrasound detection was performed and, in late August, a female horseshoe bat was caught and fitted with a radio transmitter. The results showed that hedgerows and woodlands with bushes and coppice are key foraging habitats. They also highlight the importance of the presence of a network of wooded elements connecting the maternity roost with the foraging areas. To assure long-term protection of this colony, strong habitat conservation measures should be taken in a radius of up to 1-2 km around the roost.

KEY WORDS: *Rhinolophus hipposideros*, habitat use, bat detector, radiotracking, conservation.

INTRODUCTION

Over the last 40 years, most of the bat populations in the western Paleartic region have declined (STEBBINGS & GRIFFITH, 1986) On a more local scale, several species are now considered as either extinct, endangered or at least vulnerable. In north-western Europe, the lesser horseshoe bat, *Rhinolophus hipposideros*, was formerly widespread and quite common. At present, along with the barbastelle, *Barbastella barbastellus*, it is probably the most endangered species (BEZEM et al., 1957; SLUITER et al., 1963; STEBBINGS & GRIFFITH, 1986) Extinct in the Great Duchy of Luxembourg and the Netherlands (DAAN 1980), populations of the lesser horseshoe bat are at a very low level in northern France as well as in western Germany (DUBIE & SCHWAAB, 1997; BIEDERMAN, 1997). In Belgium, its numbers are estimated at no more than 200-250 individuals whereas, in the past, it was one of the most widespread species, and one of the most ringed (FAIRON, 1977).

Aware of this overall deteriorating situation, the authorities and some conservation groups began to protect hibernation caves and nurseries from the late 1970s. Moreover, on both international and national (or regional) levels, some legal protection was progressively given to the species themselves as well as their habitat (Bern Convention, EC Directive 92/43, annex 2, Bonn Convention). However, though essential to the preservation of viable bat populations, these measures did not have the expected effects and no restoration of the most vulnerable species occurred. In the meantime, the rural landscape changed, lands were drained, hedges removed and small-scaled agricultural landscapes disappeared. It is difficult to assess the impact of landscape modifications on the population dynamics of *R. hipposideros*, as the feeding habitat requirements of the species are poorly known (GAISLER, 1963, MCANEY & FAIRLEY, 1988; MITCHELL-JONES, 1995). It became a species of special concern under the European Bats Agreement (e.g. species selected for Consistent Monitoring and proposed as a Priority Species for Autecological Studies).

By studying the feeding grounds of the last important Belgian colony of the lesser horseshoe bat, we intend to characterise the main landscape features that are of importance for the species. Then, from the observations, some management and conservation measures will be proposed.

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MATERIAL AND METHODS

Study area

The nursery is located at Revogne, a small village at the border of the karstic zone of the Famenne region (Belgium, UTM coordinates: 31 U FR 4651). The bat colony, comprising 60 adults, has been established, in the cellar of a 19th century castle-farm for 25 years. The surroundings are mainly permanent grazing meadows with a dense network of hedgerows. Deciduous or mixed pine woods are located on the top of nearby hills.

Methods

Bat detectors are widely used to record the presence of bats (BAAGOE, 1989; LIMPENS 1993) or to study their behaviour (FENTON, 1982; JONES et al., 1992). They are also used to study their habitat use (WALSH et al., 1995; BOONMAN, 1996; MOTTE, 1997; MOTTE et al., 1998). These devices transform the bat echolocation calls into audible signals and allow the detection of target individuals without disturbing them. Since the lesser horseshoe bat is considered a highly disturbance-sensitive species (MCANEY & FAIRLEY, 1988), the choice of this survey method seemed fully justified.

The apparatus (Pettersson Elektronik AB, D-980) was used in heterodyne mode, and tuned to 108-113 kHz, which is the specific frequency of the echolocation calls of the lesser horseshoe bat (BARATAUD, 1996; JONES & RAYNER, 1989; TUPINIER, 1996). This frequency is higher than those used by other bat species living in Belgium, therefore precluding any risk of confusion.

The ultrasonic calls *of R. hipposideros* are of low intensity and cannot be detected at more than 5-6 m, even with a high-performance detector working in optimal conditions: no wind, no rain, open field and fully charged batteries (pers. obs.). Furthermore the calls of rhinolophid bats could be more directional than those of vespertilionids species. As a consequence, the signal received from a flying bat is often incomplete and sometimes hardly detectable.

In order to find the precise location of the feeding grounds, the direction taken by the bats when leaving their roost was observed first. Each route was then followed until either the contact was lost or a hunting area was discovered. If lost, the contact was searched for during the next night exactly at the same place or at a distance varying from 10 to 20 meters. In that way, an area of about 300 hectares around the nursery roost (1 km radius) was explored, during 71 evenings from May to mid August 1998. Records were made from 10 min before dusk till 90 min after, while crepuscular light allowed us to identify the flight direction taken by the detected bat.

To confirm previous results, and as the colony seemed thriving, it was decided to catch only one adult female and to fit it with a radio transmitter (Holohil LB-2T; weight: 0.65 gram i.e. 10 % of the bat weight). This choice was the result of a trade-off between the pertinence of tracking more individuals and the high sensitivity of the species to disturbance. We are aware that the radio tracking results only relate to a single individual. However, with regards to the status of the species and the lack of knowledge about foraging habitat requirements, data accumulated are discussed below.

The experiment was run in August, after the juveniles had been weaned.

The animal was caught at dusk when leaving the roost. A home-made butterfly net was placed just outside the entrance of the roost. The transmitter was glued to the middle of the animal's back (Histoacryl surgical glue: Braun). The bat was then tracked for 11 nights, from 6 to 17 August 1998, using a single Stobo XR 100 receiver (GFTmbh) with a 3-element Yagi aerial using the "homing-in" method (WHITE & GARROTT, 1990). The bat was pursued either by car or on foot. The activity area of the monitored bat was estimated in accordance with the minimum area method (WHITE & GARROTT, 1990). It was then divided in 0.25 ha cells ($n = 219$) where habitats were characterised with seven variables (Table 1). Using a χ^2 test (SPIEGEL, 1961) correspondences between habitat characteristics and the presence of a foraging area were established.

RESULTS

Detection of ultrasonic calls

From 58 observation points, 51 contacts were obtained, 36 of which led to the discovery of either a flight route or a feeding place (Fig. 1).

There were two flight routes from the nursery. The most used one ($\chi^2 = 164{,}8$; 5 f.d.; $p < 0{,}001$; n = 255 bats during five counting sessions) ran along stone walls and led directly to the nearest woodland (see f 1 on Fig. 1) . The other ran along other stone walls, then hedgerows with trees and led to another wood (see f 2 on Fig. 1). Three other flight routes were found connecting woodlands (see f 3, 4, 5 on Fig.1). They ran along large hedgerows separating grazing meadows. Some tall trees were included in these hedges: *Quercus robur, Fraxinus excelsior, Acer pseudoplatanus* and very old *Crataegus monogyna*. A sixth was located along a leafy woodland path (see f 6 on Fig.1).

Although some foraging was observed along the hedgerows, the feeding grounds were deciduous woodland (*Fraxinus excelsior; Quercus sp., Carpinus betulus, Acer pseudoplatanus, Acer campestre*) with copses (*Crataegus monogyna; Corylus avellana; Cornus mas; Cornus sanguinea*) or mixed clear coniferous woodland (*Pinus nigra; Pinus sylvestris*).

Fig. 1. – Localisation of observation points (**X**), feeding grounds ( ) and flight routes ( ) discovered by ultrasonic call detection. deciduous woodland; coniferous woodland; clear-felling; grazed pasture; cutting pasture; arable land; hedgerows inferior to 2 meters height; hedgerows superior to 2 meters height; scrubs; o o o orchard; trees line; river; **R**: nursery roost; **c**: cave; **f 1 to 6**: flight route 1 to 6. Map based on IGN topographic map (59/6) and completed by our field observations.

Most of the observation points were checked both during the first (May to June) and the second (July to August) part of the study. No obvious change was observed in either the location of the flight routes or the feeding grounds.

Radio tracking experiment

The bat was released five minutes after its capture and flew directly to the next woodland, without apparent difficulty. Having flown for 6 min, it perched for 10 min on an old oak and then returned to the roost till the next night.

During subsequent nights, having left the nursery, the animal flew immediately to a woodland strip in the vicinity where it hunted in deciduous (*Quercus sp., Fraxinus excelsior, Acer pseudoplatanus*) or in mixed coniferous woodland with coppices (*Pinus sp., Quercus sp., Fraxinus excelsior, Corylus avellana, Crataegus monogyna*). Woodland paths were used as flight routes and allowed the animal to move quickly.

Every night, when either leaving the roost or returning to it, the bat was located along the same hedgerow (*Fraxinus excelsior, Corylus avellana, Crataegus monogyna, Quercus sp.*) connecting two woodland areas used as feeding grounds (see double arrow on Fig. 2).

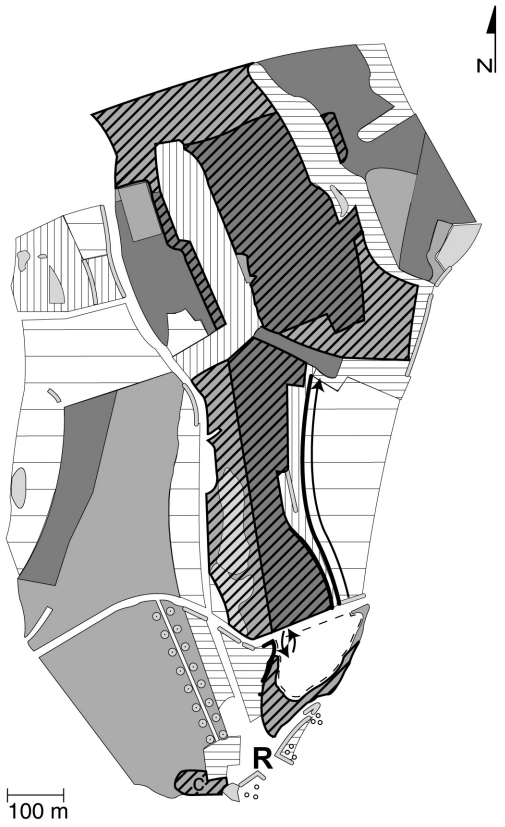

Fig. 2. – Localisation of feeding grounds ( ) and flight routes ( )exploited by the tracked bat. deciduous woodland; coniferous woodland; clear-felling; grazed pasture; cutting pasture; arable land; hedgerows inferior to 2 meters height; hedgerows superior to 2 meters height; scrubs; orchard; trees line; **R**: nursery roost; **c**: cave. Map based on IGN topographic map (59/6) and completed by our field observations.

Fig. 2 illustrates the area covered by this animal and shows its feeding grounds as well as its flight routes. The map is based on a total of 27 hours (1623 min) of observation, the radio contact having been lost during 25 h (1473 min). In fact, contact was lost when the animal went into dense woodland or was too far away from the position of the observer. The maximum observed range was 1.2 km from the roost but it was presumably more.

The results (Table 1, 2) confirm that the presence of hunting grounds was strongly associated with pine woodland (*pw*), hedgerows (*h*) and deciduous woodland (*dw*) (χ^2_1 pw = 30.37, $p < 0.999$; χ^2_1 h= 10.97, $p < 0.999$; χ^2_1 dw = 6.94, $p < 0.95$) whereas their absence was associated with arable land (*al*) and spruce woodland (*Picea abies*) (*sw*) (χ^2_1 al = 25.84, $p < 0.999$; χ^2_1 sw = 19.7, $p < 0.999$). Hay meadow (*hw*) and grazed pasture (*gp*) did not seem to have any influence (χ^2_1 hw = 0.05, $p < 0.999$; χ^2_1 gp = 0.02, $p < 0.999$).

TABLE 1

Cross-table between the presence of foraging areas in the 219 home range cells and some of their habitat characteristics. Results obtained by radiotracking.

Variables		**Number of cells with hunting ground**	**Number of cells without hunting ground**
Pine woodland	with	60	21
	without	49	89
Spruce woodland	with	2	23
	without	107	87
Deciduous woodland	with	62	43
	without	47	67
Grazed pasture	with	18	19
	without	91	91
Hay meadow	with	18	17
	without	91	93
Hedgerows	with	27	9
	without	82	101
Arable land	with	2	28
	without	102	87

TABLE 2

χ^2 values obtained for each variable using table 1.

Variable	**χ^2 (*d.f.*= 1)**	**Critical value**	***p* <**
Pine woodland	30.37	10.8	0.999
Spruce woodland	19.70	10.8	0.999
Deciduous woodland	6.94	3.84	0.95
Grazed pasture	0.02	10.8	0.999
Hay meadow	0.05	10.8	0.999
Hedgerows	10.97	10.8	0.999
Arable land	25.84	10.8	0.999

DISCUSSION AND CONCLUSION

It is interesting to note that most of our field work has been done with a bat detector. Although ultrasonic pulses of the species were difficult to detect, the particular methodology used is practical for this species and such methodology could be used by most field workers.

Nevertheless, the detection of ultrasonic calls needs to be carefully monitored because a lack of signal reception cannot necessarily be correlated with the absence of bats. Despite this drawback, most areas exploited by *R. hipposideros* in a 500-700 m radius were probably identified, since the observations were made at a large number of points close to one another.

Figs 1 and 2 clearly show that all the feeding grounds of the lesser horseshoe bats were mid-open or closed habitats. Hedgerows and woodlands therefore appear as key-habitats for the feeding of the species.

All these observations are in accordance with the observations made by previous studies. McAney & Fairley (1988) in Ireland rarely detected the ultrasonic foraging pulses of the lesser horseshoe bat over open pasture.

In Switzerland, analysis of excrements and bitten-off food remains indicated that most prey-insects (Lepidoptera, Neuroptera) were caught by bats in abundantly structured hedges, woodland and their outskirts near water (Beck et al., 1989).

In Britain, the use of a bat detector and of an infra red spot lamp by Schofield (1996), confirmed that the lesser horseshoe bat foraged in woodland, hedgerows and tree lines. In England and Wales, this author also used a Geographical Information System to characterise habitat factors and identify the areas in which the lesser horseshoe bat is found. Habitat preferences, as determined by comparisons between availability and utilisation, indicated that the bat selected areas of undulating countryside with hedgerows, tree lines and woodland, in preference to flat open areas that were intensively farmed.

Our observations with a bat detector indicated that in the woods, the horseshoe bat was hunting, swerving between branches and in the foliage of coppice, at 1 to 4 m height. This seems to indicate that the structure of the wooded habitat has more importance for the bat than the specific composition of the different layers. The density of the taller trees (either deciduous or coniferous) must be low enough to allow the development of an understorey of shrub and small coppice.

Moreover, what is striking, is that all places where a contact with a bat was obtained (Fig. 1 and 2) were linked to each other and to the roost by a network of wooded elements : tree lines, wood edges or large hedgerows with tall trees.

Therefore, a network of tall hedgerows can be considered as a key-element in a lesser horseshoe bat home range. This assumption is reinforced considering the fact that no *R. hipposideros* has ever been seen flying at a distance of more than 1 m from a wooded element and that radio tracked individual never crossed open habitats.

The intensity of use of the key-habitats was evidenced within a 500 m radius around the nursery roost with the bat detector. The radio tracking of a single individual revealed that foraging could occur at a distance of at least 1.2 km from the roost. Schofield's study (1996) showed that two *R. hipposideros* out of six marked ones (plastic cyalume light tags) were observed up to 2 km away from their site of capture.

Habitat conservation measures must therefore be taken in a minimum radius of 1-2 km from the roost. They should include a strict protection of tall hedgerows and of all the connecting wooded elements between the roost and the feeding grounds. Moreover, clear-cuttings must be avoided in the nearby woodlands unless some untouched strips are preserved. Coppice management should be maintained and must be regularly cut on small areas so as to make a rotation between stands of different age. Indeed, the importance of this management form has seriously decreased since World War II, because of the decreasing need for small firewood. Finally, a network of wooded elements must be maintained or restored in a wider perimeter to allow easier access to hibernation caves and, for eventual future exchanges with nearby colonies.

One can hope that, in the future, more large-scale European studies using radio tracking and ultrasonic call detection will be undertaken, and will help in the assessment of the foraging habitat requirements and the home range of a given colony.

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A comparison of relict versus dynamic models for tenebrionid beetles (Coleoptera: Tenebrionidae) of Aegean Islands (Greece)

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ABSTRACT. This paper analyses the species-area relationship in the Aegean tenebrionids in order to discriminate between relict and dynamic models. Using different statistical approaches, the following eco-geographic variables were tested as predictors of the tenebrionid richness on 32 Aegean Islands: latitude, longitude, area, maximum elevation, distance to the mainland, and distance to the nearest island. Area was identified as an important predictor, while neither distance to the mainland nor distance to the nearest island was identified as of any statistical importance in affecting tenebrionid richness. Thus, as proposed for other very sedentary animals inhabiting this archipelago such as land snails, a relict model is postulated. As most tenebrionids have extremely limited ability to actively disperse over the sea, ancestral species have probably colonised the Aegean Islands by means of land-bridges during Pleistocene falls in the sea level.

KEY WORDS: Island Biogeography, relict models, Greece, Coleoptera, Tenebrionidae.

INTRODUCTION

The large number and varied sizes of the Aegean Islands make this archipelago excellent material to study the species-area relationship in insular biotas. Tenebrionids (Coleoptera, Tenebrionidae) are primarily saprophagous beetles, which occur in a great variety of habitats, being ideal objects for studies of ecological biogeography in different environments, such as forests, steppes, deserts and urban habitats (see FATTORINI, 2000 and references therein). Their highly diversified ecology can be an important property, especially for the study of insular faunas, which are generally characterised by low diversity. In fact, tenebrionids can be a rare case of a group that also appears rich in species in these circumstances, allowing for a study based on a large set of species.

According to vicariance biogeographers, present distribution patterns are more dependent on the subdivision of ancestral biotas in response to changing geography than on dispersal abilities. However, an understanding of a group's vagility is a prerequisite for any biogeographic study because species with higher dispersal ability are obviously less affected by vicariance events (e. g. NOONAN, 1988). Most Tenebrionidae are characterised by aptery. As vagility of apterous beetles is greatly reduced by their inability to fly, tenebrionids are a group of great interest in historical biogeography (FATTORINI, 2000). In particular, as many tenebrionids are strictly resident organisms with extremely limited ability to actively disperse over the sea, these beetles may be of particular interest in insular biotas strongly affected by historical events.

The tenebrionid fauna of the Aegean Islands is mostly composed of apterous species. However, these apterous species also occur in the adjacent mainland areas (i. e. Greece and Anatolia) or, if endemic, belong to apterous genera (cf. FATTORINI et al., 1999). Therefore, aptery in Aegean tenebrionids evolved before island colonisation, and it is not a consequence of living on islands. Although aptery greatly reduces the beetles' ability to actively disperse, other dispersal mechanisms, such as rafting and water transport, cannot be a priori dismissed as factors assisting island colonisation by apterous tenebrionids. For example, phylogeographic data on flightless Canarian tenebrionids strongly support models of sequential invasions affected by inter-island distances (JUAN et al., 1997, 1998; VARGAS et al., 1999), thus suggesting that recent

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dispersal played an important role in determining their present distribution patterns.

The geological history of the Aegean Islands has been evoked as an important factor in their biogeography (SFENTHOURAKIS, 1996). Before the Serravallian (12-14 Ma), the Aegean area was substantially occupied by a continental environment without marine ingressions. In the Serravallian, the Anatolian block started to move westwards, originating the southward extension of the Aegean plate. During the lower Tortonian (11 Ma), massive marine ingressions occurred in the northern and southern Aegean, and in the late Pliocene (4 Ma), as a result of subduction of the African oceanic lithosphere, the southern Aegean was submitted to tectonic uplift. Around 3-2 Ma, the Cyclades land mass was submerged, thus originating the Cyclades islands (WELTER-SCHULTES & WILLIAMS, 1999). However, as a consequence of glaciations, the coastline of the Mediterranean Basin has significantly changed since the Pleistocene. Therefore, the biogeographic characteristics of the Aegean Islands during the Pleistocene (notably their size and distance to the mainland) were very different from the present. In particular, many islands were joined to each other and to the adjacent mainland (cf. DERMITZAKIS, 1990; OLIVIER, 1993; SFENTHOURAKIS, 1996; WELTER-SCHULTES & WILLIAMS, 1999).

This historical scenario can obviously be responsible for differences in distributional patterns. As a rule, one can suppose that, in the same area, sedentary taxa are strongly affected by historical processes, whereas present ecological factors may be more important in conditioning the distribution of taxa with higher dispersal abilities. Therefore, in a given area, different animal groups should conform to different biogeographic models.

Relict models postulate that present distribution patterns of insular faunas are more dependent on historical factors (such as the palaeogeographic connections between islands and mainland areas followed by vicariance events) than present conditions (such as island distance to the mainland). According to the relict models, a positive species-area relationship results from area-dependent extinction or relaxation (cf. CROWELL, 1986). By contrast, dynamic models suppose that distribution patterns are more influenced by present conditions (especially island distance to the mainland and inter-island distances) than by historical factors.

The dynamic equilibrium model of island biogeography proposed by MACARTHUR & WILSON (1967) postulates that the effect of island area on species number is due to equilibrium between immigration and extinction. According to this model, immigration rates are enhanced by an island's proximity to a source of species (e.g. a mainland area), and large islands have lower extinction rates than do smaller islands, because their larger populations are less vulnerable to stochastic events. Two main predictions follow: (1) the number of species will increase with area; (2) species richness will decrease with distance. Note that these two predictions can also be made under different assumptions. According to CONNOR & MCCOY (1979), large islands may have more species simply because they 'sample' more individuals, and hence more species, from the total pool of immigrants. CONNOR & MCCOY (1979) do not deal with isolation effects on the passive sampling. However, one can reasonably admit that far distant islands should 'sample', and hence retain, fewer species than islands closer to the mainland. In both the equilibrium and the sampling models, an area *per se* effect is supposed, and species number on islands is regarded as a consequence of present colonisation (dispersal from the mainland). Therefore, they involve only dynamic processes.

By contrast, according to WILLIAMS (1964), large areas have more species because they have more habitats than do small areas (habitat diversity model). Also in this case, distance would have a negative effect on species richness; comparing islands with the same habitat diversity, far islands are more difficult to be reached by mainland populations and they would consequentially harbour fewer species. However, the habitat diversity model is also compatible with relict models, because one can suppose that different islands were reached by immigrants in past times, and that they retained more or less species according to their habitat diversity. In this scenario, if habitat diversity is positively correlated with area, large islands are expected to have retained more species, while, as the geography of the study area changed, no relationships are expected between species richness and distance. By contrast, as distance negatively affects present colonisation processes, both the equilibrium and the sampling models should be rejected, if no negative relationships can be observed between species richness and distance. In such circumstances, a relict model should be accepted and a habitat model can be evoked to explain a positive species-area relationship. Note that a habitat model could be also evoked in non-relict faunas, when species richness is positively correlated with habitat diversity (but not with area) and negatively with distance.

In this paper, I analyse the species-area relationship in the Aegean tenebrionids in order to discriminate between relict and dynamic models.

MATERIAL AND METHODS

Study area

The Aegean Islands are located in the East Mediterranean, between the Greek and Turkish coasts. They are mainly aligned in a NW-SE direction and embrace various large islands (including Kriti, Euboea and some north-eastern islands), as well as some fringing archipelagos (i. e. Northern Sporades, Southern Sporades and the Cyclades). The climate of the Aegean Islands is typically Mediterranean. Phrygana is the dominant vege-

tation type throughout the islands, while forest and maquis habitats occur only in scattered patches on some of the largest islands. Forests are characteristically represented by oak (*Quercus coccifera*) forests, which, most probably, were the climax vegetation type of the Aegean area (POLUNIN & WALTERS, 1985), and pine (*Pinus halepensis*, *P. brutia* and *P. nigra*) forests. Habitat changes determined by human activities, especially deforestation, are an important key feature of the ecology of many of the islands. For example, the Cyclades have been inhabited for at least 4500 years and most arable land was cleared and hillsides terraced over two millennia ago. However, while forests were seriously affected by human activities, maquis habitats are relatively well preserved and the phrygana has been greatly extended as a result of deforestation (cf. RUNEMARK, 1971; SIMBERLOFF, 1986; OLIVIER, 1993), thus presumably favouring xero-geophilous tenebrionids.

On some islands, human activities may have caused extinction of a few tenebrionid species associated with forest habitats. However, xylophilous species represent a minor component of the tenebrionid fauna considered here (about 15 out of 133 species). Therefore, as for other animal groups such as terrestrial isopods and land snails (SFENTHOURAKIS, 1996; WELTER-SCHULTES & WILLIAMS, 1999), the low number of species that could be presently absent on some islands as a result of extinction, is not expected to have any substantial influence on the overall species numbers.

Data sources

A total of 32 Aegean Islands were included in this study (Fig. 1, Table 1). Due to intensive research from the beginning of the past century to the present, all these islands are faunistically well known. These islands are

TABLE 1

Tenebrionid richness and untransformed eco-geographic variables for the studied islands. *S* = Tenebrionid richness, *La* = latitude (°N), *Lo* = longitude (°E), *A* = area (Km^2); *E* = elevation (m); *Di* = distance to the nearest island (Km), *Dm* = distance to the nearest mainland (Km).

Island	*S*	*La*	*Lo*	*A*	*E*	*Di*	*Dm*
Amorgos	7	36.5	25.59	121.1	821	12.8	104
Anafi	12	36.21	25.5	38.4	582	20.8	140
Andros	12	37.45	24.42	380	994	2	57
Castellorizon	8	36.08	29.34	7.3	271	120.8	2
Chios	10	38.22	26	842	1297	20	11
Euboea	42	38.34	23.5	3658	1745	11.2	0.3
Folegandros	7	36.37	24.54	32.1	415	14.4	132
Ios	9	36.42	25.24	107.8	713	6.6	147
Karpathos	15	35.4	27.1	301	1215	1	93
Kea	6	37.34	24.22	130.6	560	8.8	20.5
Kimolos	3	36.48	24.34	35.7	358	1.2	102
Kithnos	4	37.25	24.28	99.3	306	8.8	36
Kos	26	36.5	27.1	290.3	843	4	3.5
Kriti	70	35.29	24.42	8260	2456	36.8	99
Lemnos	10	39.54	25.21	460	459	24	59
Lesvos	17	39.1	26.2	1630	968	51.2	10.5
Mikonos	10	37.29	25.25	85.5	372	2.4	114
Milos	19	36.41	24.15	150.6	751	1.2	100
Naxos	36	37.02	25.35	428	1001	5.8	130
Pano Koufonissi	12	36.56	25.59	3.8	114	0.6	147
Paros	8	37.08	25.12	194.5	705	1.6	116
Rhodos	41	36.1	28	1400	1215	8.2	21
Samos	14	37.48	26.44	476.2	1434	5.6	2.8
Santorin	26	36.24	25.29	31	586	20.8	173
Serifos	8	37.11	24.31	73.2	585	12	64
Sifnos	9	36.59	24.4	73.2	678	12	87
Sikinos	8	36.39	25.06	41	533	6.6	144
Siros	23	37.26	24.54	83.6	422	16.8	75
Skiros	11	38.53	24.32	209	792	52	75
Skopelos	7	39.1	23.4	96	680	3.2	21
Thasos	13	40.41	24.47	379	1203	57.6	6
Tinos	13	37.38	25.1	194.3	730	8	80

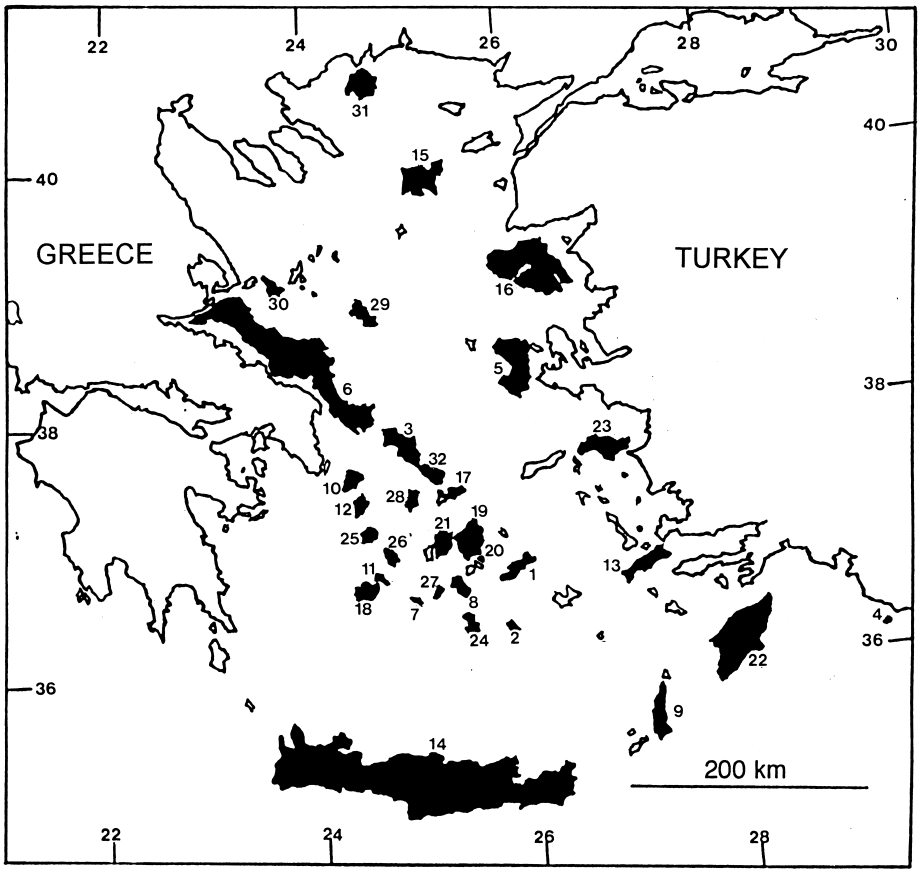

Fig. 1. – Map of the study area. Studied islands are in black. 1 Amorgos, 2 Anafi, 3 Andros, 4 Castellorizon, 5 Chios, 6 Euboea, 7 Folegandros, 8 Ios, 9 Karpathos, 10 Kea, 11 Kimolos, 12 Kithnos, 13 Kos, 14 Kriti, 15 Lemnos, 16 Lesvos, 17 Mikonos, 18 Milos, 19 Naxos, 20 Pano Koufonissi, 21 Paros, 22 Rhodos, 23 Samos, 24 Santorin, 25 Serifos, 26 Sifnos, 27 Sikinos, 28 Siros, 29 Skiros, 30 Skopelos, 31 Thasos, 32 Tinos.

also well distributed throughout the whole Aegean area and are representative of different ecological conditions. On the Aegean Islands, several species are represented by different subspecies endemic to individual islands or groups of islands. No island except Kriti has more than one subspecies of a given species. In contrast, Kriti, the largest Aegean island, harbours different subspecies of some species. It is difficult, at present, to state the actual taxonomic status of these Cretan populations. These subspecies occupy different areas on Kriti, and, probably, also different habitats. Therefore, they should be subject to the same biogeographic determinants as species in the species-area relationships. Generally speaking, large islands, having larger areas and / or more habitats, may harbour more subspecies than small islands, where reduced areas do not allow allopatric differentiation or habitat segregation. For these reasons, I have counted these populations as different taxa. As a whole, 166 species and subspecies (133 species) were ascertained to occur on the study islands as native. Note that if only the species level is used for the tenebrionid richness of Kriti, we found a value of 58 species, which is rather lower than that of 70 obtained by counting both species and subspecies. For comparison, I have performed all analyses using two sets of data: one with the value of 70 species and subspecies, the other with that of 58 species. However, both sets gave comparable results. Therefore, only results obtained by using both species and subspecies are extensively discussed. For simplicity, the term 'species' will be used in reference to tenebrionid richness, including subspecies for Kriti.

Figures reported in Table 1 are almost entirely based on data cited by Fattorini et al. (1999). Other literature records, not quoted by Fattorini et al. (1999), but included in this study, were taken from Liebegott (1982), Whitehead (1997), Grimm & Schawaller (2000), Scupola & Lo Cascio (2001) and Leo & Fattorini (in press). New records for Karpathos (an undetermined specimen of *Stenosis*, in bad condition, coll. S. Fattorini) and Rhodes (*Menephylus cylindricus* (Herbst, 1784) and *Leichenum* sp., P. Leo, personal communication) were also included. The systematic treatment of the genus *Colpotus* was updated following Grimm & Schawaller (2000). As to the genus *Pimelia*, I considered *P. subglobosa* Pallas 1781 as monotypic and synonymous with *P. sericella* Solier 1836 (Fattorini & Leo, unpublished data).

I have omitted some species, cited by various authors from the study islands, because they are synanthropic (and thus possibly introduced), transient, exotic or of

uncertain distribution. A detailed account of the criteria used to reject data can be found in FATTORINI et al. (1999). I have also omitted the whole subfamily Alleculinae, and, within the subfamily Lagrinae, the tribe Lagriini, because they are taxonomically and faunistically very poorly known. Although Lagriini and Alleculinae, both winged taxa, were omitted, other winged species, with high dispersal power, are included in the data set (e. g. *Anemia sardoa* (Géné, 1839), *Cossyphus tauricus* Steven, 1829, *Diaperis boleti* (Linné, 1758), *Hypophloeus* spp., *Nalassus plebejus* (Küster, 1850), *Pentaphyllus chrysomeloides* (Rossi, 1792), *Trachyscelis aphodioides* Latreille, 1809, etc.).

As a whole, even if some islands are better known than others (Kriti and some of the Cyclades are the best studied) and new records can be expected, the very slight differences between the numbers of taxa recorded here and those quoted several years ago by DAJOZ (1987) suggest that future increases in species numbers will not be substantial.

Data analysis

To study the relation between species and area, three different mathematical models have been used by various authors (cf. CONNOR & MCCOY, 1979; WHITTAKER, 1998): the linear function model ($S = C + zA$, where S is the number of species, A is the area and C and z are constants), the exponential function model ($S = \text{Log}C + z\text{Log}A$), and the power function model ($S = CA^z$ approximated by the double Log-transformation: $\text{Log}S = \text{Log}C + z\text{Log}A$). There is no a priori biological reason to insist either or both variables be Log-transformed, and the best-fit model for a particular species-area curve can usually only be determined empirically (cf. CONNOR & MCCOY, 1979). These three mathematical models have different properties, and they have been widely used to study the species-area relationship in a great number of archipelagos (e. g. CONNOR & MCCOY, 1979; WHITTAKER, 1998), including the Aegean Islands (cf. SFENTHOURAKIS, 1996a). Thus, for the purposes of comparison, I investigated the species-area relationship by using all these functions. The standard method of least squares linear regression has been used for all functions.

The power function model actually poses some statistical and biological problems outlined by WRIGHT (1981) and WILLIAMS (1995, 1996). WRIGHT (1981) pointed out that if the error term is multiplicative the logarithmic transformation is correct, while if the error term is additive the logarithmic transformation is incorrect. According to WRIGHT (1981), as the error in S is probably independent of A, the logarithmic transformation is incorrect and a non linear least-squares fit should be used. However, I think that the error in S can be dependent on A. It is reasonable to suppose that faunas of small islands, having fewer species, can be completely known. By contrast, large islands, harbouring a great number of species, are more difficult to be completely known. Thus, we can presume that our degree of faunistical knowledge (i. e. our estimate of S) is associated with area, and the logarithmic transformation may be appropriate. In addition, in non-linear regression, the parameter estimates are too heavily influenced by large values, causing shortcomings in the use of this technique (WELTER-SCHULTES & WILLIAMS, 1999). To avoid some biological problems of the power function, WILLIAMS (1995, 1996) proposed the cumulative extreme-value function (EVF). However, when WELTER-SCHULTES & WILLIAMS (1999) compared results obtained by using the EVF and the power function, the two functions gave substantially the same results; the same authors also found that fitting the power function model using non linear regression was of questionable utility. Therefore, as in other recent studies (e. g. MILLIEN-PARRA & JAEGER, 1999; RICKLEFS & LOVETTE, 1999; KOTZE et al., 2000), in this paper the power function in its linear form was applied instead of the EVF or a non linear function.

The influence of area has also been investigated with other procedures, together with the following other geographic parameters: latitude, longitude, elevation, distance to the mainland, and distance to the nearest island.

As in other studies (e. g. NEWMARK, 1986), maximum elevation was intended here to represent a rough estimate of potential habitat diversity. Many variables, claimed to reflect habitat diversity, do not measure this factor at all (NILSSON et al., 1988); in addition, some measures that can be appropriate for some organisms may be not appropriate for organisms with other ecological preferences. This fact may represent an important, and usually overlooked, bias when a group that includes a large set of species with different ecological preferences is studied. As to the tenebrionids, this group occurs on the Aegean Islands with species that have a great variety of ecological preferences, including both xylophilous species (which occur in rotten wood and associated cambium and subcortical spaces, feeding on wood, fungi, or even on other beetles) and geophilous species (which occur on the ground, even in littoral regions, where they can be encountered beneath dune plants or in sea weed, carrion, or other organic debris). In this case, rather than direct measures of habitat diversity, a synthetic, indirect measure, may be preferable. As elevation is correlated with temperature, precipitation, humidity, wind speed, evaporation and insolation, it may be an indirect, 'universal' measure of habitat diversity.

The Shapiro-Wilks W test was used to test if species richness and eco-geographic variables followed normal distributions. Species richness, longitude, elevation, area and distance to the nearest island showed substantial deviation from normality. Therefore, these variables were $\log_{10}$-transformed to improve normality. By contrast, both latitude and distance to mainland showed normal distributions; therefore both these variables were left untransformed.

The Pearson product-moment correlation coefficient was used in estimating the correlations among the eco-

geographic variables and between them and species richness. A sequential Bonferroni correction (RICE, 1989) was used to adjust the significance level to the number of comparisons (k) using the same data set.

A partial correlation analysis was conducted between species richness and the eco-geographic variables to identify the 'isolated effects' of each of these variables (cf. NEWMARK, 1986).

For the purposes of comparison, a forward stepwise multiple regression between species richness and eco-geographic variables was also performed. Using this type of multiple regression, the independent variables are individually added or deleted from the model at each step of the regression until the 'best' regression model is obtained. Both area (positively) and latitude (negatively) contributed significantly to the model. However, the negative correlation between species richness and latitude could be actually due to the location at low latitudes of Kriti and Rhodes, two large islands that harbour a great number of species. As multiple regression results were consistent with partial correlations, they will not be treated further in this paper.

Also, when independent variables are highly correlated (such as area and elevation in this study), a multiple regression analysis can be affected by multicollinearity problems. To remove the multicollinearity and reduce the number of predictors, the predictors can be replaced by their principal component scores. This procedure may be also appropriate when different parameters might not, taken one by one, have important effects, but they could be statistically significant when taken all together or by pairs. Thus, a Factorial Analysis using a Principal Component Analysis (PCA) was performed with a VARIMAX normalised rotation on the eco-geographic variables. The three principal components (factors) with an eigenvalue higher than one were retained and the loadings examined to find their relationships with the original variables: F1 was mainly area and elevation, F2 was mainly longitude, and F3 was mainly latitude. As the principal components were uncorrelated (orthogonal) they were used as replacements for the original eco-geographic variables into a forward stepwise regression analysis. Only F1 contributed significantly to the model, thus confirming results obtained from previous analyses, without adding extra information. Therefore, the results obtained from this analysis will not be treated further.

In all tests, a minimum probability level of $p < 0.05$ was accepted (all tests were two-tailed). Statistical analyses were performed using STATISTICA software (version 4.5, 1993).

RESULTS

Species-area relationships

Species richness was significantly correlated with area of the Aegean Islands for all the three models (Fig. 2). As to the percentage of variance explained, the linear function model was the best fit, accounting for about 70% of the total variance. The exponential function model accounted for about 40% of the variance, and the power function model for about 35%. However, it should be noted that the R^2 goodness-of-fit statistic is not strictly appropriate for estimating the relationship between observed and predicted values of S. On the contrary, the appropriateness of a model should be judged by the lack of systematic error (SUGIHARA, 1981). Therefore, the standard error of the estimate (S_{yx}), and not the correlation coefficients should be used (LOBO & MARTÍN-PIERA, 1999), to check the appropriateness of the three models. Using the standard error of estimate, the power function

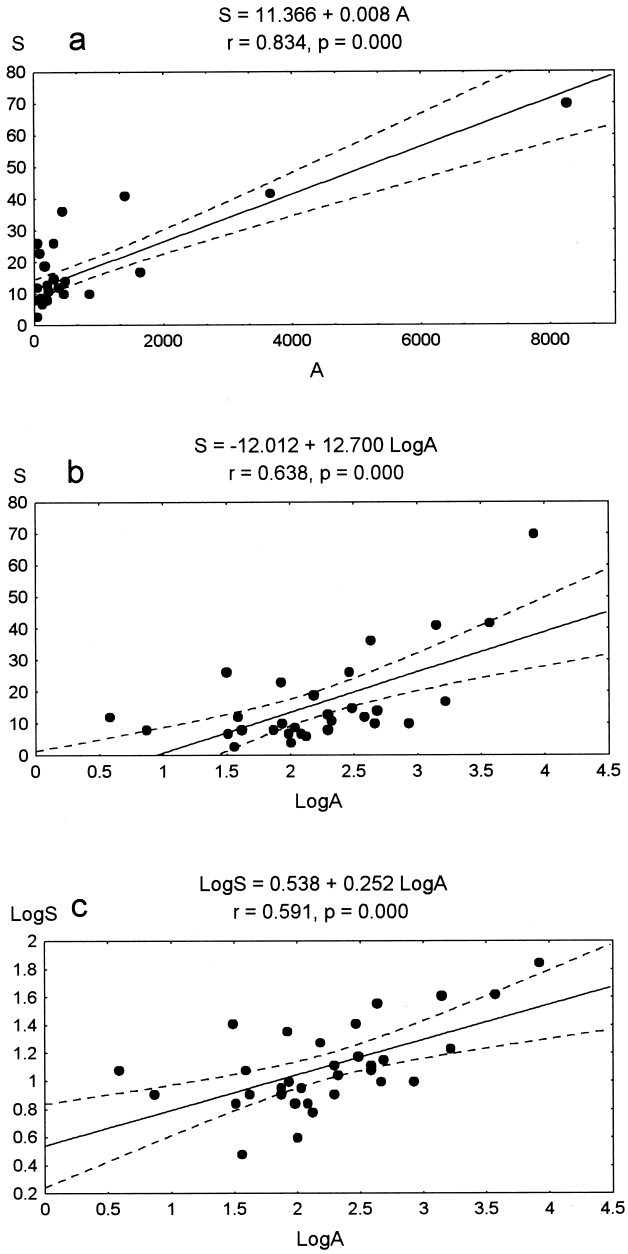

Fig. 2. – Species-area relation for tenebrionids of the Aegean Islands: a) linear function, b) exponential function, c) power function. Dotted lines correspond to 95% confidence limits.

appeared to be the best fit (S_{yx} = 0.246; actually it is the standard error of Log$S$; the standard error to be considered is that of S_{yx} = 1.761). Both the linear (S_{yx} = 7.860) and the exponential (S_{yx} = 10.966) models gave higher S_{yx}.

The species and subspecies number of Kriti (70 species and subspecies) is slightly inferior to that predicted by the linear function model (73.29), but much higher than would be expected from its area alone using the other models (33.48 with the power function, and 37.73 with the exponential model). Note that if the value of 58 species is used, the following predicted values are found: 63.66 with the linear function (S = 11.762 + 0.006A, r = 0.776, p = 0.000, S_{yx} = 8.085); 32.12 with the power function (Log$S$ = 0.556 + 0.243Log$A$, r = 0.582, p = 0.000, S_{yx} = 1.749); and 35.10 with the exponential function (S = -9.447 + 11.373Log$A$, r = 0.635, p = 0.000, S_{yx} = 9.909).

Correlation among variables

The following correlations were found among the eco-geographic variables: latitude with distance to the mainland (r = -0.513, p = 0.003), area with elevation (r = 0.874, p = 0.000), and area with distance to the mainland (r = -0.443, p = 0.011). However, the correlation of area with distance to the mainland did not resist the Bonferroni correction (k = 15).

The tenebrionid richness was positively correlated with both area (r = 0.591, p = 0.000) and elevation (r = 0.573, p = 0.000), also after the Bonferroni correction (k = 6). No correlation was found between species richness and other variables.

Partial correlation analysis

Partial correlation analysis showed that only area was significantly correlated with tenebrionid richness after holding latitude, longitude, elevation, distance to the mainland and distance to the nearest island constant (Table 2). These results suggest that only area *per se* is significantly correlated with tenebrionid richness when the effects of latitude, longitude, elevation, distance to the nearest island and distance to the mainland are removed, but that all these variables are not significantly correlated with species richness when the effect of area is removed.

TABLE 2

Partial correlations between tenebrionid richness (dependent variable) and eco-geographic variables (independent variables). The partial coefficient indicates correlation between the respective variable and the dependent variable, after controlling for all other independent variables.

Eco-geographic variables	$r_{partial}$	p-level t(26)
Latitude	-0.158	0.430
Longitude	0.231	0.245
Area	0.410	0.033
Elevation	0.023	0.909
Distance to the nearest island	0.135	0.501
Distance to the nearest mainland	0.256	0.196

DISCUSSION

A positive, statistically significant correlation between area and tenebrionid richness was found using linear, exponential and power functions. The power function model represented the best fit model. The z-value of 0.252 is very close to that (0.263) predicted by theory (e. g. SUGIHARA, 1981). However, after decades of intensive study of the species-area relationship (e. g. MACARTHUR & WILSON, 1967; MAY, 1975; CONNOR & MCCOY, 1979; GOULD, 1979; MARTIN, 1981; SUGIHARA 1981; WRIGHT, 1981; CROWELL, 1986; NEWMARK, 1986; NILSSON et al., 1988; SFENTHOURAKIS 1996a; LOBO & MARTÍN-PIERA, 1999; WELTER-SCHULTES & WILLIAMS, 1999), the question whether or not the values of z have any importance for ecological communities remains unresolved. Also, if the 0.20 to 0.40 range is considered as the null hypothesised range of slopes, only slope values deviating from this range should have biological significance (CONNOR & MCCOY, 1979). As the value observed in this study falls in the 0.2-0.4 range, the subject will not be treated further in this paper.

As to the C value, GOULD (1979) suggested that, when slopes are constant in families of related curves, C values could be compared as a size-independent invariant within a system. For the Aegean area, the C values of the power function model for land snails ($S = 9.48A^{0.18}$, WELTER-SCHULTES & WILLIAMS, 1999) and isopods ($S = 9.33A^{0.20}$, SFENTHOURAKIS, 1996a) can be compared with that observed for the tenebrionid beetles ($S = 3.45A^{0.25}$). It appears that, regardless of island size, the biota contains about three species of land snails and isopods (2.75 land snail species, 2.70 isopod species) for each tenebrionid species. This fact may suggest that (1): tenebrionid species require greater habitat extensions than do land snails or isopods (possibly because of the large size of some species such as those belonging to the genera *Tentyria*, *Akis*, *Pimelia* and *Blaps*, which can exceed 15-20 mm in length) and / or (2) tenebrionids are less able colonists (or, in other words, that they occur on islands mainly as relict species, as expected for very sedentary animals).

Distances to the nearest island and to the mainland were not identified as of any statistical importance in affecting species number. Lack of correlation between species number and island distance to the nearest island suggests that no 'stepping stone' processes are involved in determining tenebrionid species richness. According to dynamic models, species richness should be negatively related to the distance to the mainland. In this study, the distance to the mainland does not affect tenebrionid species richness. Therefore, as proposed for other very

sedentary animals such as the land snails (WELTER-SCHULTES & WILLIAMS, 1999), a relict model can be postulated for the present tenebrionid faunas on the Aegean Islands.

As tenebrionids have extremely limited ability to actively disperse over the sea, they have probably colonised the Aegean Islands by means of land-bridges during Pleistocene falls in the sea level.

During the Pleistocene regressions, most of the Cyclades were connected to each other, and their distances to the Balkan coasts were generally reduced or disappeared, while the Southern Sporades were connected to the Anatolian mainland (cf. STRID, 1970; BEUTLER, 1979; DERMITZAKIS & SONDAAR, 1981; DERMITZAKIS, 1990; SFENTHOURAKIS, 1996; WELTER-SCHULTES & WILLIAMS, 1999; and references therein). Tenebrionid species with very low dispersal abilities may have reached a continuous distribution between the mainland and present islands areas during the Pleistocene falls in the sea level. When the sea level was restored, islands became isolated and the ancestral populations occupying both mainland and island areas were subject to vicariance events, being divided into insular and mainland populations.

Palaeogeographic factors are probably involved also in the levels of endemism of the Aegean tenebrionids. During the Pleistocene lowering of the sea level, most of the remote islands were mutually connected, forming various Pleistocene groups of islands. As suggested by FATTORINI et al. (2000), these various island groupings were presumably located at different distances to the mainland, accounting for a positive correlation between proportion of sub-endemic taxa (i. e. taxa occurring on islands of the same group but not on islands of other groups) and distance to the mainland; only few islands were completely isolated, evolving strictly endemic taxa.

However, present dispersal can not be completely rejected a priori, at least for some species. Generally speaking, rafting, wind and water transport, frequently evoked as important factors that can assist island colonisation, may play an important role in determining long-distance chance dispersal especially for beetles associated with sandy shores (cf. HOWDEN, 1977). Based on data summarised by KRESTENITIS (available at http ://archimedes.civil.auth.gr/krestenitis/en/aegean.html) the complex circulation of the Aegean Sea can be sketched as follows. The prevailing winds in the Aegean Sea are summer northerly dry winds that cause a flow toward the south along the Greek coast and give rise to a two-gyre system, respectively in the west and in the east. In the summer, the North Atlantic waters enter the south Aegean Sea from the passages east and west of Kriti, forming a two-gyre system. There is also an eastward flow along the north coast of Kriti giving rise to a cyclonic circulation in the Cretan Sea. In the winter, an overall surface circulation exists with a northerly current along the Anatolian coast and a southerly current along the Greek coast. Although this type of circulation seems to be favourable to inter-island exchanges and island colonisation by mainland populations, the lack of correlation between species richness and island distance to the mainland or inter-island distance suggests that sea circulation cannot be evoked as a major factor in determining present distribution patterns. The present hydrodynamics of the Aegean Sea could be actually involved in the distribution of some strictly psammophilic species, which could be subject to over-water dispersal on 'rafts'. However, they are represented by few species, occurring on few islands, and are not expected to have any substantial influence on the general patterns here outlined.

For the Aegean tenebrionids as a whole, island area was the eco-geographic parameter accounting for most variability in species number. This agrees with the findings of WELTER-SCHULTES & WILLIAMS (1999) concerning land snails and with those of SFENTHOURAKIS (1996a) concerning land isopods. By contrast, the importance of elevation in determining tenebrionid richness is equivocal. A positive correlation between tenebrionid richness and elevation was observed by using the Pearson product-moment correlation, but not confirmed by partial correlations.

Interestingly, based on stepwise multiple regression and partial correlation analyses, SFENTHOURAKIS (1996a), who used both elevation and particular measures of habitat diversity (obtained as a sum of the presence of different types of environmental variables), found a correlation between isopod species richness and habitat diversity, but not between species richness and elevation. However, using the Pearson product-moment correlation coefficient, a positive correlation between species number and Log-transformed elevation ($r = 0.799$, $p = 0.000$) can be detected also for the SFENTHOURAKIS data.

The enhanced number of tenebrionid species associated with higher island elevations may indicate that an increased number of potential habitats may increase species richness. Actually, higher islands may contain a greater range of potential habitats, and so are richer in habitat types than low islands of the same area. On the other hand, partial correlations suggest an effect of area *per se*, while elevation appears uncorrelated with tenebrionid richness. As pointed out by NILSSON et al. (1988), to clearly differentiate between the effects of area *per se* and habitat diversity on species richness, it is necessary to study a system where these variables are uncorrelated. Unfortunately, in the Aegean Islands studied here, area and elevation were strictly correlated. Thus, it is not possible to give any definitive conclusion on this topic.

Using the power function and the exponential function, the tenebrionid richness of Kriti appears to be much higher than would be expected from its area alone. An observed value greater than predicted values has been also recognised for land snails by WELTER-SCHULTES & WILLIAMMS (1996), and probably reflects the palaeogeographic history of Kriti. During the lower Tortonian (11

Ma), massive marine ingressions in the northern and southern Aegean caused the separation of six or more islands in the region of present-day Kriti, which were joined only 3-2 Ma. This long isolation time allowed the species' populations on the palaeoislands to diverge sufficiently to become different species or subspecies (cf. WELTER-SCHULTES & WILLIAMMS, 1996). The high species and subspecies number for Kriti (22 of which are endemic, see FATTORINI et al., 2000), thus reinforces that the Aegean tenebrionids are relictual, not equilibrial.

CONCLUSIONS

As suggested by CROWELL (1986), the essential feature of equilibrium is continued immigration and extinction. Whereas enhanced distances affect species number in dynamic models by reducing immigration rates, distance is not an important determinant of species richness for relict faunas undergoing relaxation. As observed for land snails (WELTER-SCHULTES & WILLIAMS, 1999), the tenebrionid fauna of the Aegean Islands is not affected by distance effects. Therefore, this study rejects dynamic models as a principal or important cause of tenebrionid species richness on the Aegean Islands. Also, in accordance with the findings of WELTER-SCHULTES & WILLIAMS (1999) on Aegean land snails, area and possibly island elevation are identified as the most important factors affecting species number. As these two variables are highly correlated, it is not possible at present to discriminate between habitat diversity and area *per se* effects.

Although area and / or habitat availability together with historical effects have been the major factors in determining the species-area relationships, many other factors such as vegetation type and extent, anthropic pressures, or the presence of sandy beaches, may have concurred to determine the present-day island tenebrionid richness on each island. However, for most of the Aegean tenebrionids the effects of these factors are as yet unknown. For example, the maximum elevation of the island is an obviously important factor for some orophilic species such as *Asida fairmairei* Boieldieu, 1865 or *Pedinus subdepressus* Brullé, 1832. The occurrence of wooded areas (presently strongly reduced by man) is an important factor for those species either directly associated with trees, such as *Helops coeruleus* (Linné, 1758), *H. rossii* Germar, 1817 and *Nalassus plebejus* (Küster, 1850), or indirectly associated with this habitat, such as *Hypophloeus fraxini* Kugel, 1794 and *H. pini* Panzer, 1799 (both likely predators on scolitid beetles). Presence of well-preserved sandy shores and dunes is crucial for the life of psammophilic species such as *Ammobius rufus* Lucas, 1849, *Phaleria acuminata* Küster, 1852, *P. bimaculata* (Linné, 1767), *Trachyscelis aphodioides* Latreille, 1809, *Xanthomus graecus* Dajoz, 1984, and *X. ovulum* (Seidlitz, 1898).

However, new insights and more investigation are needed to ascertain the importance of present-day ecological factors in determining the tenebrionid distribution patterns in the Aegean. Further knowledge of the species' ecology may also clarify if area acts indirectly on species number through presence and absence of particular habitat types.

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S.E.M. and cytofluorimetric characterization of *Dinocras cephalotes* haemocytes (Plecoptera, Perlidae)

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ABSTRACT. Haemocytes of the stonefly *Dinocras cephalotes* have been characterized by light and scanning electron microscopy (SEM), adherence to a plastic surface, and phagocytosis of latex particles. Haemocytes appear to consist of at least two cell populations: granulocytes and plasmatocytes, which can be distinguished by morphological and functional criteria. Morphologically, granulocytes are rounded and vesiculated, while plasmatocytes have an irregular shape, with many filopodia, and a slightly vesiculated content. Culturing haemocytes "in vitro" resulted in the spreading onto the surface of an adherent population (plasmatocytes), and in a loosely attached population (granulocytes). Phagocytosis assay showed the capacity of *D. cephalotes* plasmatocytes to engulf exogenous particles. Haemocytes of adults and nymphs of *D. cephalotes* were also analysed by flow cytometry and compared with those of *Perla grandis*, another species of Perlidae. We observed a slight difference in haemocyte morphology among nymphs and adults, and a shift of cell populations was also detected.

KEY WORDS: haemocytes, stoneflies, plasmatocytes, granulocytes, phagocytosis, flow cytometry.

INTRODUCTION

The insect circulatory system is responsible for moving fluids through body cavities and appendages, but, as is well known, is not involved in respiration. Insect haemolymph is composed of a liquid part, or plasma, and of a cellular fraction with cells having different morphologies, collectively called haemocytes (RATCLIFFE et al., 1985; GUPTA, 1986). Haemocytes display many important physiological functions, such as wound healing during repair of the exoskeleton, in transferring molecules and nutrients (GUPTA, 1986), and in the defence response against pathogens, parasites and foreign substances (ANDERSON & CHAIN 1986; HUNG et al., 1993). These defence functions performed by haemocytes are exerted by means of cellular and humoral activities (DRIF & BREHÉLIN, 1989; BULET et al., 1991; MARMARAS et al., 1994; CHARALAMBIDIS et al., 1995; SCAPIGLIATI et al., 1997; SCAPIGLIATI et al., 1998; BULET et al., 1999).

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In Plecoptera, studies on *Acroneuria* and *Diura* species (ARNOLD, 1966; PRICE & RATCLIFFE, 1974) showed that the haemolymph contains 20-40000 cells/mm^3 and that haemolymph coagulation is very fast. Also, it was pointed out that blood composition in the adults changed with age (ARNOLD, 1966), although this can be due to the cell fixation as well as to cytolysis processes (In: ZWICK, 1980). There is no general agreement in the literature on the existence of different haemocytes. GUPTA (1985) stressed the existence of five haemocyte types in Plecoptera: prohaemocyte, plasmatocyte, granulocyte, spherulocyte and coagulocyte. PRICE & RATCLIFFE (1974) also considered the adipohaemocytes as a kind of haemocyte.

SUTCLIFFE (1962) gave some information on the chemical composition of *Dinocras cephalotes* (Curtis 1827) and *Perla bipunctata* (Pictet, 1833) haemolymph (osmolarity, conductivity, free amino acids, etc.), however, this study did not give information on the haemocyte morphology.

In the Plecoptera, as in other minor insect orders, the knowledge about haemocytes is scarce and more studies

on the morphology and the function of these cells are needed. The aim of the present study was to add new insight about Plecoptera haemocytes by using light and scanning electron microscopy (SEM), phagocytosis test and, for the first time in this insect group, flow cytometry analysis.

MATERIAL AND METHODS

Insects

Dinocras cephalotes and *Perla grandis* (Panzer, 1799) were collected in the River Nera (Italy) in June and July 1998 and in April and May 2000. Nymphs were collected with a net from the river. Adults were collected directly from vegetation or from the river bank rocks with entomological tweezers or by an entomological net. They were transported alive to the laboratory in cooled containers and used for the morphological and functional studies.

Haemocyte collection

Haemocytes were collected from specimens by dissection of the abdomen. The haemolymph was collected by carefully washing each insect with 0.15 M NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 and 8 mM Na_2HPO_4, pH 7.4 (PBS), opening the insect over a Petri dish in PBS containing 10 mM EDTA to prevent coagulation. Haemocytes were harvested by centrifuging at 450g for 5 min, washed in 500 µl of PBS, stained with 0.2% Trypan blue in PBS to assess cell viability and counted in a haemocytometer.

SEM analysis

Haemocytes were placed on coverslips in a Petri dish containing a culture medium (Grace's insect medium - GIBCO Europe, Paisley, Scotland) at 25-28°C for one hour, washed three times in PBS and fixed in 1ml/well of 5% glutaraldehyde and 4% paraformaldehyde buffered with 0.1M sodium cacodylate (pH 7.2) for 2 hr at 4°C (Karnovsky, 1965). After rinsing overnight in the same buffer, the samples were post-fixed in 1% OsO_4 in the same buffer for 1 hr at 4°C and dehydrated in a graded ethanol series (50% to 100%). Afterwards, the coverslips were dried by the critical point method with liquid CO_2 in a Balzer CPD 020 apparatus, attached to specimen stubs, coated with gold in a Balzer Union MED 010 evaporator, and observed with a 5200 Jeol JMS scanning electron microscope. Pictures were taken on Kodak T-max film exposed at 200 ISO.

Phagocytosis test

Haemocytes were placed on coverslips in a Petri dish containing culture medium at 25–28°C for one hour. After that, they were incubated in 2 ml of culture medium containing 5 µl of polystyrene latex particles of 0.8 µm diameter, for half an hour. Then they were washed in PBS and prepared for SEM observation as described above.

Cell culture and flow cytometry

Where necessary, cells were cultured for 2 hours at 25°C in 3-cm Petri dishes containing 3 ml of Grace's insect medium to allow adherent cells to attach and spread. Non-adherent cells were removed by washing the plate with PBS, and collected by centrifugation.

Cells were washed in PBS and fixed with 0.5 ml of 2% paraformaldehyde in PBS for 15 min at 4°C. Flow cytometry of non adherent cells was performed on an FACScalibur flow cytometer, and between 3,000 to 10,000 cells were counted for each experiment without the selection of a particular population during acquisition, unless indicated. Cells were analysed by the following parameters: side scatter (SSC), forward scatter (FSC), and spontaneous fluorescence channel (FITC) (Tierno de Figueroa et al., 2001).

RESULTS AND DISCUSSION

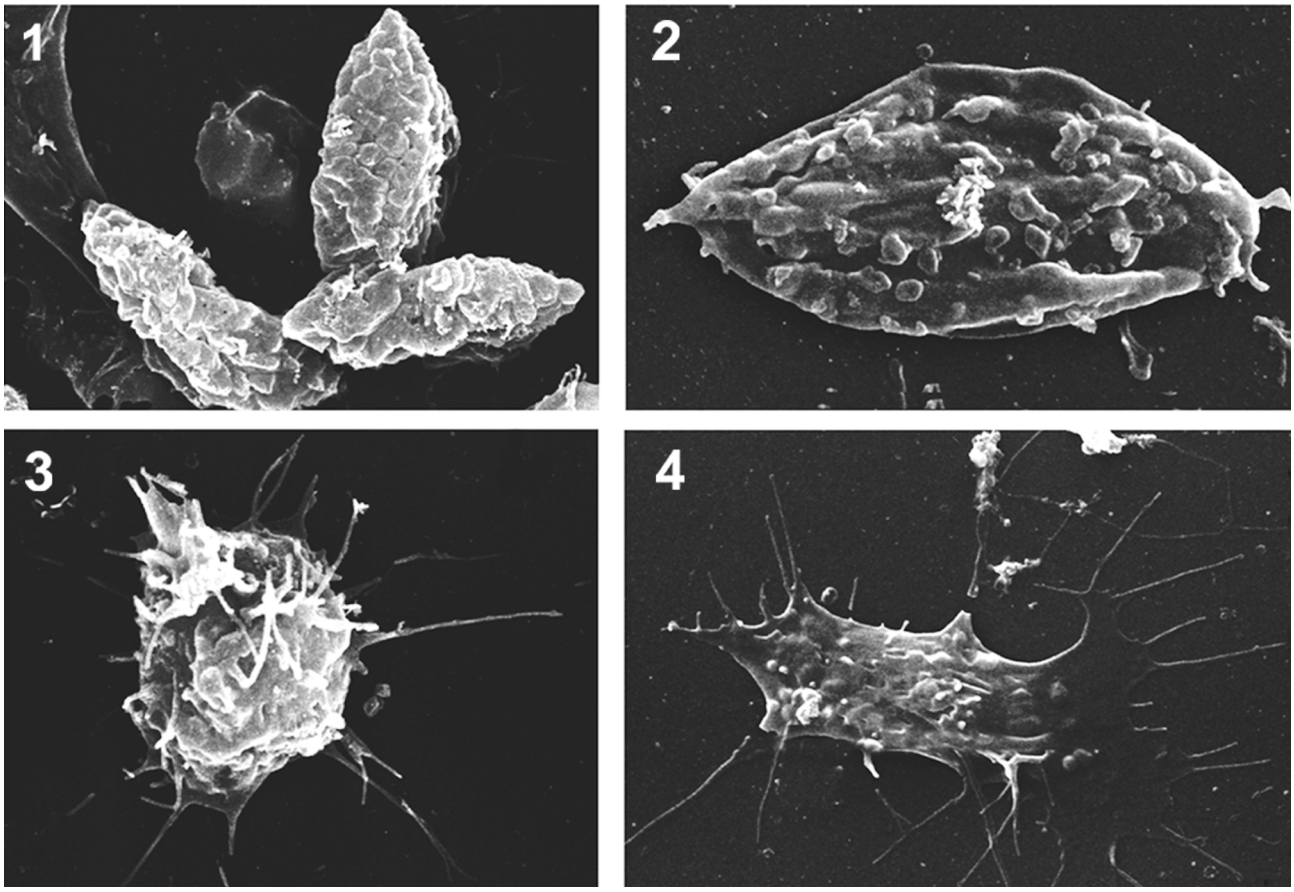
In this study we investigated cell composition of the haemolymph of the stonefly *Dinocras cephalotes*. SEM observations showed the presence of at least two different haemocyte populations: plasmatocytes and granulocytes (Figs 1-6), probably belonging to the two more widespread cell types found in the haemolymph of studied insect species (Gupta, 1985, Scapigliati & Mazzini, 1992).

Morphologically, granulocytes contain many granules and do not seem to have evident filopodia (Fig. 1), whereas plasmatocytes have an irregular shape, few or many filopodia, and little granular content (Fig. 1-6). These morphological differences could be related to different physiological properties, with plasmatocytes mainly involved in cellular reactions (phagocytosis and encapsulation), and granulocytes in humoral reactions (secretion), as previously shown in stick insects (Scapigliati et al., 1997).

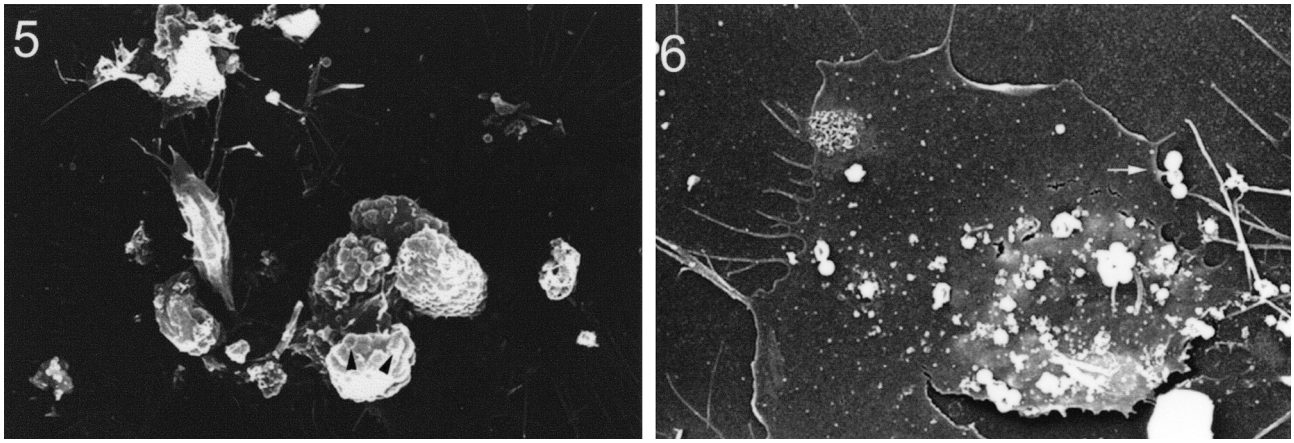
In relation to adherence capacity, during "in vitro" culturing, both cell types showed some adherence to a glass surface, but washing of culture wells with PBS showed that plasmatocytes were more strongly attached.

When adherent, insect plasmatocytes can have different morphologies, and *D. cephalotes* "spindle shaped" adherent plasmatocytes displayed high similarity with those of *Bacillus rossius* previously described (Scapigliati et al., 1993).

The phagocytosis of foreign particles was employed to distinguish between plasmatocytes and granulocytes (Scapigliati & Mazzini, 1994). In *D. cephalotes,* adherent haemocytes were able to phagocytose exogenous particles (Figs 5-6). These preliminary observations seem to



Figs 1-4. – SEM images of haemocytes of *Dinocras cephalotes*. 1. Granulocytes (x3000). 2. A "Spindle shaped" plasmatocyte (x5000). 3. A "sea urchin-like" plasmatocyte (x4500). 4. A completely adherent plasmatocyte (x3000).



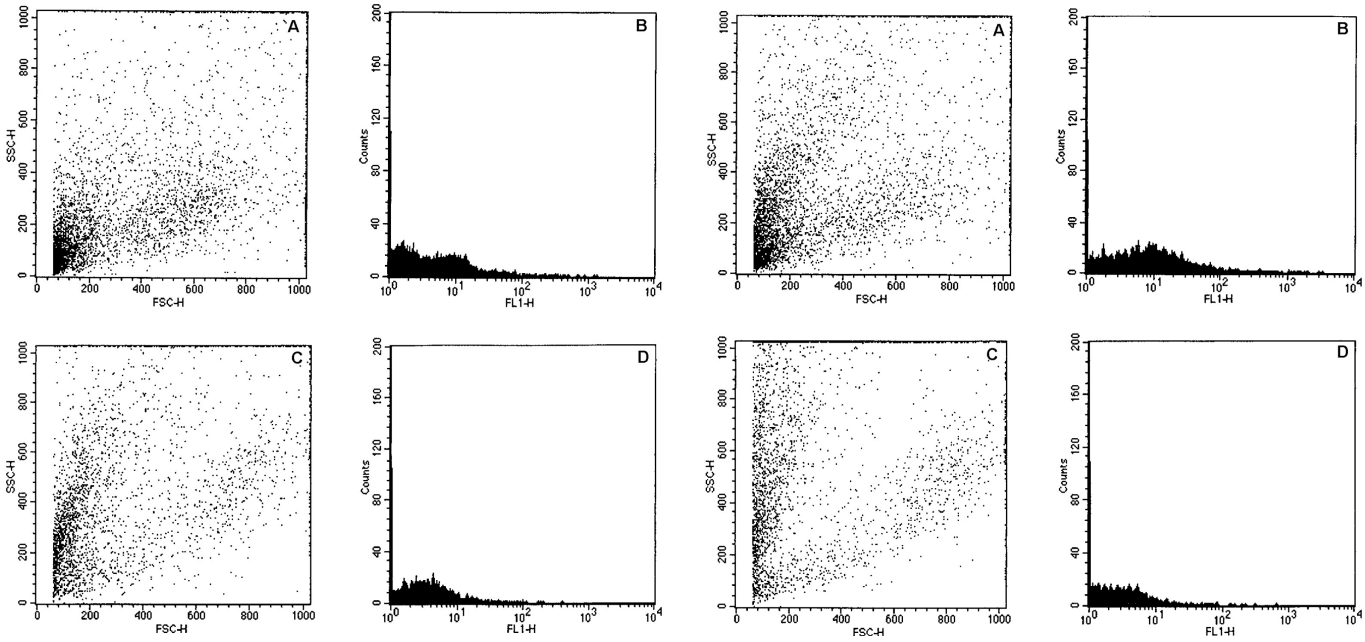
Figs 5-6. – Phagocytosis test with latex particles in *Dinocras cephalotes* (Fig. 5 x2000, arrowheads indicate latex particles engulfed; Fig. 6 x 2500, arrow indicates latex particles).

confirm that also in Plecoptera the phagocytic activity is restricted to plasmatocytes, as it has been pointed in other insects (Scapigliati & Mazzini, 1994).

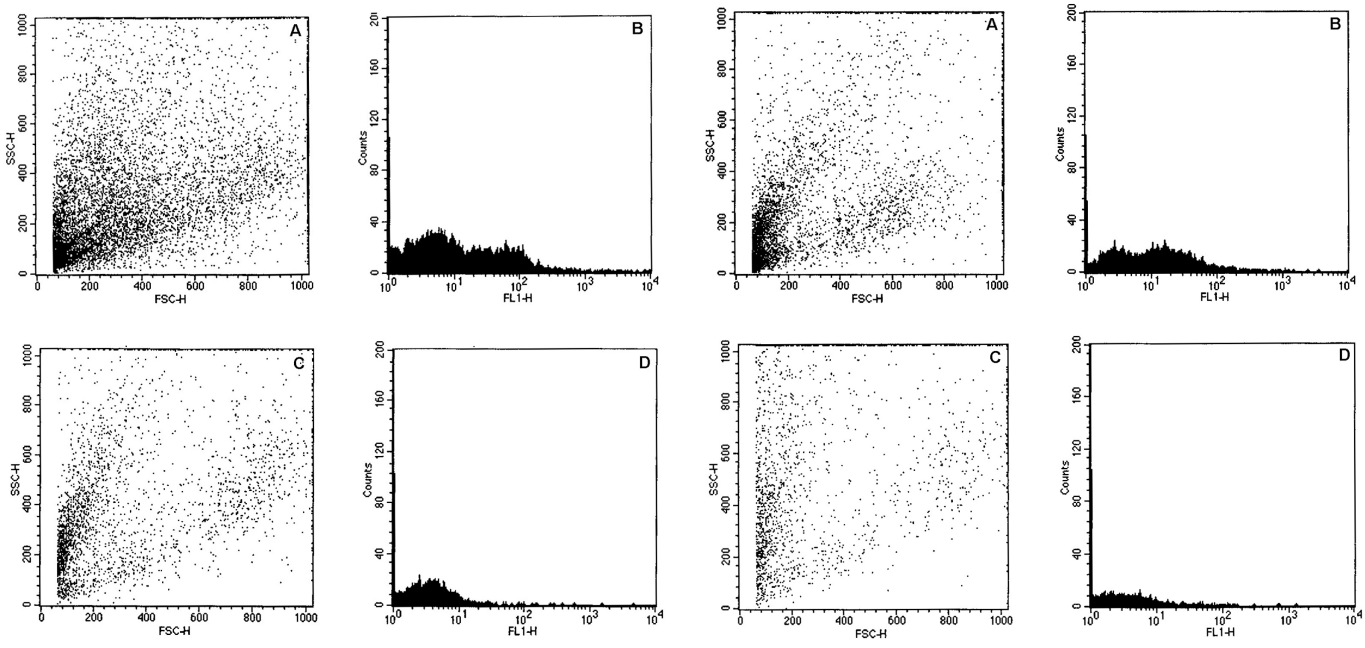
Other haemocyte types, such as prohaemocyte, spherulocyte or coagulocyte, present in other Plecoptera species and in other insect orders (Gupta, 1985), have not been detected in our study on *D. cephalotes* haemolymph.

Flow cytometry is a powerful technique to analyse the overall morphology of a great number of cells. FSC parameters describe the size of cells, increasing from low to high values, and SSC the "granularity", or the intracellular organ content. Analysing a mixed cell population will result in grouping cells having similar morphology in a same group with similar FSC and SSC coordinates. The flow cytometric analysis of haemolymph of *D. cephalotes* and *P. grandis* (the latter is a related species belonging to the same family Perlidae), showed certain homogeneity between the cell populations of the nymphs (young and mature) and the

adults (also when male and females are studied separately). Nevertheless, in the adult there is a decrease of cells having higher values of SSC and FSC. This decrease is observed in total haemocytes as well as in the adherent populations, and in both species (Figs 7-10, a, c), and it can be also appreciated by spontaneous fluorescence of cell populations (FL1 values) (Figs 7-10, b, d). These observations may be related to the species life cycle, because the adults do not feed and have a short life, in which they only mate and oviposit. It can be speculated that the change of relative number of cell types circulating in haemolymph during the imaginal life could be due to a progressive loss of some physiological functions related to the circulatory activity. Thus, a cytolysis process could be involved in this cell shift, confirming previous hypotheses by ZWICK (1980). Nevertheless, ARNOLD (1966) pointed out that in the Pteronarcyidae *Acroneuria arenosa* there is a progressive decrease in relative numbers of circulating plasmatocytes with age (in adults), due to their adherence to the walls of wing veins and presumably to others tissues within the body. In fact, JONES (1962) showed that in some cases in insects there is a decrease of the total haemocyte count due to adhesion of



Figs 7-8. – Flow cytometric analysis of *Dinocras cephalotes* haemocytes. Total (7) and non adherent (8) haemocytes were analysed for their FSC and SSC parameters (A, C), or for their fluorescence (B, D). Nymphs (A, B) and adults (C, D).



Figs 9-10. – Flow cytometric analysis of *Perla grandis* haemocytes. Total (9) and non adherent (10) haemocytes were analysed for their FSC and SSC parameters (A, C), or for their fluorescence (B, D). Nymphs (A, B) and adults (C, D).

haemocytes to the tissue surfaces, and that this change could be accompanied by significant changes in types of haemocytes. Taken together, our results and previous observations confirm that insect haemocyte cell populations are morphologically rather homogeneous. Also, it is possible that an active apoptotic process is involved in the shift of haemocyte populations during insect metamorphosis.

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Immunoreactivity of alpha- and beta-layers in lizard epidermis

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ABSTRACT. Reptilian epidermis may share some mechanisms of keratinization with those of mammalian and avian epidermis. The expression of keratins and proteins associated with the process of keratinization (filaggrin) and formation of the cell corneus envelope (loricrin) has been analysed at the light microscopic and ultrastructural level in the complex normal and regenerating lizard epidermis. The localization of alpha and beta-keratins, and of filaggrin-like and loricrin-like immunoreactivities has confirmed that the epidermis of lizards consists of two different layers, an alpha-layer localizing alpha-keratins and showing weak filaggrin- and loricrin-like immunoreactivities, as in the mammalian epidermis, and a beta-layer that localizes a beta-keratin immunoreactivity, such as in scutate scales and feathers of birds. The present study suggests that the segregation of alpha- from beta-keratin synthesis is correlated with the evolution of an intraepidermal shedding layer that allows the epidermal molt.

KEY WORDS: lizard, epidermis, keratins, loricrin, filaggrin, immunocytochemistry.

INTRODUCTION

The cell renewal of mammalian epidermis, apteric avian epidermis, crocodilian and turtle epidermises is determined by a more or less continuous production of cells from the germinative (basal) layer (ALEXANDER, 1970; FLAXMANN, 1972; MATOLTSY, 1987). Instead, in amphibian anurans (frogs and toads) and in lepidosaurian reptiles (lizards and snakes), a cyclical shedding of the outermost epidermal layers takes place and produces a molt (BUDZ & LARSEN, 1973, 1975; MADERSON, 1985; MADERSON et al., 1998). In relation to the periodic shedding process, epidermal cells progressively differentiate into specific cell layers that constitute cyclically-renewed epidermal generations. The most complex alternance of epidermal generations is present in the lepidosaurian epidermis where a specific and broadly accepted terminology has been established to describe this shedding cycle (MADERSON et al., 1972, 1998; MADERSON, 1985; LANDMANN, 1979; 1986; ALIBARDI, 2002; see Fig. 1).

In particular, an epidermal generation (outer epidermal generation) of lizards morphologically consists of six different layers: oberhautchen, beta-, mesos-, alpha-, lacunar-, and clear layer, the latter forming a shedding complex with the next oberhautchen of the following epidermal generation (Fig. 1A). During their terminal differentiation the lacunar cells, and in particular the cells of the clear layer, accumulate keratohyalin-like granules, which resemble mammalian keratohyalin, and which coalesce with keratin bundles during the terminal differentiation of these cells (ALIBARDI, 1998, 1999, 2000a). Instead, the clear layer of snakes does not accumulate these granules (Fig. 1B).

While the oberhautchen and beta-layer produce a hard form of keratinization (beta-keratin, see BADEN & MADERSON, 1970; BADEN et al., 1974; WYLD & BRUSH, 1979, 1983), the remaining layers produce a softer keratin (alpha-). The hard keratins present in oberhautchen cells form the microornamentation typical of the surfaces of lepidosaurian scales (IRISH et al., 1988) and of the long setae of the climbing pads of some geckos and lizards (MADERSON, 1970; ALIBARDI, 1997; see Fig. 1C). So far, scarce immunocytochemical and biochemical studies on keratin distribution have confirmed the above morphological, ultrastructural and biophysical data (CARVER & SAWYER, 1987; ALIBARDI et al., 2000, 2001). The knowledge of the molecules implicated in the process of keratinization in lower amniotes (reptiles) may give some indications on the evolution of the process of keratiniza-

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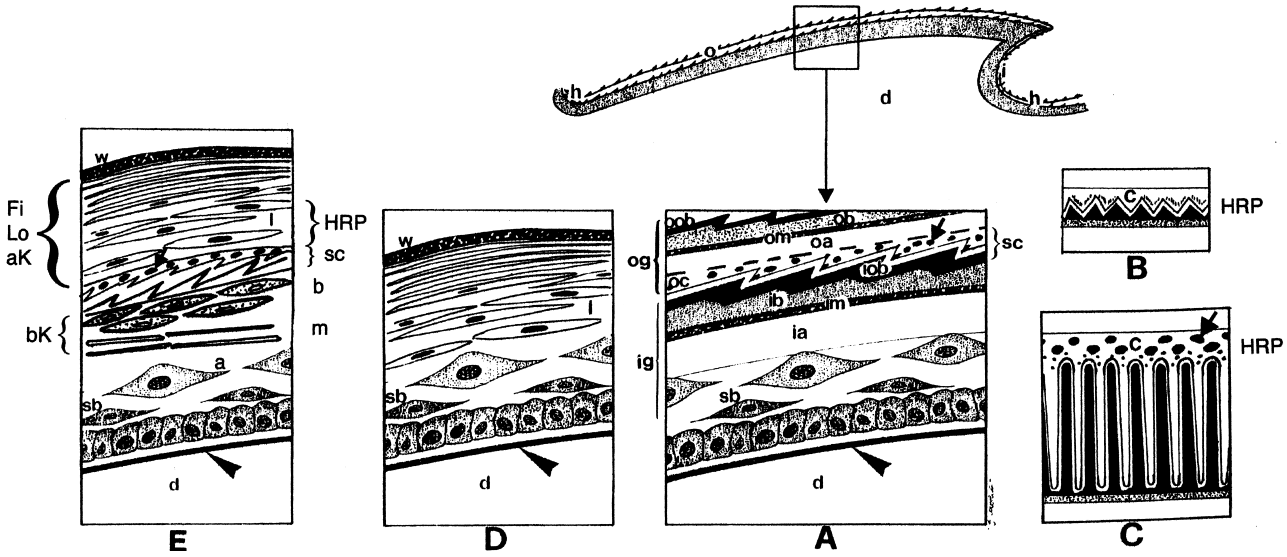

Fig. 1. – Schematic drawing illustrating the histology of lizard and snake scale. **A**, details of the epidermal stratification. **B**, aspect of the shedding complex in snakes (black areas represent the oberhautchen). **C**, aspect of the setae (black rods) of the shedding complex of gecko with keratohyalin-like granules in the clear layer (c, arrow). **D**, aspect of the initial phase of epidermal regeneration. **E**, later phase of epidermal regeneration with formation of the shedding complex and production of beta-keratin over alpha-keratin in oberhautchen and beta-layers. Legends: a, alpha layer; aK, alpha keratin production; b, beta layer; bK, beta keratin production; c, clear layer (containing keratohyalin-like granules, arrow); d, dermis; Fi, filaggrin-like immunoreactivity; h, hinge region; HRP, histidin-rich protein production; i, inner scale surface; ia, inner alpha-layer; ib, inner beta-layer; ig, inner epidermal generation; im, inner mesos layer; iob, inner oberhautchen; l, lacunar tissue; Lo, loricrin-like immunoreactivity; m, mesos layer; o, outer scale surface; oa, outer alpha-layer (incorporating also the lacunar layer); ob, outer beta-layer; oc, outer clear layer (containing keratohyalin-like granules, arrow); og, outer epidermal generation; om, outer mesos layer; oob, outer oberhautchen; sc, shedding complex. sb, suprabasal cells; w, wound epidermis. The arrowhead points to the basement membrane.

tion present in mammals (ALIBARDI, 2002; MADERSON & ALIBARDI, 2002).

Aside from alpha- and beta-keratins, the presence of filaggrin (a histidin-rich protein implicated in keratin aggregation in mammalian keratinocytes, see RESING & DALE, 1991; ISHIDA-YAMAMOTO et al., 2000), and loricrin (a sulphur-rich protein implicated in the formation of the mammalian cell corneus envelope, see MEHREL et al., 1990) has been presently analysed. The study on the expression of the last mentioned proteins was selected according to previous histochemical and autoradiographic studies on sulphur-rich and histidin-rich epidermal layers in lepidosaurian reptiles (SPEARMAN, 1966; BANERJEE & MITTAL, 1978; MITTAL & SINGH, 1987; ALIBARDI, 2001). No comprehensive study is presently available on the molecular characterization of these two types of keratinization (alpha- and beta-) in lizard epidermis. Since the process of epidermal differentiation in both normal and regenerating epidermis of lizards is similar (ALIBARDI, 1998, 2000a,b; ALIBARDI et al., 2000, 2001), both normal and regenerating epidermis can be used to study the process of keratinization.

The present immunohistochemical study attempts to: 1) summarise what is presently known in terms of distribution of some specific proteins in lizard epidermis, 2) address future molecular studies on the modalities of keratinization in reptilian epidermis, 3) compare the modality of keratinization in lizards with those of other vertebrates.

MATERIAL AND METHODS

Ten adult wall lizards (*Podarcis muralis*, Laurentii 1768) with normal and regenerating tails were used in this study. Six geckoes (*Hemidactylus turcicus*, Linnaeus 1758) were used to study the skin of the climbing pads of the arms and feet. Skin pieces, 2-5 mm long, were collected and immediately fixed in Carnoy's fluid (9 parts of ethanol and 1 part of acetic acid) or in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 4-6 hours, dehydrated in ethanol, and embedded in Bioacryl resin under UV light at 0-4°C (SCALA et al., 1992).

Immunocytochemistry was performed on 1-4 μm thick sections to localize keratins, filaggrin and loricrin as previously reported (ALIBARDI, 2000a,b). The beta-1 antibody, produced in rabbit against a chick scale beta-keratin was a kind gift of Dr. RH Sawyer (Biological Science Department, University of South Carolina, Columbia, USA, see SAWYER et al., 2000). The anti-alpha keratin antibodies (AE2, AE3, from mouse hybridoma lines), were purchased from Progen (Heidelberg, Germany): they recognize most keratinization-specific and basic alpha-keratins (SUN et al., 1983; O'GUIN et al., 1987). The anti-filaggrin antibody was characterized and kindly sup-

plied by Dr. B.A. Dale (Department of Oral Biology, University of Washington, Seattle, USA). It is a polyclonal antibody (#466) produced in rabbit and directed against rat filaggrin (DALE B.A., personal communication; RESING & DALE, 1991). It specifically stains keratohyalin-like granules of the granular layer of both human and rat epidermis, and a rat filaggrin band isolated by electrophoresis and immunoblotting (Alibardi & Maurizii, unpublished observations). The anti-loricrin antibody, produced in rabbit, was kindly supplied by Dr. E. Fuchs (Howard Hughes Medical Institute, University of Chicago, USA), and recognises a 15-amino acid sequence toward the C-terminal of mouse loricrin (MEHREL et al., 1990).

Tissues were preincubated for 20 minutes in 5% normal goat serum in 2% BSA in 0.05 M Tris/HCl buffer at pH 7.6, in order to neutralize aspecific antigenic sites on the sections, then incubated overnight at 4°C in the same medium (without the goat serum) containing the primary antibody (dilutions 1: 100-200 for beta-1, 1: 50-100 for alpha-antibodies, 1: 500-1000 for filaggrin, and 1: 200 for loricrin). After prolonged rinsing in the medium, the sections were incubated for 1 hour at room temperature in the same medium containing 1:50 of anti-mouse-IgG (for AE2 and AE3 detection) or anti-rabbit-IgG (for beta-keratin, filaggrin, loricrin detection) FITC conjugated secondary antibodies. After extensive rinsing, sections were mounted in Fluoromount (EM Sciences, USA), and observed under a Zeiss epifluorescence microscope equipped with a fluorescein filter.

For immunoelectron microscopy, 40-90 nm thick sections were collected on nickel grids, and immunostained with the primary antibody as above, but using 1% Cold Water Fish Gelatin in the Tris/HCl buffer to saturate non-specific bindings sites. An anti-mouse or anti-rabbit IgG conjugated to 10 nm large gold particles (Sigma, USA, or Biocell, UK) was used as the secondary antibody. Sections were studied, unstained or lightly stained with uranyl acetate, under a CM-100 Philips electron microscope.

RESULTS

The epidermis in lizards during post-shedding (resting) condition consists of a keratinized pale beta-layer and a darker alpha-layer underneath, followed by suprabasal cells and a basal layer (Fig. 2A). The epidermis of a regenerating tail initially consists of a pluristratified epithelium with a keratinized alpha-layer: the wound epidermis (see Fig. 1D). The AE2 antibody labels essentially the keratinized alpha-layer of normal and regenerating epidermis, a pattern also seen after application of the filaggrin- (Fig. 2C, D), and loricrin-antibodies (Fig. 2E, F). All the other layers (beta and living layers) remain unlabelled with these antibodies.

The ultrastructural analysis shows that while the AE2 antibody specifically labels the external alpha-keratinocytes of both normal and regenerating epidermis, the filaggrin antibody barely, if at all, immunolabels the external keratinocytes (Fig. 2G, H). The keratohyalin-like granules of the clear layer (see Fig. 1A, C) are not labelled with both anti-keratin (AE2 and AE3) and anti-filaggrin antibodies, while they are diffusely but specifically labelled with the anti-loricrin antibody (Fig. 2I). The alpha-keratinocytes of the alpha-layer or of the wound epidermis are diffusely but significantly labelled with the antiloricrin-antibody (Fig. 2J).

The distribution of the beta-1 antibody shows that it recognises antigens exclusively localized in the oberhautchen (weak labelling) and beta-layer (stronger labelling) of both normal and regenerating scales, and over the elongated oberhautchen spinulae (the setae) and the thin beta-layer of the digital pads of gecko (Fig. 3A-F). A weaker labelling is also seen in the first cells of the mesos layer (Fig. 3A) and in the basal-most part of the wound epithelium of the regenerating tail, although the latter labelling was not constantly seen (Fig. 3D). The beta-keratin labelling is substituted by alpha-keratin labelling in the lowermost mesos and alpha-layers (Fig. 3G, H).

The detailed ultrastructural analysis of lizard epidermis shows that, initially the beta-keratin packets of the oberhautchen cells (the small bundles of beta-keratin) are barely, if at all, labelled with the beta-1 antibody (Fig. 3I). Conversely, the compact matrix of the mature beta-layer (Fig. 4A) or beta-keratin bundles within the differentiating beta-cells of the inner beta-layer are intensely labelled with the beta-1 antibody but not with the AE3 antibody (Fig. 4B-D). Keratohyalin-like granules and tonofilament bundles in lacunar and clear cells are also unlabelled with the beta-1 antibody (Fig. 4D). Also the forming setae of the climbing pads of geckos show a specific immunolabelling of the growing and mature setae of the oberhautchen layer while no tonofilaments of the clear cells are labelled (Figs. 3F, 5A-C). At the beginning of setae formation only the pale keratin filaments of the core are labelled while the external fibrous material is not labelled (Fig. 5A). In the growing setae, the more external and denser bundles of beta-keratin are also less labelled than those located more centrally, which are electron-paler (Fig. 5B).

The small bundles of beta-keratin in differentiating beta-cells progressively merge and form larger beta-keratin masses, which are intensely labelled. This contrasts with the unlabelled tonofilaments of the undifferentiated cells present underneath the beta-layer (pre-beta and mesos-cells, Fig. 6A). In early differentiating beta-cells the labelling is also located over few roundish dark granules, sparse among the beta-keratin filaments (Fig. 6B). The latter granules are more frequent in presumptive, undifferentiated mesos-cells underneath the layer of beta-cells, and are located among unlabelled tonofilaments and vacuoles occupied by electron-pale lipid-like material (Fig. 6C). This further indicates that early in their differentiation the first mesos-cells contain some beta-keratin material.

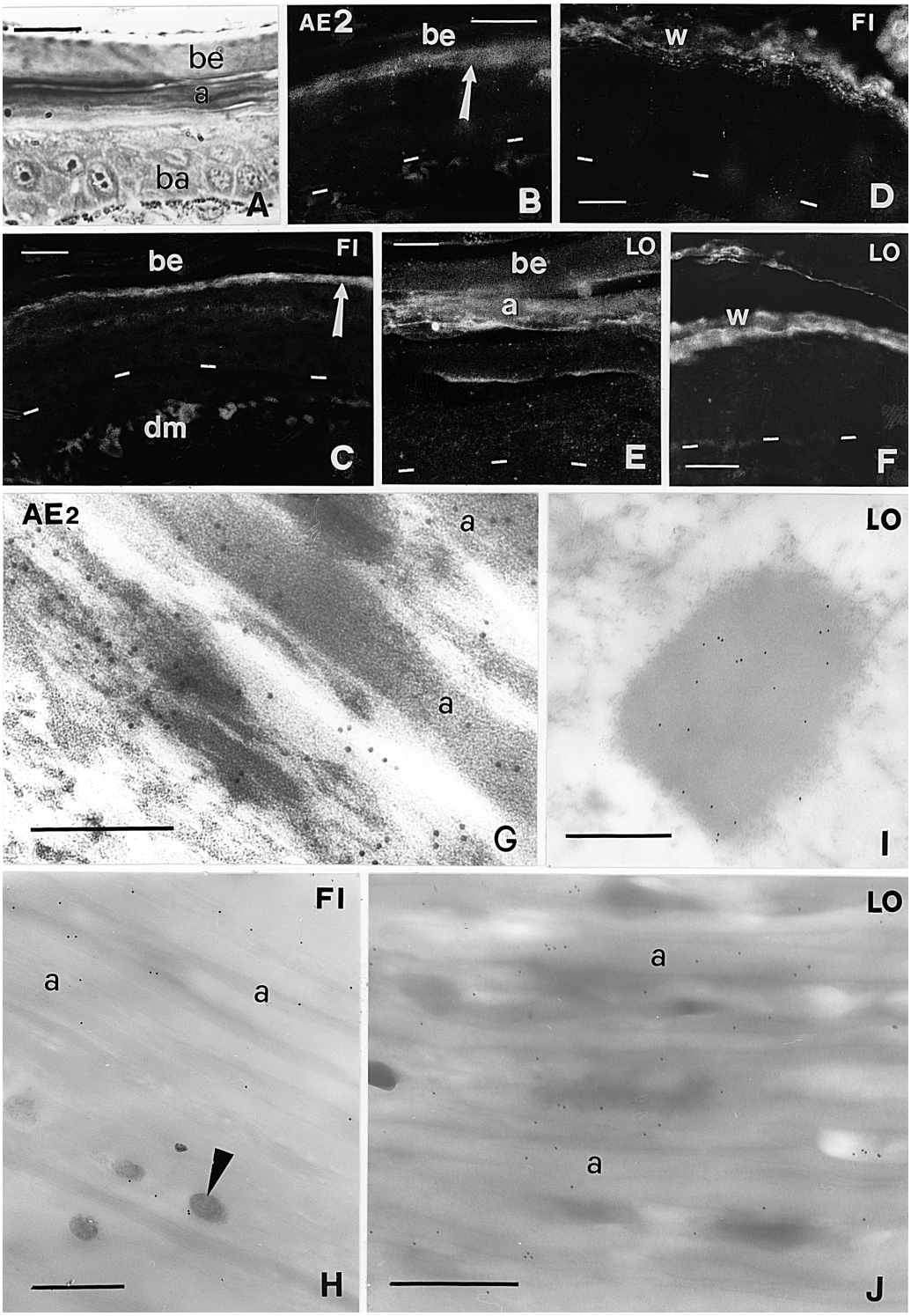

Fig. 2. – **A**, toluidine blue staining of a longitudinal section of normal lizard epidermis. Bar, 20 μm. **B**, AE2 immunofluorescence present in the alpha-layer of normal epidermis only (arrow). Bar, 20 μm. **C**, Filaggrin-like immunofluorescence in the alpha-layer (arrow) of normal epidermis but not in the other tissues. Bar, 20 μm. **D**, filaggrin-like labelled wound epidermis of regenerating epidermis. Bar, 20 μm. **E**, loricrine labelling of the alpha-layer only (a, artifactually detached from the underlying living layers) of the normal epidermis. Bar, 10 μm. **F**, loricrine immunolabelling of the wound epidermis of regenerating epidermis. Bar, 20 μm. **G**, alpha-keratinocytes (a) of the wound epidermis of regenerating epidermis decorated with gold particles after AE2 antibody staining. Bar, 200 nm. **H**, regenerating epidermis. Diffuse labelling for filaggrin of the alpha-keratinocytes (a) of the wound epidermis. The arrowhead points to a melanosome incorporated into the corneus layer. Bar, 500 nm. **I**, loricrine-labelled keratohyaline-like granule in the clear layer of regenerating epidermis. Bar, 500 nm. **J**, regenerating epidermis. Diffuse loricrine labelling of alpha keratinocytes (a) of wound epidermis. Bar,500 nm. **Legends**: a, alpha layer; AE2, AE2 immunolabelling; ba, basal layer; be, beta layer; dm, dermal melanophores (non-specific yellow stain); FI, filaggrine immunolabelling. LO, loricrine immunolabelling; w, wound epidermis; Dashes underline the basal layer.

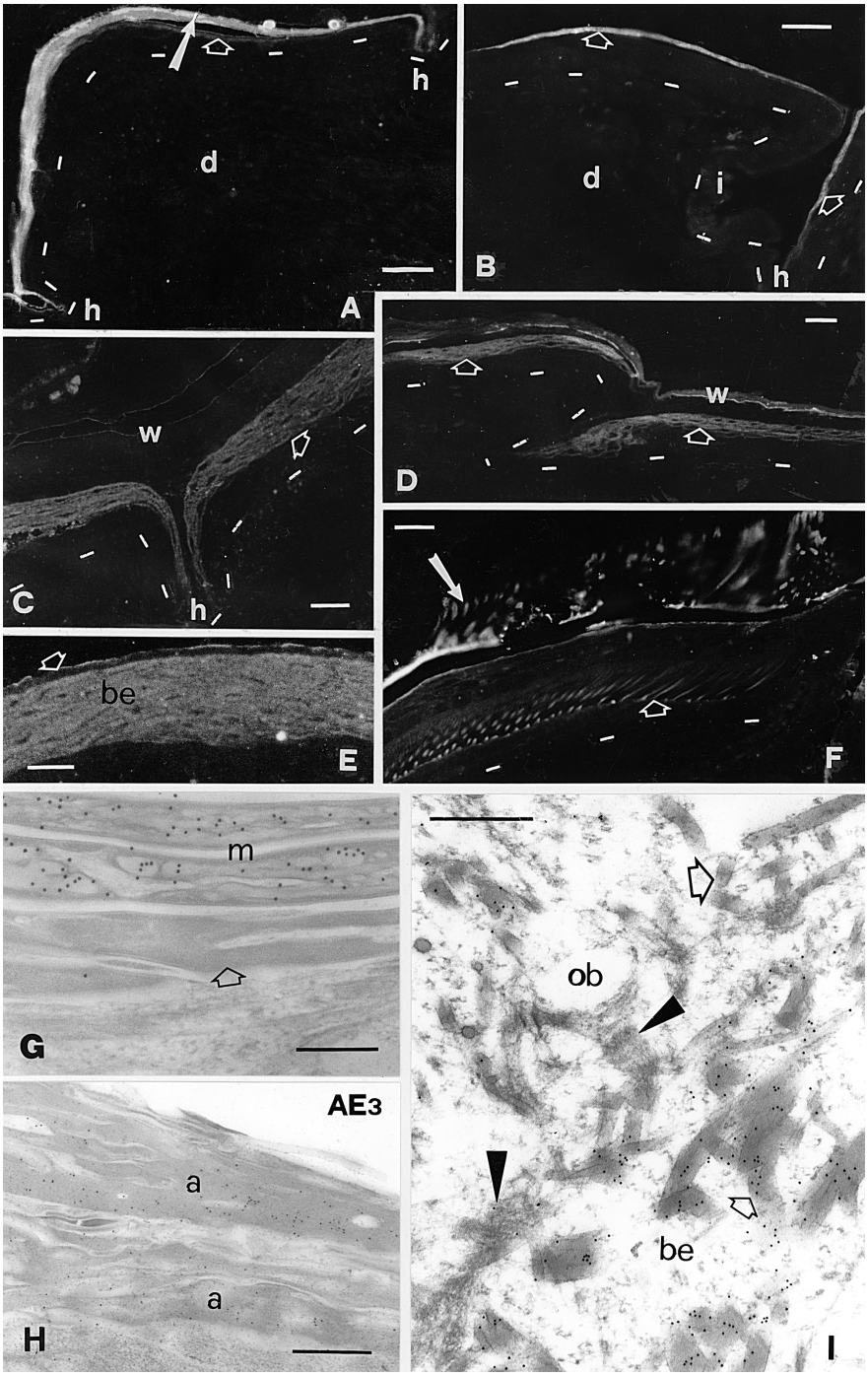

Fig. 3. – **A**, cross section of lizard scale in post-shedding condition (normal epidermis) showing beta-1 labelling in the outer beta-layer (arrow) and less in the mesos-layer (empty arrow). Bar, 20 μm. **B**, longitudinal section of gecko scale in post-shedding condition (normal epidermis) showing beta-1 labelling only in the beta layer of the outer scale surface (empty arrows), but not in the inner scale surface and hinge region. Bar, 20 μm. **C**, cross section of lizard regenerating scale showing beta-1 immunolabelling only in the inner beta-layer (empty arrow). Bar, 20 μm. **D**, longitudinal section of lizard regenerating scale showing beta-1 immunolabelling only in the inner beta layer (empty arrows) and in lowermost wound epidermis (w). Bar, 20 μm. **E**, detail of beta-1 labelling of inner beta layer of regenerating epidermis. The empty arrow points to the weakly positive oberhautchen. Bar, 10 μm. **F**, normal epidermis. Tip of modified scale of gecko climbing pads stained with the beta-1 antibody. Only the setae and the oberhautchen-beta layer of the outer (arrow) and those of the inner (empty arrow) generation are labelled. Bar, 20 μm. **G**, normal epidermis. Ultrastructural detail of mesos-layer of gecko scale showing beta-1-labelling gold particles in the upper mesos cells but not in the lower mesos cells (empty arrow). Bar, 200 nm. **H**, normal epidermis of gecko scale. Other cells of the mesos and alpha-layer (a) are labelled with the AE3 antibody. Bar, 500 nm. **I**, regenerating epidermis of lizard. Intensely beta-1-labelled beta-keratin packets (small empty arrow) in beta cell (be) beneath the cytoplasm of an oberhautchen cell (ob), which contains fibrous material (arrowheads) and unlabelled (large empty arrow) or little-labelled beta-packets. Bar, 500 nm. **Legends**: a, alpha-layer; AE3, AE3 labelling; be, beta cells; d, dermis; h, hinge region; i, inner scale surface; m, mesos layer; ob, oberhautchen cell; w, wound epidermis. Dashes underline the basal layer.

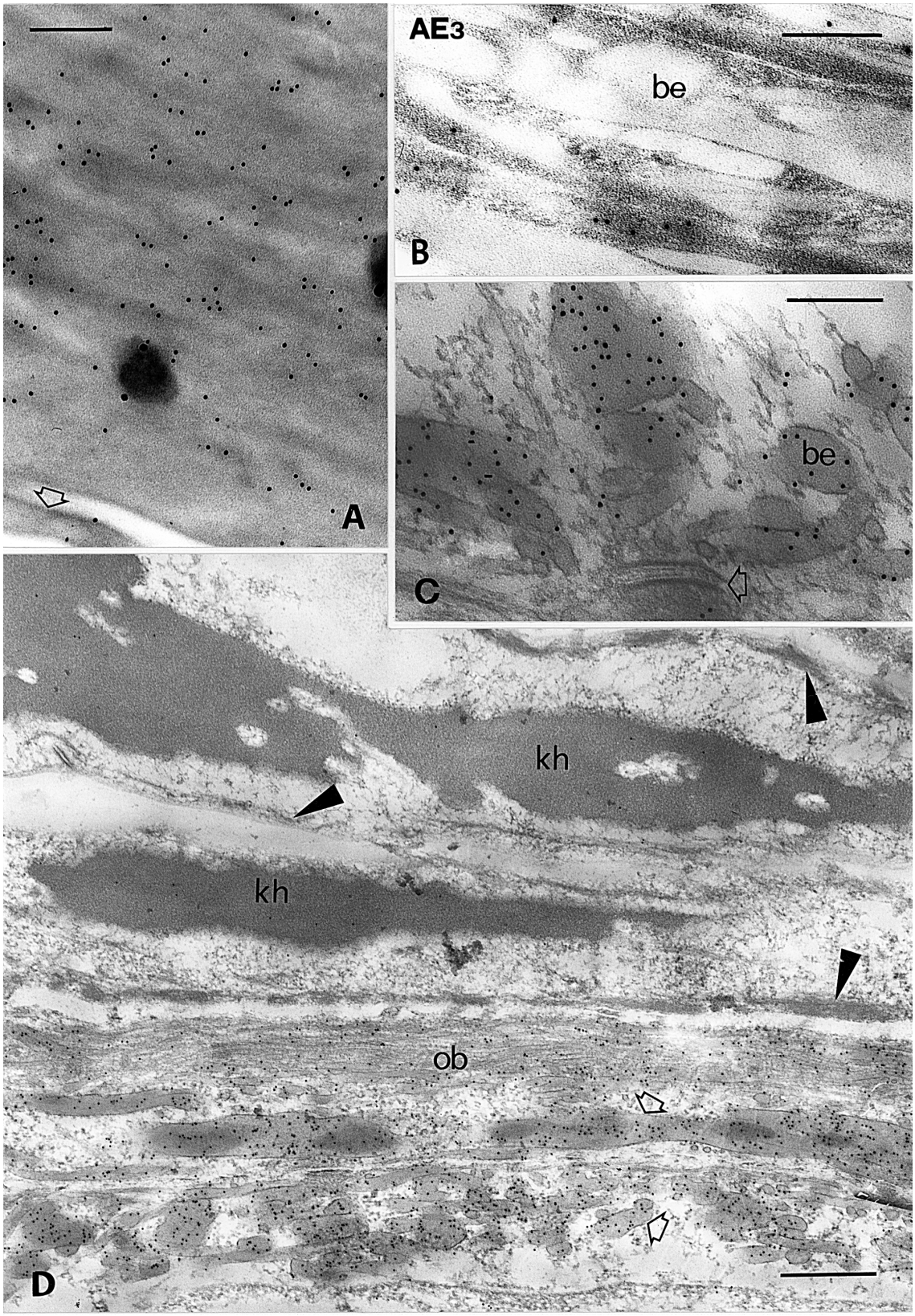

Fig. 4. – **A**, normal lizard epidermis. Uniformly-labelled outer beta-layer with the beta-1 antibody, contacting a narrow mesos cell (empty arrow). Bar, 200 nm. **B**, absence of AE3 immunolabelling over beta-packets (be) of differentiating beta-cell in regenerated scale. Bar, 200 nm. **C**, regenerating epidermis. Beta-1 immunolabelled beta-packets (be) of forming beta-cell, which contacts an unlabelled desmosome (empty arrow). Bar, 200 nm. **D**, regenerating epidermis. Detail of passage region between the clear layer and the underlying oberhautchen (ob) immunostained with the beta-1 antibody. No labelling is present over tonofilaments (arrowheads) and the large keratohyalin-like granules (kh) in the clear layer. Over the oberhautchen cytoplasm is seen a diffuse labelling, which becomes intense over the large beta-packets at the bottom (empty arrows). Bar, 500 nm.

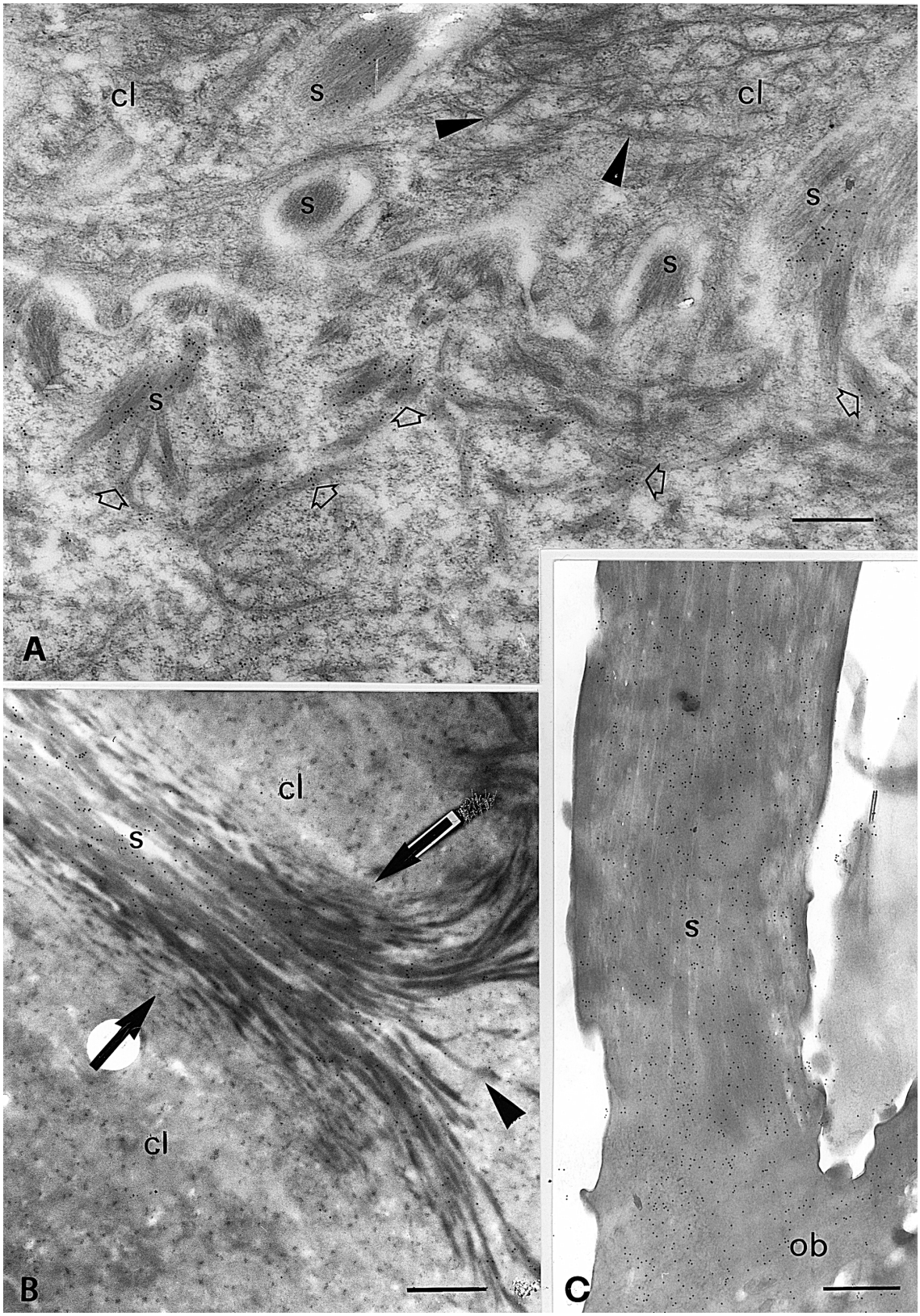

Fig. 5. – **A**, normal epidermis of gecko. Detail of forming setae of gecko; shedding complex stained with beta-1 antibody. Only the beta packets (empty arrows) at the base of the setae (s) are decorated with gold particles but not the tonofilaments (arrowheads) in the cytoplasm of clear cells (cl). Bar, 500 nm. **B**, normal epidermis of gecko. Growing setae of inner generation (s, the large arrows indicate its lateral boundaries, and the arrowhead its base) containing labelled central beta-keratin filaments. No structure is labelled in the cytoplasm of surrounding clear cells (cl). Bar, 500 nm. **C**, normal epidermis of gecko. Mature outer setae (s) in continuity with the oberhautchen (ob), which are homogeneously labelled with the beta-1 antibody. Bar, 500 nm.

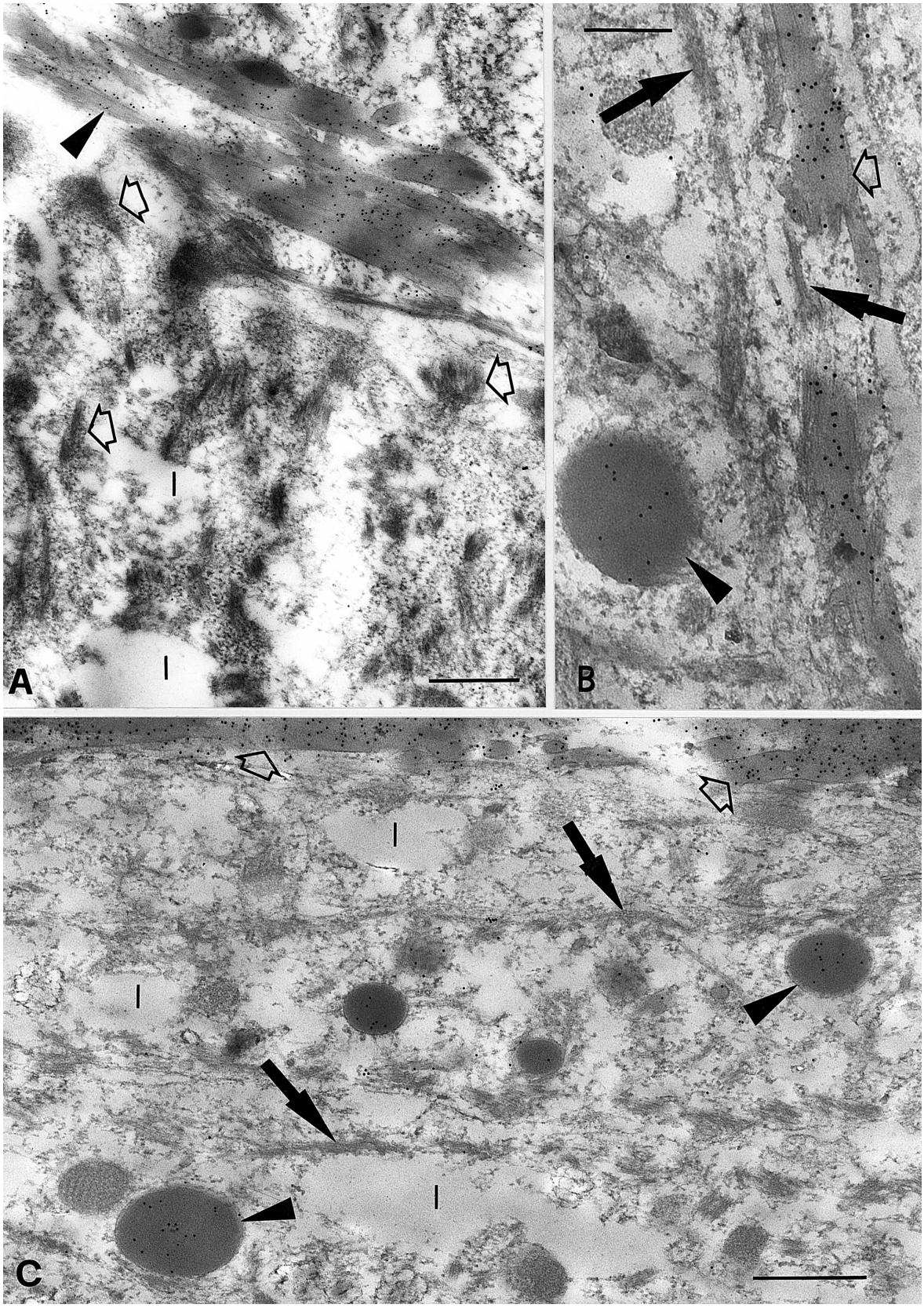

Fig. 6. – **A**, regenerating epidermis of lizard. Detail of beta-1-immunolabelled beta-filaments (arrowheads) in maturing beta cell as compared with the unlabelled tonofilaments (empty arrows) of undifferentiated beta cell at the bottom containing lipidic vacuoles (l). Bar, 500 nm. **B**, regenerating epidermis of lizard. Detail of beta-1-labelled, pale beta-filaments (empty arrows) and of a denser roundish granule (arrowhead) in beta cells. Fibrous tonofilaments are unlabelled (large arrows). Bar, 500 nm. **C**, regenerating epidermis of lizard. Detail of a forming mesos cell beneath the beta-layer (empty arrows), which contains lipid droplets (l) and unlabelled tonofilaments (large arrows). Only few dense granules are labelled (arrowheads). Bar, 500 nm.

TABLE 1

Summary of the immunoreactivities of different epidermal layers (of both normal and regenerating epidermis) to the antibodies utilized in this study (AE2, AE3, Beta-1; Filaggrin; Loricrin). -, negative; -/+, negative or low to diffuse; +, positive. ++, strongly positive. (forming)= early differentiation; (mature)= complete differentiation; one single indication refers to both forming and mature layers

Epidermal Layers	AE2	AE3	Beta-1	Filaggrin	Loricrin
Oberhautchen	-	+ (forming) -/+ (mature)	-/+ (forming) + (mature)	-	-
Beta	-/+	+ (forming) -/+ (mature)	++	-	-
Mesos	-	-/+	-/+	-	-
Alpha	+ or ++	+ or ++	-	-/+ or +	+
Lacunar	- (forming) + (mature)	+	-	-/+ or +	-/+ or +
Clear	- (forming) + (mature)	+	-	-/+ or +	-/+ or +

Table 1 summarises the immunoreactivities of the different epidermal layers of both normal and regenerating epidermis.

DISCUSSION

The present study confirms previous data (ALEXANDER & PARAKKAL, 1969; BADEN & MADERSON, 1970; MADERSON et al., 1972; LANDMANN, 1979), and indicates that lizard epidermis consists of two superimposed, alternating, modalities of keratinization, alpha- that resembles mammalian keratinization, and beta- that resembles avian keratinization.

Also, the observations of the present study complete previous analyses on the distribution of alpha-keratins and keratin-associated proteins in the epidermis of lizards (ALIBARDI, 2000 a,b). Beta-keratin substitutes for alpha-keratin, and is packed into hard and compact beta-cells that merge into a resistant, electron-pale beta-layer containing sparse electron-denser areas. The similarity of some antigenic epitopes of lizard beta-keratin with those of chick beta-keratin is demonstrated by the specific immunolocalization with this antibody produced against chick scale beta-keratin (CARVER & SAWYER, 1987; SHAMES et al., 1988, 1989; SAWYER et al., 2000). The labelling further suggests a phylogenetic affinity between reptilian and avian hard (beta) keratins (SAWYER et al., 2000). This immunoreactivity is well manifested in the compact form of beta-keratin of the mature beta-layer, within the thick beta-packets and in the thick filaments of compacting beta-cells.

The present observations also indicate that the two layers of passage between alpha to beta (oberhautchen, see Fig. 1A) and beta to alpha (mesos), contain small quantities of beta-keratin. While the mesos layer (and the wound epidermis of regenerating tail) rapidly looses beta-keratin as alpha-keratogenic cells are generated underneath, the oberhautchen layer rapidly accumulates beta-keratin as it merges with the underlying beta-cells.

Therefore the epidermis of lizards seems to utilise the mechanism of alpha-keratinization for the production of a softer layer where the extensibility of this keratin may be used for keeping elasticity in the hinge region among scales. The rigid, hard (beta) keratin covering of the outer scale surface also protects the underlying mesos and alpha-layers where the water-barrier is located (MENON et al., 1996; MADERSON et al., 1998). In fact, the mesos layer and also the alpha-layer accumulate lipids that form the barrier, limiting water loss. However, because these layers have a limited extensibility, they must be periodically shed in order to allow somatic growth, and are replaced by a new epidermal generation (MADERSON et al., 1998).

Without biochemical data it remains uncertain whether filaggrin and loricrin molecules are really present in the compact alpha-layer of lizard epidermis (ALIBARDI, 2001). The case of filaggrin in particular remains enigmatic, as this protein shows very poor cross-reactivity even in the granular layer of epidermis of different mammals (RESING & DALE, 1991). Although the observed immunoreactivity may or may not be due to a mammalian-like filaggrin, previous autoradiographic studies have, however, indicated that histidin-rich proteins (HRP) are present in lizard and snake epidermis (ALIBARDI, 2001, 2002, and unpublished observations, see Fig. 1E). As in the epidermis of turtles, crocodilians, and snakes, in lizards weak filaggrin- and loricrin-like immunoreactivities are associated with AE2 immunoreactivity and epidermal regions rich in sulfhydryl groups (BANERJEE & MITTAL, 1978; MITTAL & SING, 1987; ALIBARDI, 2001). It is known that in mammalian epidermis, antibodies that recognise both AE2-positive keratins and filaggrin, seem to recognise a common, uncharacterized, antigenic sequence (DALE & SUN, 1983). It is, therefore, possible that the overlap of immunoreactivity observed in lizard epidermis may be due to the presence of

an antigenic determinant that is recognised by both the AE2 and anti-filaggrin antibody. Histochemical reactions have also indicated that the alpha layer contains more sulfhydrylic groups than the beta-layer, as in the latter most sulphur is probably under a disulfide bond (MITTAL & SINGH, 1987; ALIBARDI, 2001). The association of these groups with non completely polymerized alpha-keratins or with other sulphur-rich proteins may explain the immunoreactivity to loricrin, although the labelling is diffuse within keratinocytes and not linearly associated with the cell corneus membrane as in mammalian keratinocytes (MEHREL et al., 1990; STEVEN et al., 1990; ISHIDA-YAMAMOTO et al., 2000). However, a marginal layer (the thin but dense material associated with the plasmalemma of maturing keratinocytes (LANDMANN, 1979)) is not constantly found in mesos and alpha-keratinocytes of lizard epidermis. Only future biochemical studies will allow clarification of the true identity of the histidin-rich and sulphur-rich molecules present in reptilian epidermis, and comparison of the amino acidic sequence of these putative proteins with those present in mammalian epidermis.

We hypothesise that in lepidosaurian and archosaurian progenitors, cells of the stratum corneum were initially capable of producing alpha-keratin, and later also beta-keratin (MADERSON & ALIBARDI, 2000; SAWYER et al., 2000). Although not constantly seen, the presence of some immunoreactivity for beta-keratin at the base of the wound epidermis suggests that a small quantity of beta-keratin may also be present in this repairing epithelium.

Whatever the case, it has probably been a specific trend in lepidosaurian evolution to segregate cells capable of synthesizing alpha from those capable of producing beta-keratin. This aspect is presently visible in the intermediate region between the alpha- and beta-layer of the epidermis of the living fossil *Sphenodon punctatus* (ALIBARDI, 1999; ALIBARDI & MADERSON, personal observations). In this region beneath the alpha- and beta-layer, cells with alpha- and beta-characteristics are present. It may be speculated that a further cell segregation produced a final alpha-layer (the clear layer) contacting the first beta-layer (the oberhautchen), so that a shedding complex was formed. The different consistency of these two layers, together with the enzymatic process for the degradation of the junctions between clear layer and oberhautchen, produces the detachment of the two layers and the molt (GOSLAR, 1964; LANDMANN, 1979; ALIBARDI, 1998; MADERSON et al., 1998).

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