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BELGIAN JOURNAL OF ZOOLOGY

Belgian Journal of Zoology

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Révision de *Niobrara encarsia*, téléostéen (Osteichthyes, Tselfatiiformes) du Crétacé supérieur marin du Kansas (Etats-Unis)

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RÉSUMÉ. L'auteur étudie l'ostéologie de *Niobrara encarsia*, un genre monospécifique de téléostéen fossile du Crétacé supérieur marin du Kansas. Il redéfinit le genre et l'espèce, précise les plésiomorphies et apomorphies de ce poisson et confirme son appartenance aux Tselfatiiformes (= Bananogmiiformes). *Niobrara* diffère de tous les autres Tselfatiiformes par l'architecture particulière de son toit crânien où les ptérotiques sont rejetés en arrière des pariétaux. Il est l'un des genres les plus primitifs de Tselfatiiformes et, au sein de l'ordre, s'apparente plus particulièrement au genre *Bananogmius*.

MOTS CLEFS: *Niobrara encarsia*, Teleostei, Tselfatiiformes, Crétacé supérieur marin, Kansas, ostéologie.

Revision of *Niobrara encarsia*, teleost (Osteichthyes, Tselfatiiformes) from the marine Upper Cretaceous of Kansas (United States)

ABSTRACT. The author studies the osteology of *Niobrara encarsia*, a monospecific genus of fossil teleost from the marine Upper Cretaceous of Kansas. He redefines the genus and the species, emphasizes the plesiomorphies and apomorphies of this fish, and confirms its belonging to the Tselfatiiformes (= Bananogmiiformes). *Niobrara* differs from all the other Tselfatiiformes by the peculiar pattern of its skull roof where the pterotics are rejected posterior to the parietals. It is one of the most primitive genus inside the Tselfatiiformes and, within the order, is more particularly related to the genus *Bananogmius*.

KEY WORDS: *Niobrara encarsia*, Teleostei, Tselfatiiformes, marine Upper Cretaceous, Kansas, osteology.

INTRODUCTION

Niobrara encarsia JORDAN, 1924 est un genre monospécifique de téléostéen provenant du Coniacien ou du Santonien (Crétacé supérieur) marin du Kansas, Etats-Unis (SCHULTZE et al., 1982: 35). Il n'est connu que par le spécimen holotype, pratiquement complet et bien conservé. Seules quelques-unes des dernières neurépines et hémépines ainsi que les deux lobes de la nageoire caudale manquent mais ont été sculptés dans le substrat et colorés pour faire croire à leur présence.

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JORDAN (1924) a rapporté ce fossile à une nouvelle famille, les Niobraridae, mise depuis en synonymie avec celle des Plethodidae, au sein de l'ordre des Tselfatiiformes (=Bananogmiiformes) (PATTERSON, 1993: 627; NELSON, 1994: 90). JORDAN (1924) attribue également aux Niobraridae quatre autres genres monospécifiques du Crétacé supérieur du Kansas, *Zanclites* Jordan, 1924, *Kansanus* Jordan, 1924, *Luxilites* Jordan, 1924 et *Ferrifrons* Jordan, 1924. *Zanclites xenurus* Jordan, 1924 a fait l'objet d'une révision récente qui a confirmé qu'il s'agissait bien d'un représentant des Tselfatiiformes (TAVERNE, 1999). SCHULTZE et al. (1982: 35) ont montré que *Kansanus martini* Jordan, 1924 n'appartenait pas aux Plethodidae mais aux Pachyrhizodontidae, ce que mes propres observations ont confirmé. *Luxilites striolatus* Jordan,

1924 est en cours de révision et doit également se ranger parmi les Tselfatiiformes (obs. pers., travail en cours). Quant à *Ferrifrons rugosus* Jordan, 1924, il s'agit d'un acanthomorphe archaïque (ARRATIA & CHORN, 1998).

JORDAN (1924: 223) n'a donné qu'une description très succincte de *Niobrara encarsia*. Il n'en décrit pas le crâne, expliquant que le spécimen est «well preserved, except for the disintegration of the bones of the head, which cannot be made out in detail». En fait, malgré de multiples brisures, la tête est bien conservée. Les deux faces en sont accessibles. Une immersion dans l'alcool permet de visualiser convenablement les sutures entre les os céphaliques, de les différencier des lignes de brisures et de reconstituer ce crâne de façon presque parfaite. L'auteur ne dit rien non plus du squelette caudal du poisson pourtant bien visible lui aussi. Ces éléments rendent nécessaire la révision de *N. encarsia* afin de le mieux faire connaître, de le définir de façon précise, de prouver ou de réfuter son appartenance aux Tselfatiiformes et, enfin, de préciser éventuellement sa position phylogénétique au sein de cet ordre. C'est là le but du présent article qui s'inscrit dans la série de travaux que je consacre à l'étude des Tselfatiiformes (TAVERNE, 1975, 1983, 1999, 2000a, 2000b, 2000c).

Rappelons encore une fois que les Tselfatiiformes ou Bananogmiiformes forment 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, le Paléoatlantique et la mer intérieure nord-américaine. Ils n'ont plus de descendants actuels. Comme beaucoup d'autres téléostéens marins de la fin du Secondaire, ils n'ont pas survécu aux événements qui ont marqué le passage du Crétacé au Tertiaire.

La morphologie des Tselfatiiformes est proche de celle des Scombridae et des Coryphaenidae, indiquant des poissons à nage rapide qui menaient probablement, au Crétacé, le même type de vie que ces deux familles mènent dans les océans d'aujourd'hui. Le crâne est presque toujours médio-pariétal. Le prémaxillaire et le maxillaire bordent la mâchoire supérieure. Il n'y a qu'un seul supramaxillaire. Les dents 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 s'étirant sur 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 réduites. Le squelette caudal comporte une large plaque hypurale soudée à une vertèbre urale I et II réduite. Le parhypural a disparu. La nageoire caudale est grande, bilobée et compte 19 rayons principaux. L'hypurostégie est prononcée. Les écailles sont cycloïdes.

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

MATÉRIEL ET MÉTHODES

L'holotype et unique exemplaire de *Niobrara encarsia* est conservé dans les collections paléontologiques de l'Université du Kansas à Lawrence (Kansas, U.S.A.) sous le N° KUVP 179. Il a été découvert à ½ mille au sud de Banner, dans le comté de Trego, au Kansas. Il provient du Smoky Hill Member de la Niobrara Formation et est d'âge coniacien ou santonien (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).

ÉTUDE DU MATERIÉL

Généralités (Fig. 1, 2)

Les principales proportions du poisson s'établissent comme suit:

En % de la longueur standard (62 cm)

Longueur de la tête (région operculaire comprise).	25,0%
Hauteur maximum de la tête	22,0%
Hauteur maximum du corps	26,5%
Longueur prédorsale (augmentée par le léger recul de la nageoire dorsale)	37,9%
Longueur de la nageoire dorsale	60,6%
Longueur prépelvienne	61,4%
Longueur de la nageoire pelvienne	9,8%
Longueur préanale	69,7%
Longueur de la nageoire anale	30,3%
Longueur du pédoncule caudal	2,3%
Hauteur du pédoncule caudal	2,3%

Le crâne (Fig. 3, 4, 5A, B)

Les os dermiques crâniens sont ornementés de fines ridules. Cette ornementation n'est cependant pas très prononcée.

Le mésethmoïde est long, large et à peu près d'égale largeur sur toute sa longueur. Son bord antérieur est légèrement concave et laisse voir l'extrémité du vomer sous-jacent. L'épaisseur de ce mésethmoïde laisse penser qu'il résulte de la fusion d'un dermethmoïde (= rostral) et d'un supraethmoïde. Les ethmoïdes latéraux et les nasaux ne sont pas conservés. Le vomer est petit, plus large que long et sa face orale est entièrement couverte de denticules et de minuscules puits, restes de l'implantation de denticules disparus. Une cassure dans le parasphénoïde qui sépare la partie antérieure du reste de l'os peut faire croire que le vomer est beaucoup plus allongé et que sa partie postérieure est éden-

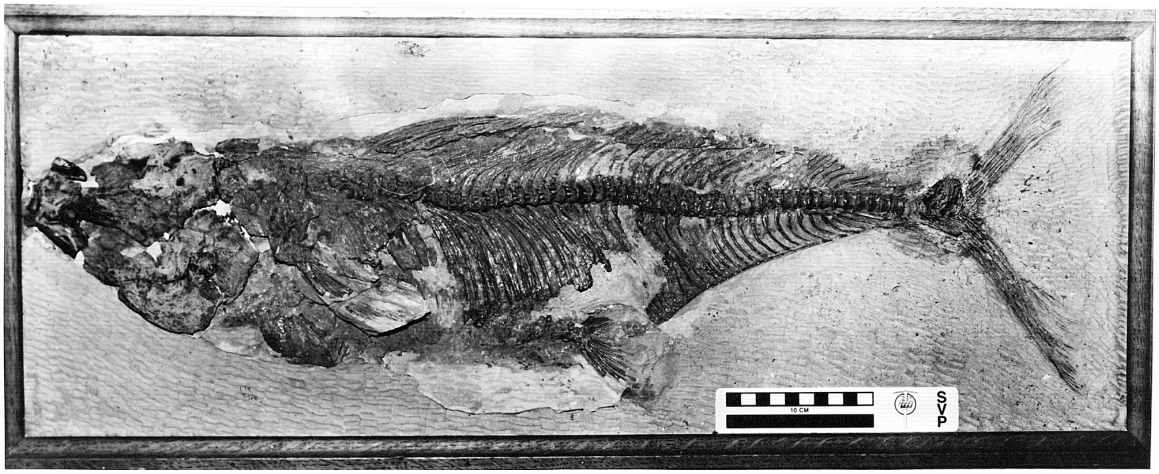

Fig. 1. – *Niobrara encarsia* JORDAN, 1924. Le spécimen holotype N° 179 (photographie due à la courtoisie du Dr. John CHORN de l'Université du Kansas à Lawrence).

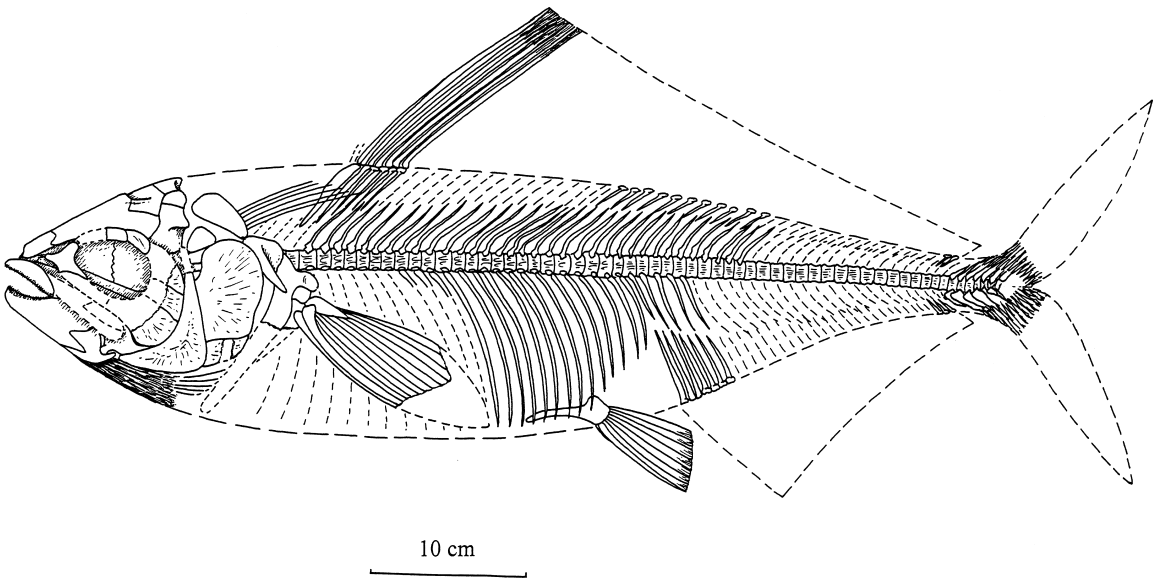

Fig. 2. – *Niobrara encarsia* JORDAN, 1924. Reconstitution du squelette complet d'après l'holotype N° KUVP 179.

tée. Un examen minutieux montre néanmoins qu'il y a bien eu brisure d'os et que cette portion appartient au parasphénoïde et non au vomer (Fig. 4, au-dessus).

Le toit crânien est très large, surtout dans sa partie postérieure, et presque plat. Les frontaux sont vastes et forment l'essentiel de ce toit. Les pariétaux sont grands, anguleux et jointifs, déterminant un crâne de type médio-pariétal. C'est une vaste avancée postérieure du frontal qui borde latéralement le pariétal et non pas le ptérotique qui, lui, est rejeté en arrière du pariétal (Fig. 5A, B au-dessus). Cette architecture diffère de celle de tous les autres Tselfatiiformes à toit crânien plus ou moins plat où le frontal s'arrête à la limite du pariétal et où le ptérotique longe latéralement le pariétal (Fig. 5B, en dessous), ce qui est aussi le cas des téléostéens primitifs (RAYNER, 1937: fig. 2; FOREY, 1973: nombr. fig.; TAVERNE, 1977: nombr. fig.). La dépression fronto-pariétale, courte, large et de forme triangulaire, est délimitée par une crête osseuse bien marquée. Cette crête s'ébauche à la limite postérieure des frontaux et se continue sur les pariétaux qui supportent la dépression fronto-pariétale dans sa totalité. Les ptérotiques sont courts, hauts et traversés d'une crête horizontale qui détermine la limite supérieure de la *dilatator fossa*, elle aussi assez haute. Les sphénotiques sont volumineux, allongés et développent un large processus postorbitaire qui marque la limite antérieure de la *dilatator fossa*. En vue dorsale, le sphénotique est nettement mieux visible que chez la plupart des autres Tselfatiiformes car le bord du frontal subit un retrait à ce niveau et le recouvre moins (Fig. 5B). Le supraoccipital

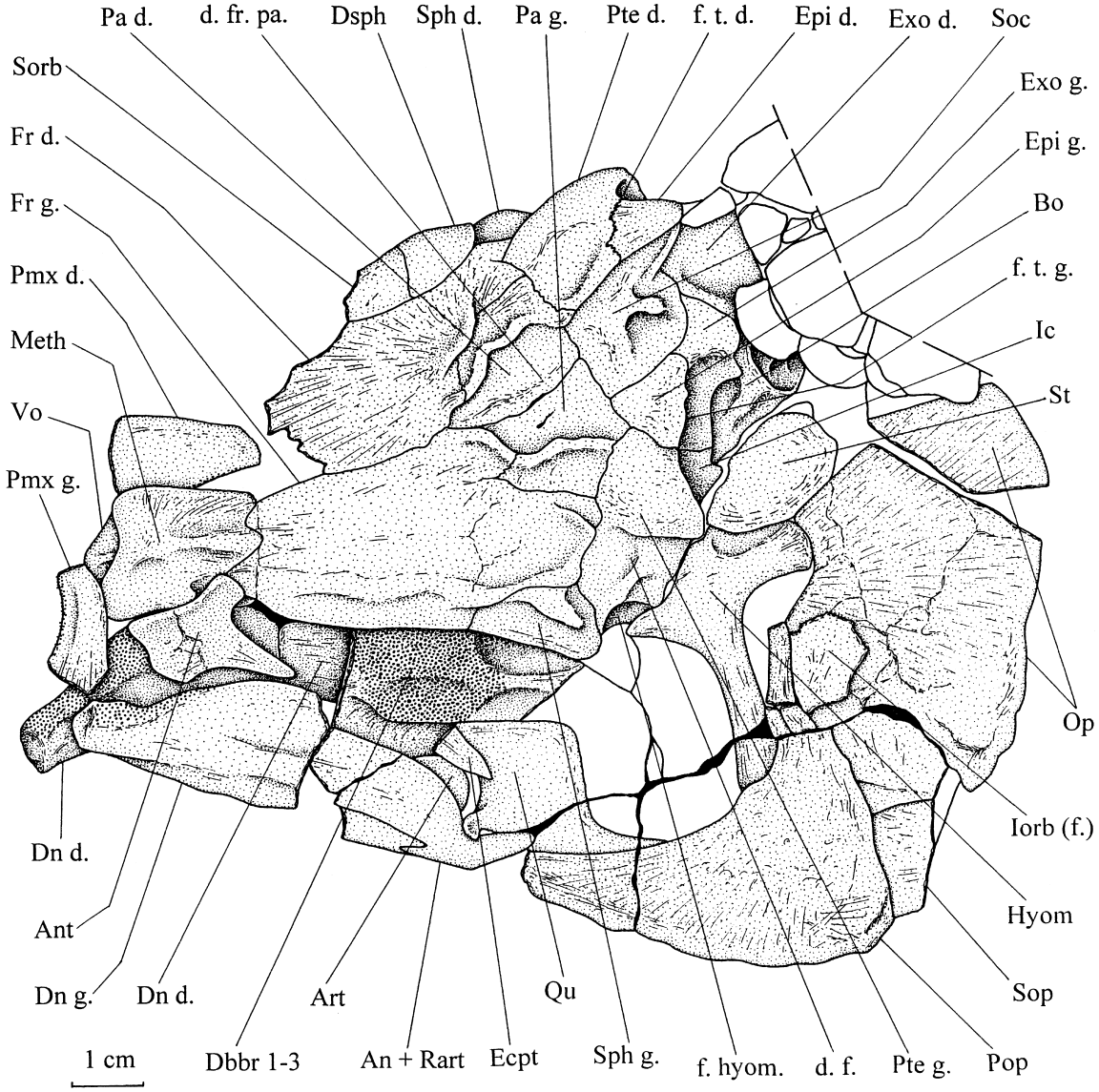

Fig. 3. – *Niobrara encarsia* JORDAN, 1924. La tête de l'holotype N° KUVP 179 en vue latérale gauche. Le toit crânien a été rabattu sur le neurocrâne par la fossilisation. Le substrat est laissé en blanc. Les pièces du squelette axial et de la ceinture scapulaire reprises telles quelles sur la figure 6 ne sont pas ombrées ni légendées.

est bien développé mais sa crête médiane est petite. Les épiotiques sont petits et encadrent le supraoccipital.

La fosse temporale (= posttemporale) s'ouvre à l'arrière du crâne entre le ptérotique, l'épiotique, l'exoccipital et l'intercalaire, juste en dessous du supratemporal. Latéralement, la fosse est couverte par le ptérotique. Seul le supratemporal gauche est conservé. Il est réduit à sa partie latérale et ne rejoint donc pas son homologue sur la ligne médiane du crâne. Toutefois, le développement de son composant membranodermique reste important et donne à cet os un aspect à peu près ovale.

Le septum interorbitaire osseux est complet et formé par un immense orbitosphénoïde, une paire de pleurosphénoïdes bien développés et la crête médio-dorsale du parasphénoïde, elle aussi très développée. La région trabéculaire du parasphénoïde est longue et large, avec une courte partie antérieure édentée et une longue partie postérieure creusée de puits minuscules, restes de l'implantation de denticules. Le *processus ascendens* existe mais est peu développé.

Le prootique droit est le seul visible. C'est un os plus haut que long. Son bord antérieur est brisé. Le complexe de la chambre trigémino-faciale et de la *pars jugularis* ne peut donc être décrit. Les exoccipitaux sont très vastes. A la face arrière du neurocrâne, ils délimitent le *foramen magnum*. La fossette articulaire pour l'hyomandibulaire

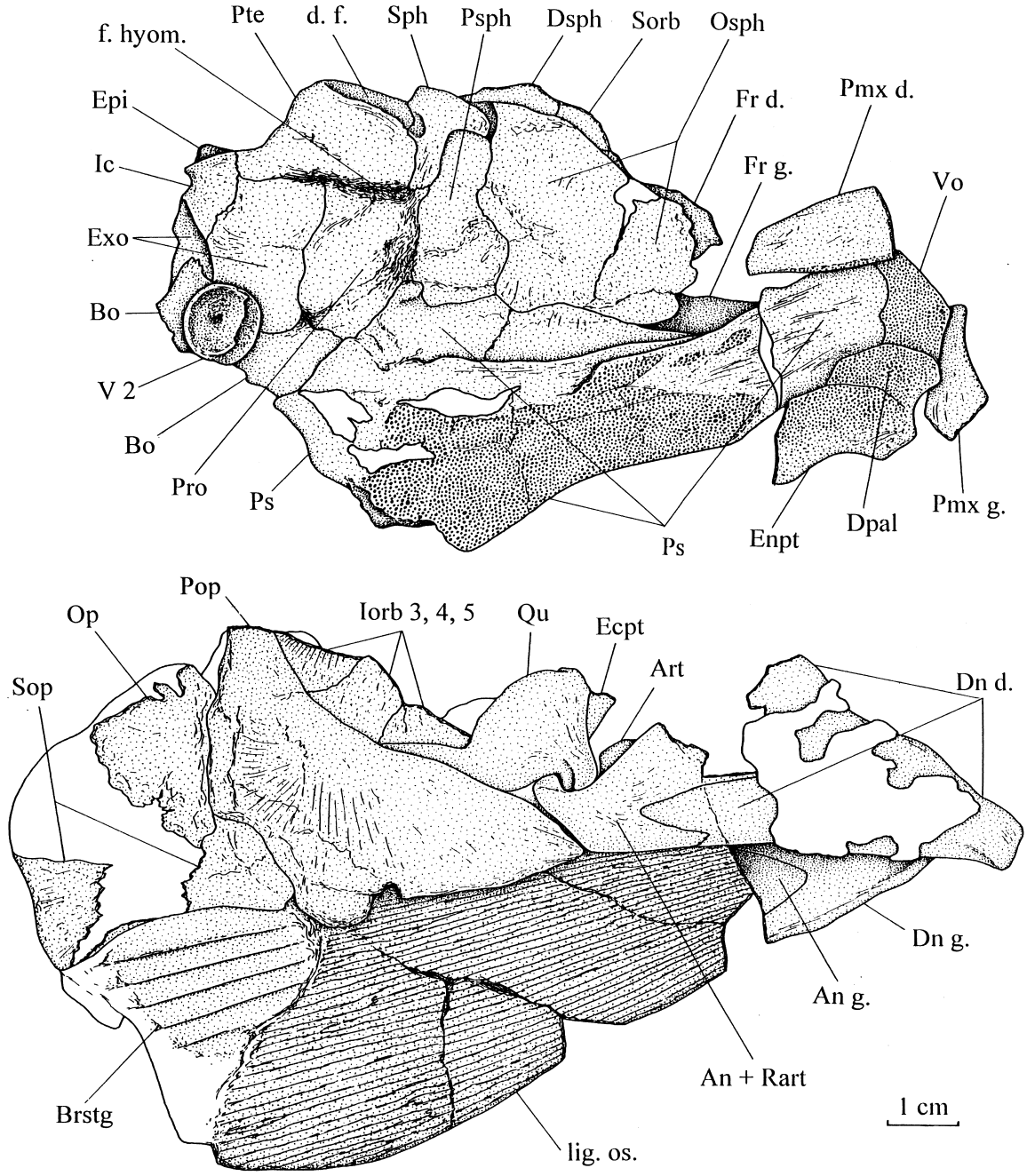

Fig. 4. – *Niobrara encarsia* JORDAN, 1924. Le neurocrâne (au-dessus) et le suspensorium (en dessous) de l'holotype N° KUVP 179 en vue latérale droite. Le substrat est laissé en blanc.

est creusée à la limite du ptérotique, de l'exoccipital et du prootique et touche le sphénotique. Le basioccipital est court et massif, formant à lui seul le condyle articulaire pour l'axe vertébral. Les intercalaires sont bien développés.

Les os circumorbitaires sont incomplètement conservés. L'antorbitaire droit est visible; c'est un grand os, haut et long, qui porte un très long processus postéro-ventral pointu. Les premier et deuxième infraorbitaires (= lacrymal et jugal) sont perdus. Du côté gauche du crâne, on remarque un fragment d'un vaste infraorbitaire postérieur qui chevauche l'operculaire. Du côté droit de la tête, on observe des morceaux des trois grands infraorbitaire postérieurs. Du côté droit, également, on trouve le dermosphénotique et la partie postérieure du supraorbitaire.

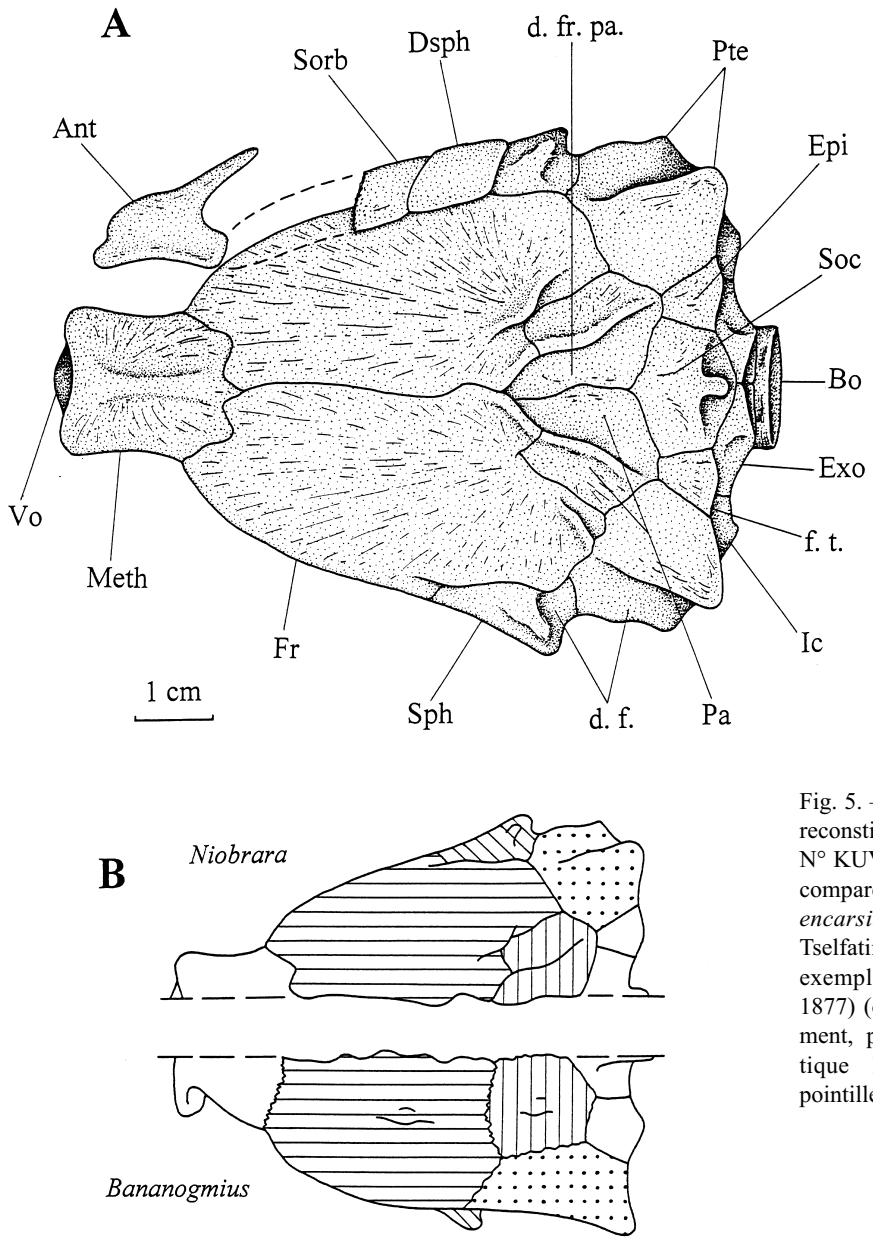

Fig. 5. – *Niobrara encarsia* JORDAN, 1924. (A): reconstitution du toit crânien d'après l'holotype N° KUVP 179. (B): représentation schématique comparée entre le toit crânien de *Niobrara encarsia* JORDAN, 1924 et celui des autres Tselfatiiformes à toit crânien plus ou moins plat exemplifié par *Bananogmius aratus* (COPS, 1877) (obs. pers.). Frontal hachuré horizontalement, pariétal hachuré verticalement, sphénotique hachuré obliquement et ptérotique pointillé.

Ces deux os sont articulés entre eux et avec le bord du frontal; ils n'atteignent pas le niveau du ptérotique.

Le prémaxillaire gauche a perdu sa partie postérieure et le prémaxillaire droit sa partie antérieure. Des deux fragments conservés, on peut déduire que cet os est allongé, assez élevé mais sans qu'un véritable processus dorsal ne s'individualise. Le bord oral de l'os porte une étroite bande de denticules. Le maxillaire et le supramaxillaire sont perdus. La mandibule est longue, moyennement haute et dépourvue de processus coronoïde clairement marqué. Le bord oral du dentaire porte une large plage denticulée qui déborde nettement sur la face interne de l'os. Vers la symphyse, cette plage déborde aussi quelque peu sur la face externe de l'os. L'angulaire et le rétroarticulaire sont fusionnés et l'os ainsi composé montre un processus postarticulaire bien développé. 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 dermopalatin gauche est visible par sa face orale. C'est un petit os, coincé entre le vomer, le parasphénoïde et l'endoptérygoïde. Il est entièrement denticulé. L'extrémité antérieure de l'endoptérygoïde gauche est préservée. C'est un os large, probablement long et à sur-

face orale denticulée. Seules les extrémités postérieures édentées des deux ectoptérygoïdes sont conservées. La couverture complète par des denticules, y compris sur l'extrémité arrière de l'os, est la condition primitive de l'ectoptérygoïde des Tselfatiiformes (LOOMIS, 1900: pl. XXII, fig. 6, erronément appelé quatrième cératobranchial; APPLEGATE, 1970: fig. 196E, G; TAVERNE, 2000b: fig. 8C). La partie antérieure de l'ectoptérygoïde de *Niobrara encarsia*, n'étant pas connue, il est donc possible que cet os soit édenté ou incomplètement denté. Le métaptérygoïde n'est pas conservé. Le carré est grand, 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. Seuls les six derniers rayons branchiostèges ont laissé des traces. Ils sont larges, contigus et en contact étroit avec le sous-operculaire.

Le squelette hyoïdeo-branchial (Fig. 3)

L'hyomandibulaire gauche est presqu'entièrement conservé. Il est élargi dorsalement dans sa zone articulaire avec le neurocrâne et se rétrécit ventralement en une épaisse tige osseuse. Le *processus opercularis* est très allongé. Le symplectique et les autres pièces de l'arc hyoïdien ne sont pas connus.

Du squelette branchial, seul s'observe un grand fragment du dermobasibranchial des trois premiers arcs branchiaux. C'est un os long et large dont la région denticulée occupe la portion centrale légèrement convexe et surélevée. En fait, les denticules ont disparu et seuls subsistent les petits orifices des canalicules qui traversent l'os, donnant à la surface de ce dernier un aspect ponctué caractéristique. Vers l'arrière, la zone denticulée surélevée de l'os se prolonge par une petite crête médiane.

La condition primitive chez les Tselfatiiformes est d'avoir trois plaques denticulées linguales: 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 *Niobrara* (voir discussion), il est possible que son squelette branchial comportait également les trois plaques denticulées en question.

Les ceintures (Fig. 1, 2, 6)

La ceinture scapulaire est complète mais il n'y a pas de postcleithrum. Le posttemporal est une vaste plaque

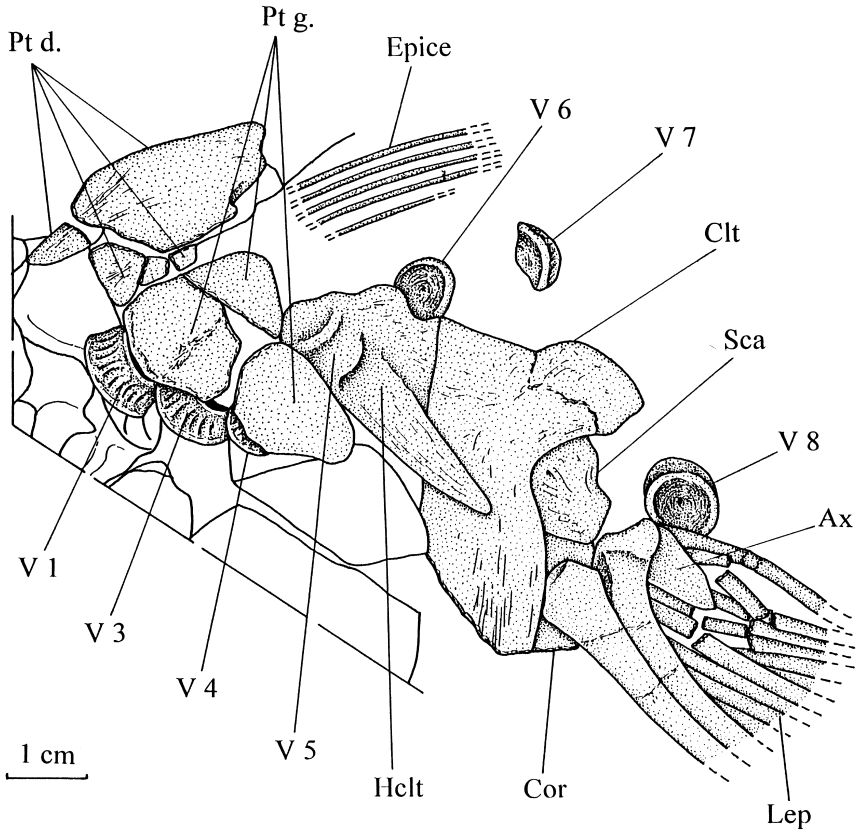

Fig. 6. – *Niobrara encarsia* JORDAN, 1924. La ceinture scapulaire en vue latérale gauche et les premières vertèbres de l'holotype N° KUVP 179. Les os de l'arrière du crâne repris tels quels de la figure 3 ne sont pas ombrés ni légendés.

osseuse triangulaire. L'hypercleithrum (= supracleithrum), très large dorsalement, se rétrécit ventralement en une longue pointe. Seule la région dorso-postérieure du cleithrum est conservée. Le long bras ventral de l'os est brisé. La branche dorsale est quasi inexistante. La partie préservée est large, épaisse et montre un vaste processus arrière qui se recourbe au-dessus de la scapula (= hypercoracoïde). Celle-ci est un petit os massif et globuleux. La région postéro-dorsale du coracoïde (= hypocoracoïde) est présente mais la région antéro-ventrale de l'os est brisée et perdue. La nageoire pectorale est longue, large et insérée haut sur le flanc. Elle s'étire presque jusqu'à la mi-longueur du *situs viscerum* malgré que son extrémité distale soit brisée et manquante. Elle comporte 11 rayons. Il n'y a pas de petite épine initiale impaire. Le premier rayon de la série est de beaucoup le plus épais et l'extrémité proximale articulaire de ses deux hémi-lépidotriches est très renflée. JORDAN (1924 : 223) mentionne 12 rayons pectoraux. Il semble avoir confondu chaque hémi-lépidotriche du premier rayon avec un rayon complet. Les ptérygophores pectoraux ne sont pas visibles. Une écaille axillaire triangulaire est associée avec la nageoire pectorale.

On observe une masse très importante de ligaments ossifiés qui font la jonction entre la région antéro-ventrale de la mandibule et la ceinture scapulaire. FIELITZ & SHIMADA (1999) pensent que ces ligaments dérivent de l'ossification des muscles protracteurs hyoïdiens et que leur attache antérieure se situent à l'extrémité antérieure des barres hyoïdiennes et non pas à la symphyse mandibulaire.

La ceinture et les nageoires pelviennes sont de grande taille et situées en position abdominale sous la partie postérieure du *situs viscerum*. Les os pelviens sont longs, épais et élargis à la base. L'origine des nageoires pelviennes se situe au niveau de la vingt-septième vertèbre abdominale. Chaque nageoire compte 8 rayons. Ceux-ci sont abondamment branchus, sauf le premier.

Le squelette axial (Fig. 1, 2, 4, 6, 7)

JORDAN (1924 : 222) signale 52 vertèbres chez *Niobrara*. Ce chiffre est erroné car l'auteur ne paraît pas avoir remarqué les premières vertèbres abdominales détachées du reste de l'axe vertébral et éparpillées juste derrière la tête et au-dessus et sous la ceinture scapulaire (Fig. 3, 4), ni la petite vertèbre préurale 1et la minuscule vertèbre terminale. En fait, on compte 63 vertèbres dont 29 abdominales et 34 caudales, y compris le petit centre terminal ural 1 + 2. Toutes ces vertèbres sont plus hautes que longues. Les vertèbres abdominales, sauf les premières, sont cependant plus longues mais aussi moins hautes que les vertèbres caudales. Les faces latérales des premières vertèbres abdominales sont ornées de nombreuses crêtes horizontales qui séparent autant de petites fossettes. Les faces latérales des vertèbres abdominales suivantes ne montrent plus que deux fortes crêtes séparant trois fossettes plus vastes. Les faces latérales des dernières vertèbres abdominales ne s'ornent plus que d'une seule crête qui sépare deux fossettes. Ces reliefs s'estompent au niveau des faces latérales des vertèbres caudales où l'on observe parfois pourtant de fines striations horizontales.

Tout au long du squelette axial, les arcs neuraux et hémaux sont articulés par gomphose avec les corps vertébraux correspondants. Ces arcs demeurent étroits sauf dans la région du pédoncule caudal et du complexe urophore. Les neurépines qui prolongent les arcs neuraux sont longues, fines et recourbées vers l'arrière. Les dernières neurépines se raccourcissent mais deviennent aussi beaucoup plus robustes. Dans la région abdominale, les

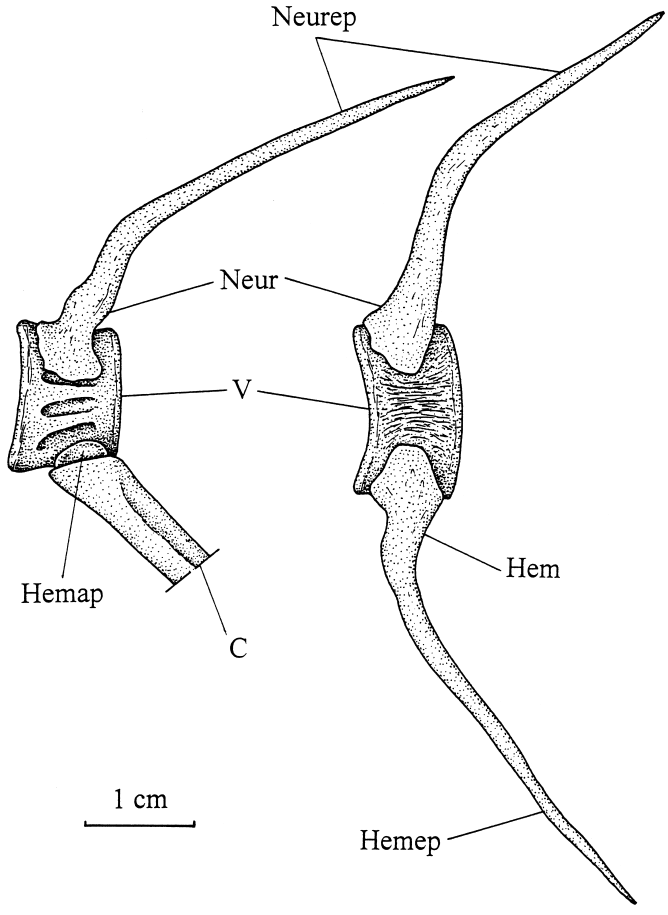

Fig. 7. – *Niobrara encarsia* JORDAN, 1924. Reconstitution d'une vertèbre abdominale (à gauche) et d'une vertèbre caudale (à droite).

arcs hémaux sont réduits à des paires d'hémapophyses en forme de petits nodules osseux. Bon nombre de ces petites hémapophyses ont disparu suite aux aléas de la fossilisation. Dans la région caudale, l'arc hémal se referme sur lui-même et se prolonge par une longue et fine hémépine également recourbée vers l'arrière. La première hémépine est notablement plus courte que les suivantes. Les dernières hémépines sont plus courtes et deviennent très épaisses.

Les côtes associées à la région abdominale du squelette axial sont longues, fortes, creusées dans leur région proximale d'un petit sillon vertical et articulées sur les petites hémapophyses. Elles descendent presque jusqu'au bord ventral du *situs viscerum* sauf les dernières qui se raccourcissent quelque peu.

On remarque des restes fragmentaires d'arêtes dans quelques endroits de la partie dorsale de la région abdominale. Les premières sont très longues, très fines et situées juste en arrière de la tête. Ce sont des épicentraux. J'ai pu observer, en effet, chez d'autres Tselfatiiformes, que ces premières et très longues arêtes s'articulent sur les centres vertébraux correspondants et non pas sur les arcs neuraux (PATTERSON & JOHNSON, 1995). De courts morceaux d'arêtes s'observent également plus en arrière sur l'axe vertébral associés à quelques neurépines. Il s'agit cette fois d'épineuraux. On n'observe ni épineuraux ni épipleuraux dans la région caudale. Cela s'explique sans doute par le mauvais état de conservation de cette région où quelques neurépines et beaucoup d'hémépines manquent et ont été sculptées dans le substrat et colorées pour imiter la réalité.

Les nageoires dorsale et anale (Fig. 1, 2)

La nageoire dorsale est très incomplètement conservée et il n'est guère possible d'en dénombrer ni les rayons ni les ptérygophores. Elle s'étire sur presque toute la longueur du dos et est haute dans sa partie antérieure. Tel que le spécimen se présente, la nageoire dorsale paraît débuter au niveau de la mi-longueur de la nageoire pectorale et de la onzième vertèbre abdominale. En fait, l'origine de la nageoire dorsale devait être antérieure à ce point et se situer plus près de l'arrière de la tête, mais la fossilisation a rabattu le début de cette nageoire dorsale dans une direction postéro-ventrale, ce qui amène la base des premiers lépidotriches à recouvrir le sommet des neurépines et ce qui donne une orientation presque horizontale aux premiers ptérygophores. Les premiers rayons, seuls bien visibles, demeurent insegmentés. Le premier de la série est moitié moins long que le suivant. Ces deux premiers rayons ne sont pas dichotomisés. Les rayons suivants sont légèrement branchus à leurs extrémités distales. Il y avait probablement deux ou trois très petits rayons épineux au début de la nageoire, comme chez *Tselfatia formosa* ARAMBOURG, 1943 (TAVERNE, 1983 : fig. 1) mais ils ne sont pas conservés. Le premier ptérygophore est plus large et un peu plus long que les suivants. On remarque aussi quelques débris de ptérygophores situés juste en avant du pédoncule caudal. Au niveau des premiers ptérygophores et à partir du deuxième de la série, on remarque que l'extrémité supérieure de l'axonoste est élargie, formant une sorte de petit plateau nettement moins étendu, toutefois, que chez *Tselfatia formosa* ou chez *Zanclites xenurus* (TAVERNE, 1975 : fig. 1, 1999 : fig. 4C).

De la nageoire anale, seuls quelques fragments de ptérygophores sont conservés. Tous les rayons ont disparus. Cette nageoire s'étire tout le long du bord ventral de la partie caudale du poisson, depuis le niveau de la trente-quatrième vertèbre jusqu'au début du pédoncule caudal. Le premier ptérygophore anal est le plus long de la série. Il est incliné vers l'avant et se dirige vers l'arrière de l'hémépine de la trentième vertèbre, qui est aussi la première vertèbre caudale, mais sans qu'il y ait un contact intime entre ce ptérygophore et cette hémépine. Cela correspond donc à ce que BLOT (1968 : fig. 1 I) appelle un complexe hémaxanal de type I, c'est-à-dire le type le plus primitif rencontré chez les téléostéens. L'extrémité ventrale des axonostes est légèrement élargie en un petit plateau.

Le squelette caudal (Fig. 8)

Quoique le complexe urophore soit en partie recouvert par les lépidotriches caudaux, sa région médiane demeure parfaitement visible et permet d'en comprendre la structure. Les dernières vertèbres deviennent de plus en plus petites et de plus en plus étroites. Jusque et y compris la vertèbre préurale 2, le corps vertébral garde cependant une forme classique. La vertèbre préurale 1, par contre, est déformée en un petit bloc osseux irrégulier. Les vertèbres urales 1 et 2 sont fusionnées en un petit centre terminal de forme irrégulière également. Tous les arcs neuraux et hémaux associés aux dernières vertèbres sont articulés sur ces dernières par gomphose et non pas soudés aux corps vertébraux correspondants. Ces arcs couvrent une portion importante des faces latérales des vertèbres concernées. Les ultimes neurépines et hémépines sont épaisses et les hémépines plus encore que les neurépines. La dernière neurépine complète est portée par la vertèbre préurale 1. La dernière hémépine est associée à la vertèbre préurale 2. La vertèbre préurale 1 supporte un arc hémal réduit mais le parhypural manque.

Ce petit arc hémal préural 1 s'articule dans une encoche creusée dans la partie proximale du bord postérieur de l'hémépine préurale 2. Les quatre premiers hypuraux sont fusionnés en une plaque, elle-même soudée à la petite vertèbre terminale urale 1 + 2. Au-dessus de cette plaque, on observe un cinquième hypural bien développé, autogène et dont l'extrémité proximale élargie vient s'articuler sur le sommet de la vertèbre terminale. La région qui surplombe le cinquième hypural est entièrement cachée par les lépidotriches caudaux, ce qui ne permet pas de voir s'il y avait un éventuel sixième hypural autogène. Cependant, *Niobrara encarsia* étant une espèce particulièrement primitive au sein de l'ordre (voir discussion), il est permis de

penser qu'un sixième hypural y existait bien, puisque telle est la condition plésiomorphe chez les Tselfatiiformes (NELSON, 1973 : fig. 8B ; TAVERNE, 2000a : fig. 2). Un autre fait renforce cette manière de voir. Le dernier hypural des Tselfatiiformes, qu'il soit le cinquième ou le sixième, entre en contact avec la neurépine qui le précède (NELSON, 1973 : fig. 8B, C ; TAVERNE, 1999 : fig. 5, 2000a : fig. 2). Or, chez *N. encarsia*, un large espace subsite entre la dernière neurépine et le cinquième hypural (Fig. 8), laissant ainsi toute la place voulue pour un sixième hypural. Il n'y a pas d'uroneuraux. La région épurale est couverte par les lépidotriches caudaux, mais on peut penser que les épuraux étaient perdus, comme c'est le cas chez les autres Tselfatiiformes (NELSON, 1973 : fig. 8B, C ; TAVERNE, 1999 : fig. 5, 2000a : fig. 2).

La nageoire caudale n'est que très partiellement conservée. Seules les bases de ses deux lobes sont visibles et montrent une hypurostégie importante. Les rayons caudaux principaux des deux lobes sont précédés de plusieurs petits rayons pointus. Les parties médianes et distales des grands lépidotriches caudaux, telles que le fossile les montre, sont faux. Elles ont été sculptées dans le substrat et colorées pour leur donner un aspect de réalité. Cette nageoire comptait vraisemblablement 19 rayons principaux dont 17 branchus comme chez les autres Tselfatiiformes où elle est connue dans sa totalité (TAVERNE, 1983 : 175, 1999 : 432). Les chiffres de 20 rayons principaux dont 18 branchus cités par BARDACK & TELLER-MARSHALL (1980 : 1076) chez *Tselfatia* ARAMBOURG, 1943 sont probablement dus à un artefact de fossilisation, à un cas individuel ou à une erreur d'interprétation ; ils ne correspondent pas à mes propres observations sur de nombreux spécimens de ce genre (TAVERNE, 1975, 1983).

L'écaillure

L'écaillure est en très grande partie perdue. Seuls des fragments d'écailles subsistent au-dessus et en dessous de la nageoire pectorale. Ces écailles sont grandes, cycloïdes et à surface lisse. On distingue par endroits de fins *circuli* à disposition concentrique et serrés les uns contre les autres. On n'observe guère de *radii* mais c'est peut-être dû au très mauvais état de conservation des morceaux d'écailles présents.

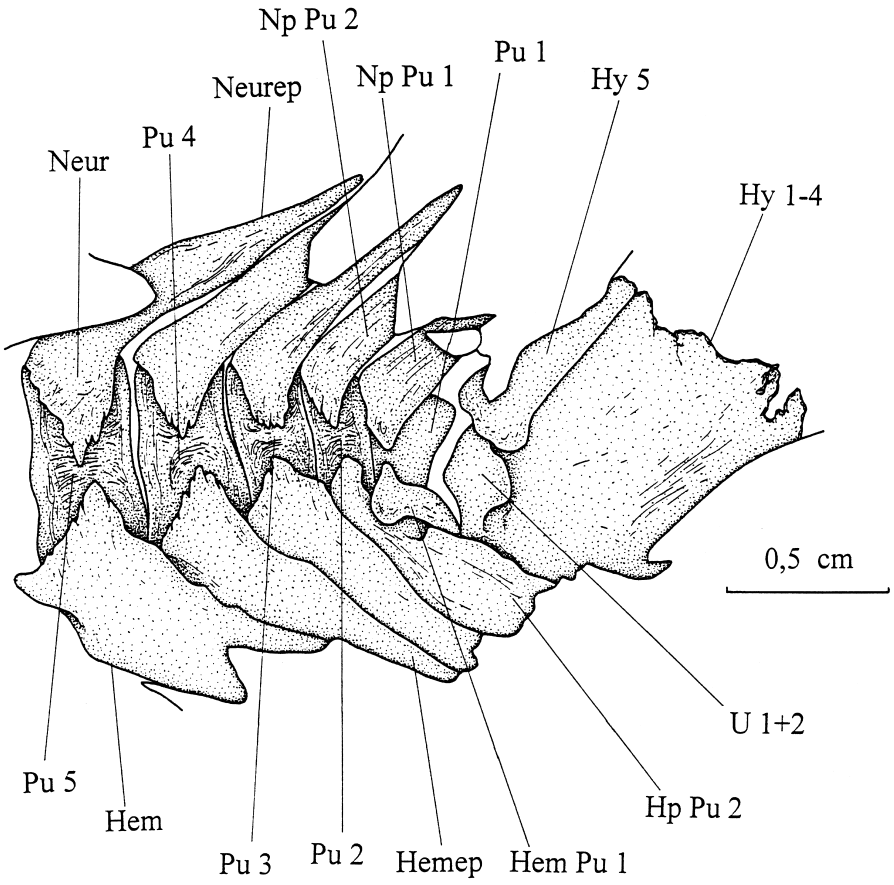

Fig. 8. – *Niobrara encarsia* JORDAN, 1924. Le squelette caudal de l'holotype N° KUVP 179.

DISCUSSION

Niobrara encarsia et les Tselfatiiformes

La forme générale et les proportions du poisson, les dimensions et la position de ses nageoires, son très large toit crânien assez plat, ses grands pariétaux jointifs, sa dépression médiane fronto-pariétale, son supraorbitaire et son dermosphénotique articulés entre eux et avec le frontal, son septum interorbitaire osseux, ses 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 présence de ligaments ossifiés mandibulo-cleithraux, les arcs neuraux et hémaux articulés par gomphose sur les centres vertébraux et l'anatomie si particulière du squelette caudal sont autant d'éléments qui, associés, attestent sans doute possible que *Niobrara encarsia* appartient bien à l'ordre des Tselfatiiformes.

Niobrara encarsia au sein des Tselfatiiformes

On remarque que ce poisson appartient au groupe des Tselfatiiformes à toit crânien plus ou moins plat et non pas à celui des formes spécialisées où le toit crânien s'incurve fortement le long de la ligne médiane (Taverne, 1983, 2000a). On note aussi que *Niobrara* est l'un des Tselfatiiformes les plus primitifs, puisqu'il conserve à l'état plésiomorphe quatre caractères qui existent à l'état apomorphe chez la plupart des autres genres :

(1) Les deux branches du préoperculaire sont bien développées et de longueurs subégales. Chez beaucoup de Tselfatiiformes, la branche ventrale du préoperculaire est devenue nettement plus courte que la branche dorsale (Loomis, 1900 : fig. 6 ; Bardack, 1965 : fig. 2 ; Bardack & Teller-Marshall, 1980 : fig. 4 ; Taverne, 1983 : fig. 2, 2000b : fig. 1, 9, 2000c : fig. 3). Dans un seul cas, celui de *Zanclites xenurus* Jordan, 1924, la branche ventrale du préoperculaire s'est, au contraire, considérablement allongée (Taverne, 1999 : fig. 2).

(2) La ceinture et les nageoires pelviennes sont de grande taille. Chez la plupart des autres formes de l'ordre, elles sont atrophiées (Bardack, 1965 : fig. 1 ; Patterson, 1967 : fig. 6 ; Bardack & Teller-Marshall, 1980 : fig. 4, 5 ; Taverne, 1983 : fig. 1, 1999 : fig. 1a, b).

(3) Le complexe hémaxanal (Blot, 1968) est de type 1, alors qu'il est presque toujours de type 3 chez les autres Tselfatiiformes (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). Les caractères (2) et (3) sont probablement en partie liés. C'est vraisemblablement l'atrophie de la ceinture et des nageoires pelviennes qui favorise l'inversion de l'orientation des ptérygophores anaux, assurant ainsi un meilleur soutien de la région postéro-ventrale du *situs viscerum* et provoquant par la même occasion le passage du complexe hémaxanal du type 1 vers le type 3.

(4) La vertèbre préurale I réduite porte encore un arc petit hémal qui a complètement disparu chez presque tous les autres membres de l'ordre (Taverne, 1975 : fig. 3, 1983 : fig. 5, 1999 : fig. 5 ; 2000a : fig. 2).

Quant aux apomorphies de *Niobrara encarsia*, elles sont au nombre de huit :

(1) Le coin postéro-ventral de l'antorbitaire 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).

(2) La dépression fronto-pariétale est courte et large, en forme de triangle à pointe dirigée vers l'avant, limitée antéro-latéralement par une forte crête osseuse qui forme la limite entre les frontaux et les pariétaux et qui se prolonge sur les pariétaux. Chez les autres Tselfatiiformes à toit crânien relativement plat, cette dépression est soit presque inexistante (Loomis, 1900 : pl. XXI, fig. 1 ; Stewart, 1900 : pl. LXV, fig. 1 ; Hay, 1903 : fig. 23 ; Applegate, 1970 : fig. 197R), soit étroite et allongée (HAY, 1903 : fig. 27 ; Taverne, 1999 : fig. 2, 2000b : fig. 2, 4).

(3) Le ptérotique est étroit mais aussi très haut et marqué d'une courte mais forte crête délimitant dorsalement la *dilatator fossa*, elle aussi très haute. Chez les autres Tselfatiiformes, le ptérotique est plus allongé et moins élevé (Loomis, 1900 : fig. 6 ; Taverne, 1983 : fig. 2, 3, 1999 : fig. 2, 2000a : fig. 10, 2000b : fig. 1, 2, 4, 9, 2000c : fig. 3, 4). *Enischnorhynchus* Bardack, 1965 du Crétacé supérieur marin du Texas est le seul autre représentant de l'ordre à montrer un ptérotique haut et court (Bardack, 1965 : fig. 2). Toutefois, ce genre est apparenté au groupe des Tselfatiiformes à toit crânien incurvé (Taverne, 2000c) et diffère donc nettement de *Niobrara*.

(4) Les ptérotiques sont situés postérieurement par rapport aux pariétaux et non pas latéralement. C'est, au contraire, la partie postéro-latérale du frontal qui encadre latéralement le pariétal. Cela différencie *Niobrara encarsia* de tous les autres Tselfatiiformes à toit crânien plus ou moins plat où le frontal reste totalement antérieur au pariétal, lequel est bordé latéralement par le ptérotique (Fig. 5B ; Loomis, 1900 : pl. XXI, fig. 1 ; Stewart, 1900 : pl. LXV, fig. 1 ; Hay, 1903 : fig. 23, 27 ; Taverne, 1999 : fig. 2, 2000a : fig. 10, 2000b : fig. 1, 2, 4, 9 ; Fielitz & Shimada, 1999 : fig. 2). Les caractères évolués (3) et (4) sont vraisemblablement liés.

(5) L'ectoptérygoïde paraît édenté ou incomplètement denté. Chez les Tselfatiiformes, la condition primitive

est d'avoir l'ectoptérygoïde complètement couvert de dents, y compris dans la partie arrière de l'os (LOOMIS, 1900 : pl. XXII, fig. 6, erronément appelé quatrième cératobranchial ; APPLEGATE, 1970 : fig. 196E, G ; TAVERNE, 2000b : fig. 8C). Chez *Zanclites xenurus*, l'ectoptérygoïde est édenté mais c'est suite à sa transformation en une épaisse tige osseuse destinée à soutenir un gros palatin (TAVERNE, 1999 : fig. 2).

(6) L'hypercleithrum est fortement élargi dans sa partie dorsale et s'étire en une longue pointe ventrale. Chez les autres Tselfatiiformes où cet os est connu, l'hypercleithrum est de grande taille mais ne montre pas d'élargissement très important dans sa portion supérieure (HAY, 1903 : pl. II ; TAVERNE, 1983 : fig. 2, 1999 : fig. 3).

(7) La branche dorsale du cleithrum est presque inexistante et l'os forme un gros processus postérieur qui surplombe l'hypercoracoïde. Chez les autres Tselfatiiformes, un tel processus n'existe pas (HAY, 1903 : pl. II ; TAVERNE, 1983 : fig. 2, 1999 : fig. 3).

(8) Les faces latérales des vertèbres abdominales sont ornées de deux ou trois crêtes qui encadrent une ou deux fossettes. Primitivement, les faces latérales des vertèbres abdominales sont décorées de fines ridules horizontales (obs. pers.).

Les quatre caractères primitifs évoqués plus haut, déjà perdus chez presque tous les autres Tselfatiiformes et qui attestent que *Niobrara* est l'un des membres les plus primitifs de l'ordre, se retrouvent également chez *Bananogmius* WHITLEY, 1940. Ce genre partage aussi avec *Niobrara* les apomorphies (1) et (5) (HAY, 1903 : pl. II ; WOODWARD, 1923, fig. p. 299 ; obs. pers.), inconnues chez les autres Tselfatiiformes. Cela indique assurément un lien de parenté étroit entre les deux genres. Toutefois, *Niobrara* se distingue clairement de *Bananogmius* par ses apomorphies (2), (3), (4), (6), (7) et (8). Ces six caractères évolués et surtout le (4), unique au sein des Tselfatiiformes, justifient amplement l'érection d'un genre particulier pour *Niobrara encarsia*.

La diagnose amendée de *Niobrara*

Le présent travail permet de définir *Niobrara* d'une manière beaucoup plus précise que ne l'avait fait JORDAN (1924 : 222). La nouvelle diagnose du genre devient donc : tselfatiiforme de taille moyenne ; corps allongé, modérément élevé et comprimé ; mésethmoïde long, large et d'égale largeur sur toute sa longueur ; toit crânien large et presque plat ; grands pariétaux jointifs, encadrés latéralement par les frontaux ; ptérotiques hauts et courts, situés en arrière des pariétaux ; dépression fronto-pariétale courte, large, de forme triangulaire, cernée par une crête osseuse, limitée antérieurement par les frontaux et supportée par les pariétaux ; crête du supraoccipital peu marquée ; *dilatator fossa* haute et bien délimitée par une crête du ptérotique ; fosse temporale ouverte à l'arrière du neurocrâne et couverte latéralement par le ptérotique ; antorbitaire très développé et muni d'un long processus postéro-ventral pointu ; supraorbitaire et dermosphénotique articulés entre eux et avec le frontal ; les trois infraorbitaires postérieurs bien développés ; basioccipital formant seul le condyle pour l'axe vertébral ; septum interorbitaire osseux complet formé par l'orbitosphénoïde, les pleurosphénoïdes et le parasphénoïde ; vomer court et large, entièrement denticulé ; région denticulée du parasphénoïde large, très légèrement concave et perte des denticules dans la partie antérieure de l'os ; supratemporal réduit à sa partie latérale mais conservant un vaste composant membranodermique ; dermopalatin petit et entièrement denticulé ; endoptérygoïde denticulé sur toute sa surface ; ectoptérygïde édenté ou partiellement denticulé ; 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 ; prémaxillaire long et haut, sans processus dorsal différencié et portant une étroite bande de denticule le long de son bord oral ; mandibule allongée et modérément élevée, dépourvue de processus coronoïde individualisé ; dentaire garni d'une plage de denticules qui déborde sur les deux faces de l'os ; angulaire et rétroarticulaire fusionnés ; articulaire autogène ; operculaire et sous-operculaire bien développés ; rayons branchiostèges de grande taille et accolés les uns aux autres ; dermobasibranchial denticulé ; hyomandibulaire large dorsalement, prolongé par une forte tige ventrale et garni d'un *processus opercularis* allongé ; ligaments ossifiés mandibulo-cleithraux présents ; posttemporal grand et de forme triangulaire ; hypercleithrum très large dorsalement, étiré en une longue pointe ventrale ; cleithrum à longue branche ventrale et branche dorsale très courte mais montrant un vaste processus postérieur ; nageoire pectorale insérée haut sur les flancs et comptant 11 rayons ; ceinture et nageoires pelviennes de grande taille et en position abdominale ; nageoire pelvienne comportant 8 rayons ; nageoire dorsale allongée sur tout le dos et débutant un peu en arrière de la tête ; nageoire anale longue ; têtes des axonostes des ptérygophores dorsaux et anaux élargies en petits plateaux ; complexe hémaxanal de type I ; 63 vertèbres dont 29 abdominales et 34 caudales ; vertèbres plus hautes que larges, ornées d'une ou deux fortes crêtes dans la région abdominale et de fines stries dans la région caudale ; arcs neuraux et hémaux autogènes ; hémapophyses petites ; côtes fortes et longues ; épicentraux et épineuraux présents ; pédoncule caudal court ; complexe urophore avec de fortes neurépines et hémépines autogènes ; vertèbre préurale 1 réduite portant une neurépine complète autogène et un arc hémal réduit autogène ; vertèbres urales 1 et 2 fusionnées en un petit centre terminal soudé à une large plaque hypurale formée des quatre premiers hypuraux ; un cinquième hypural autogène (un sixième hypural autogène est possible, voir le paragraphe sur le squelette caudal) ; nageoire caudale bilobée ; grandes écailles cycloïdes à surface lisse.

Le genre étant monospécifique, la nouvelle diagnose du genre *Niobrara* vaut également pour l'espèce *Niobrara encarsia*.

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LISTE DES ABRÉVIATIONS DES FIGURES

An (g.):	angulaire (= angulo-splénial) (gauche)
Ant:	antorbitaire
Art:	articulaire
Ax:	écaille axillaire pectorale
Bo:	basioccipital
Brstg:	rayons branchiostèges
C:	côte
Clt:	cleithrum
Cor:	coracoïde (= hypocoracoïde)
Dbbr 1-3:	dermobasibranchial des trois premiers arcs branchiaux
Dn (d., g.):	dentaire (= dento-splénial, dentalo-splénial) (droit, gauche)
Dpal:	dermopalatin
Dsph:	dermosphénotique
Ecpt:	ectoptérygoïde
Enpt:	entoptérygoïde
Epi (d., g.):	épiotique (= épioccipital) (droit, gauche)
Epice:	épicentraux
Exo (d., g.):	exoccipital (droit, gauche)
Fr (d., g.):	frontal (droit, gauche)
Hclt:	hypercleithrum (= supracleithrum)
Hem:	arc hémal (= hémarcual)
Hemap:	hémapophyse
Hemep:	hémépine (= hémacanthe)
Hem Pu 1:	arc hémal de la vertèbre préurale 1
Hp Pu 2:	hémépine de la vertèbre préurale 2
Hy 1-4:	plaque hypurale formée des quatre premiers hypuraux soudés
Hy 5:	cinquième hypural
Hyom:	hyomandibulaire
Ic:	intercalaire
Iorb 3, 4, 5:	infraorbitaires 3, 4, 5
Iorb (f.):	fragment d'un infraorbitaire postérieur
Lep:	lépidotriche
Meth:	mésethmoïde
Neur:	arc neural (= neurarcual)
Neurep:	neurépine (= neuracanthe)
Np Pu 1:	neurépine de la vertèbre préurale 1
Np Pu 2:	neurépine de la vertèbre préurale 2
Op:	operculaire
Osph:	orbitosphénoïde
Pa (d., g.):	pariétal (droit, gauche)
Pmx (d., g.):	prémaxillaire (droit, gauche)
Pop:	préoperculaire
Pro:	prootique
Ps:	parasphénoïde
Psph:	pleurosphénoïde (= ptérosphénoïde)
Pt (d., g.):	posttemporal (droit, gauche)
Pte (d., g.):	ptérotique (droit, gauche)
Pu 1, 2, 3, 4, 5:	vertèbres préurales 1, 2, 3, 4, 5
Qu:	carré (= quadratique)
Rart:	rétroarticulaire
Sca:	scapula (= hypercoracoïde)
Soc:	supraoccipital
Sop:	sous-operculaire
Sorb:	supraorbitaire
Sph (d., g.):	sphénotique (= autosphénotique) (droit, gauche)
St:	supratemporal (= extrascapulaire)
U 1 + 2:	centre vertébral terminal ural 1 et 2
V:	corps vertébral
V 1 à 8:	les huit premières vertèbres abdominales
Vo:	vomer (= prévomer)
d.f.:	*dilatator fossa*
d. fr. pa.:	dépression fronto-pariétale
f. hyom.:	fossette articulaire pour l'hyomandibulaire
f. t. (d., g.):	fosse temporale (= posttemporale) (droite, gauche)
lig. os.:	ligaments ossifiés mandibulo-cleithraux

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Variations histologiques de l'ovaire au cours du cycle reproductif annuel chez *Agama impalearis* Boettger, 1874 (Reptilia : Agamidae)

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RÉSUMÉ. Ce travail est consacré à l'étude du cycle sexuel femelle de l'agamidé nord africain *Agama impalearis*. L'examen histologique de l'ovaire a permis de suivre l'évolution des ovocytes et de leurs enveloppes folliculaires depuis leur formation jusqu'à la ponte ovulaire et l'apparition des corps jaunes (corpora lutea). La folliculogénèse et la croissance des ovocytes suit le modèle général décrit chez les lézards. Un seul lit germinatif a été mis en évidence dans chaque ovaire. Durant le repos sexuel, seuls des follicules prévitellogéniques sont observés et la vitellogenèse commence juste après l'émergence de l'hivernage. On note l'existence de deux générations de follicules vitellogéniques donnant vraisemblablement lieu à deux pontes successives durant la saison de reproduction. Le nombre de follicules vitellogéniques peut atteindre 12 par ovaire et par génération. La croissance ovocytaire est accompagnée par des modifications de la thèque, de la granulosa et des zones pellucide et radiata. Les corpora lutea sont observés jusqu'au début du second cycle vitellogénique et disparaissent rapidement par la suite. Deux types d'atrésie folliculaire sont décrits ; atrésie en phase d'hydratation et atrésie en phase de vitellogenèse. L'atrésie apparaît peu fréquente chez *Agama impalearis* et frappe le plus souvent les follicules en vitellogenèse.

MOTS CLÉS : Cycle ovarien, histologie, reproduction, *Agama*.

Histological changes in the ovary during the annual breeding cycle in *Agama impalearis* Boettger, 1874 (Reptilia : Agamidae)

ABSTRACT. The aim of the present work is to investigate the female reproductive cycle of the North African agamid, *Agama impalearis*. Histological changes of the whole ovarian cycle were described. Folliculogenesis and oocyte growth were comparable to the general lizard pattern. A single germinal bed per ovary was found. Only previtellogenic follicles were present during the non reproductive period and the onset of vitellogenesis was observed just after emergence from winter dormancy. Two vitellogenic cycles which probably produce two successive clutches per breeding season were observed. The number of vitellogenic follicles can reach 12 per ovarian vitellogenic cycle. The oocyte growth was accompagnied by changes in the theca, granulosa, zona pellucida and zona radiata layers. The postovulatory follicles (corpora lutea) were observed until the onset of the second vitellogenic cycle and disappeared rapidly after them. Two kinds of atretic follicles, hydratation stage and vitellogenic stage atretic follicles, were described. Atresia was less frequent in *Agama impalearis* and particularly concerned with larger vitellogenic follicles.

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INTRODUCTION

L'étude de la reproduction a été depuis longtemps un outil important dans la compréhension de la stratégie d'adaptation des espèces à leur milieu (Guillette & Mandez de La Cruz, 1993). Une importante indication de la biologie de reproduction d'une espèce est la caractérisation de son cycle sexuel (Fitch, 1970). A cet égard, les études effectuées chez la plupart des reptiles des zones tempérées montrent que la saison de reproduction est généralement bien définie et mettent en évidence des variations saisonnières de la taille et de l'activité des gonades (voir Fitch, 1970; Duvall et al., 1982; Saint Girons, 1984 pour revue). Bien que de nombreux travaux aient traité le cycle reproductif chez diverses espèces de lézards (e.g., Saint Girons & Duguy, 1970; Saint Girons, 1984; Castilla & Bauwens, 1992), les données relatives à la famille des Agamidés restent toutefois peu nombreuses. Ainsi, les seuls agamidés des zones tempérées dont le cycle reproductif a été suffisamment étudié en détail sont *Laudakia stellio* (Linnaeus, 1758) (Loumbourdis & Kattoulas, 1982a; 1982b), *Calotes versicolor* (Daudin, 1802) (Varma, 1970; Varma & Guraya, 1973), *Agama atra* (Daudin, 1802) (Van Wyk, 1983; 1984a; 1984b) *Ctenophorus nuchalis* (Devis, 1884) et *Ctenophorus caudicinctus* (Günther, 1875) (Bradshaw et al., 1991). En Afrique du Nord le cycle sexuel d'*Uromastix acanthinurus* Bell, 1825, a fait l'objet d'études morpho-histologiques relativement détaillées (Courrier, 1929; Kehl, 1944; Hammouche et al., 1994; Hammouche & Gernigon-Spychalowicz, 1996). Chez *A. impalearis*, Boettger, 1874, seul le cycle reproductif mâle a été étudié à ce point de vue (Saint Girons, 1967; Znari & El Mouden, 1997). En revanche, le tractus génital femelle, étudié du point de vue anatomique (Znari & El Mouden, 1997), demeure encore non étudié sur le plan histologique.

Outre l'influence des facteurs écologiques (e.g., climat, latitude, altitude et disponibilité trophique) (Fitch, 1985; Seigel & Ford, 1991), la taille de la ponte qui représente un aspect important de la stratégie reproductive d'un reptile, est sous l'influence d'autres facteurs d'ordre anatomique et/ou physiologique. Ainsi, d'une manière générale, la fécondité potentielle des lézards femelles dépend du nombre de lits germinatifs dans l'ovaire (Jones et al., 1982), de la vascularisation ovarienne (Jones, 1975) et du nombre de follicules en croissance par rapport à celui des follicules atrésiques lequel est influencé par les concentrations de gonadotropines et de stéroïdes circulants (Jones et al., 1976).

De point de vue structure histologique de l'ovaire, les différents stades de la folliculogenèse, depuis la différenciation de l'ovocyte primaire jusqu'à l'ovulation, ont été décrits chez plusieurs espèces de reptiles (e.g., Bons, 1972; Filosa, 1973; Sherbrooke, 1975; Jones et al.,1978; Laughran et al., 1981; Van wyk, 1984a; Hammouche & Gernigon-Spychalowicz, 1996). Par ailleurs, les follicules postovulatoires sont généralement transformés en corps jaune (ou corpora lutea) dont la signification fonctionnelle reste encore inconnue. Les follicules ovariens ne se développent pas tous jusqu'à l'ovulation. Ce phénomène connu sous le nom d'atrésie, a été décrit chez toutes les classes des vertébrés et est commun chez la plupart des reptiles. Les ovocytes peuvent devenir atrésiques à n'importe quel stade de leur développement, toutefois, l'atrésie est plus fréquente dans les gros follicules vitéllogéniques (Varma, 1970; Van Wyk, 1984a).

Le but de la présente étude est de caractériser le cycle ovarien d'*A. impalearis*. Nous décrirons la structure histologique de l'ovaire ainsi que ses variations au cours du cycle annuel. Nous proposerons une série de stades sur le développement de l'ovocyte et des différents constituants de la paroi folliculaire. Ces analyses histologiques compléteront ainsi les observations morpho-anatomiques déjà réalisées sur l'ovaire chez cette espèce (Znari & El Mouden, 1997).

MATÉRIEL ET MÉTHODES

A. impalearis est un lézard diurne de taille moyenne (Longueur totale moyenne d'environ 250 mm) et endémique d'Afrique du Nord (Schleish et al., 1996) . Il est particulièrement commun au Maroc où il occupe les milieux les plus variés (Bons & Geniez, 1996). Son rythme annuel est marqué par une période d'hivernage de novembre à février (Znari & El Mouden, 1997). Les individus des deux sexes atteignent leur maturité sexuelle à l'âge d'un an à une taille museau-cloaque d'environ 90 mm (El Mouden et al., 1997; Znari & El Mouden, 1997). La période des accouplements s'étend d'avril à juillet et les pontes sont produites entre mi-mai et début septembre.

L'étude histologique de l'appareil reproductif femelle a été conduite entre mars et novembre 1996 sur des femelles adultes collectées dans la région des Jbilets centrales à 25 Km au Nord de Marrakech (31°37'N, 8°02'W, 580 m d'altitude). Le climat de la région est de type aride avec une pluviométrie annuelle moyenne d'environ 240 mm. La température moyenne maximale du mois le plus chaud (juillet) peut atteindre 39°C et la température moyenne minimale est habituellement située autour de 0°C (Le Houérou, 1989). Les caractéristiques du milieu ont été décrites en détails dans d'autres travaux (Znari & El Mouden, 1997; 1998; Znari et al., 1998).

L'étude a porté sur 56 femelles capturées à différentes périodes de l'année à raison de 4 à 12 spécimens par mois. Les animaux ont été sacrifiés dans les 24 heures après leur capture par anesthésie profonde au Diéthyléther. Les ovaires droits sont fixés au liquide de Bouin en solution aqueuse. Après déshydratation et passage prolongé à l'alcool butylique, les pièces sont incluses dans la paraffine et débitées en coupes sériées de 7,5m d'épaisseur. Les coupes histologiques sont ensuite colorées par l'hémalun et

par le Picro indigo carmin (PIC) (MARTOJA & MARTOJA, 1967).

Du fait que les conditions reproductives des femelles collectées durant la saison de reproduction ne sont pas synchrones, ces femelles seront alors groupées selon les stades de leur activité reproductrice plutôt que la date de leur capture. Ainsi, nous proposerons une série de stades sur le développement de l'ovocyte et des différents constituants de la paroi folliculaire.

Nous avons également mesuré le grand axe des ovocytes sur des dessins réalisés au moyen d'une chambre claire. Les mesures sont prises sur des coupes voisines de l'équateur de l'ovocyte.

RÉSULTATS

L'ovaire d'*A. impalearis* adulte présente des ovocytes à différents niveaux de développement, depuis l'ovogonie jusqu'aux stades les plus avancés. Les stades jeunes peuvent être observés dans tous les ovaires quelque soit le moment de l'année. Cependant, les œufs sur le point d'atteindre leur complète maturité ne se rencontrent qu'à partir de fin avril et jusqu'au début septembre. l'examen des ovaires a montré que le dépôt du vitellus commence vers la fin du mois de Mars sur de petits follicules dont la diamètre ne dépasse guère 2 mm. Pendant la vitellogenèse, l'accroissement du diamètre des ovocytes est important et peut atteindre une valeur maximale de 8 mm.

Le diamètre moyen des cinq plus gros follicules de chaque ovaire durant le cycle sexuel montre une variation annuelle significative (ANOVA, $F_{(8, 37)} = 2,83$; $P = 0,019$) (Fig. 1). Il commence à augmenter dés le début mars pour atteindre une valeur maximale durant la période de mai-août. En se basant sur la répartition des tailles des ovocytes, l'analyse du graphique montre que l'accroissement lent dure jusqu'à une dimension voisine de 2 mm, auquel succède le grand accroissement conduisant à la ponte ovulaire. Le diamètre des ovocytes passe ainsi de 2 mm à un maximum pouvant atteindre 8 mm en l'espace d'un mois

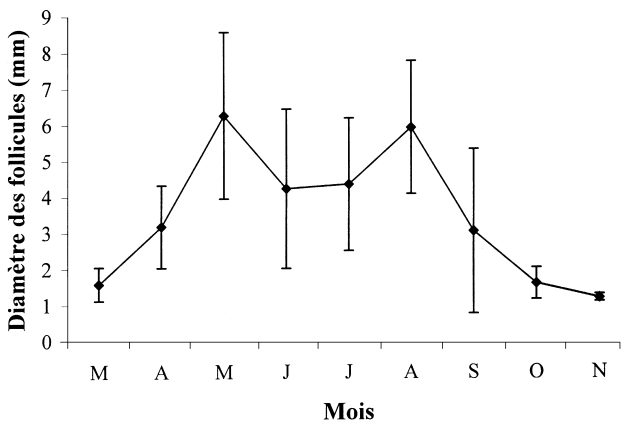


Fig. 1. – Variations mensuelles (moyenne ± SD) du diamètre moyen des cinq plus gros follicules de chaque ovaire chez *Agama impalearis* durant l'année 1996.

voire moins. Entre septembre et mars de l'année suivante, l'ovaire est caractérisé par des follicules immatures de petite taille.

Au début du mois de mars, chaque ovaire renferme 10 à 13 ovocytes faiblement opaques d'un diamètre inférieur à 1,3 mm. Lorsqu'ils dépassent cette taille, ils deviennent blancs opaques et prennent ensuite une couleur jaune pâle qui devient très vive au terme de la fin de la vitellogenèse. Au mois d'octobre, l'aspect des ovaires est similaire à celui des femelles au mois de mars. Le nombre d'ovocytes en vitellogenèse est variable ; il est parfois égal dans les deux ovaires, sans dépasser toutefois 12 par ovaire.

La preuve histologique que certaines femelles peuvent produire plus d'une ponte par an est bien confirmée par la présence sur certaines coupes de gros follicules en vitellogenèse en même temps que des corpora lutea récents.

Stroma ovarien et épithélium germinatif (Fig. 2, 3 et 4)

Des coupes sériées montrent que chaque ovaire ne possède qu'un seul lit germinatif placé vers la paroi dorsale de l'ovaire. Ce lit germinatif a la forme d'un petit monticule allongé suivant une direction parallèle à l'axe du corps. Il se compose d'un cortex médio-dorsal unique et d'une zone centrale composée de cellules ne présentant pas d'orientation définie à laquelle sont reliés les très jeunes ovocytes en cours d'accroissement. Cette apparence histologique rend l'épithélium germinatif facilement repérable (Fig. 3). En outre, l'échelonnement régulier de la taille des ovocytes confirme l'existence d'un seul lit germinatif par ovaire.

Durant le cycle reproductif, l'ovaire subit de nombreux changements avec la production et la croissance des follicules. Les éléments ovariens comportent ainsi un nombre variable de follicules en développement, des corpora lutea, des follicules atrésiques et un seul lit germinatif. Les ovogonies évoluent dans l'épithélium germinatif. Les ovocytes primaires de forme sphérique sont ensuite projetés dans les couches plus internes du lit germinatif au niveau duquel ils s'entourent d'une rangée de cellules aplaties disposées d'une manière irrégulière (Fig. 4). Les cellules placées autour de chaque ovocyte deviennent de plus en plus nombreuses pour constituer ensuite plusieurs enveloppes. Le follicule ovarien ainsi formé quitte le lit germinatif et se dirige vers la cavité de l'ovaire. Les ovocytes à ce stade ont un diamètre de 0,2 ± 0,04 mm.

Dans l'évolution de l'ovocyte primaire et de ses enveloppes, on peut distinguer plusieurs stades dont chacun est défini par des modifications précises d'un ou de plusieurs constituants :

Stade I: Ovocyte de diamètre compris entre 0,1-0,3 mm (Fig. 5)

Les follicules de cette taille viennent de quitter l'épithélium germinatif. Ils se caractérisent par un ooplasme

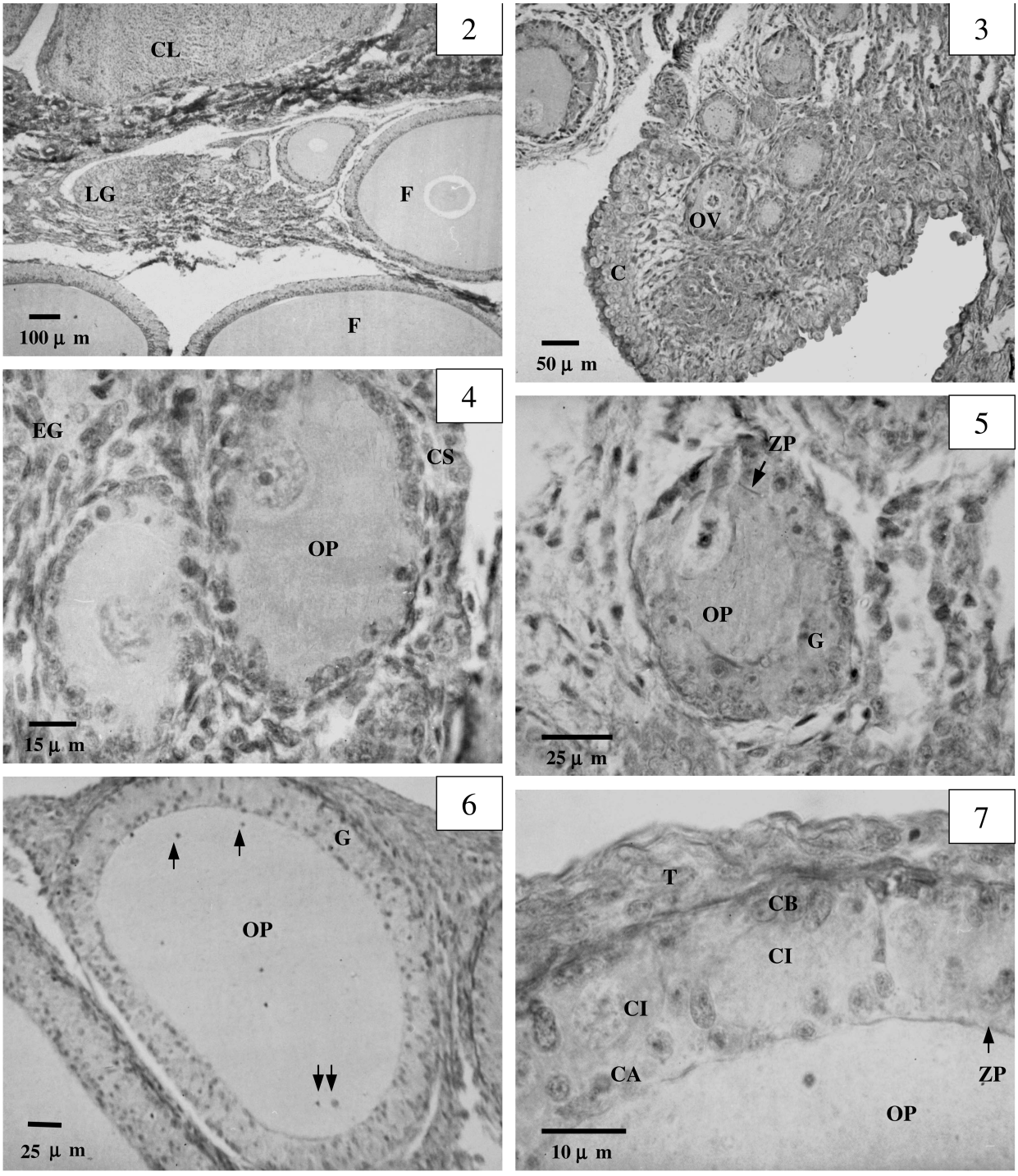


Fig. 2. – Coupe transversale de l'ovaire au niveau du lit germinatif (LG). (CL : corpora lutea ; F : follicule en croissance).

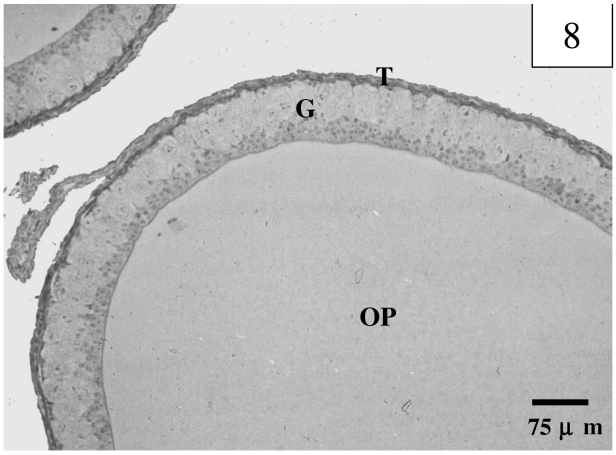
Fig. 3. – Lit germinatif montrant des ovocytes primaires (OV). (C : cortex).

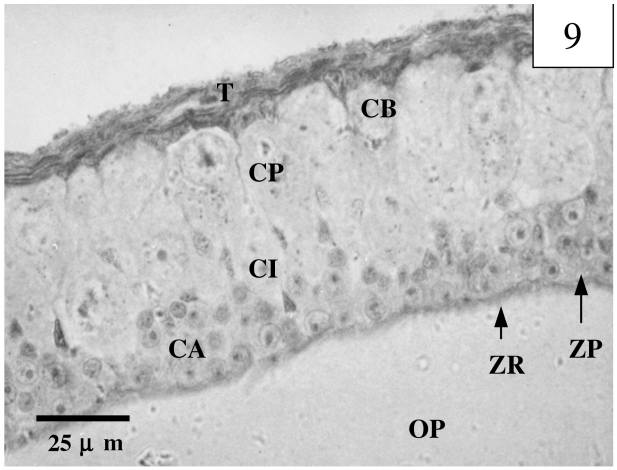
Fig. 4. – Epithélium germinatif avec des ovocytes primaires entourés de cellules stromales (CS). (EG : épithélium germinatif ; OP : ooplasme).

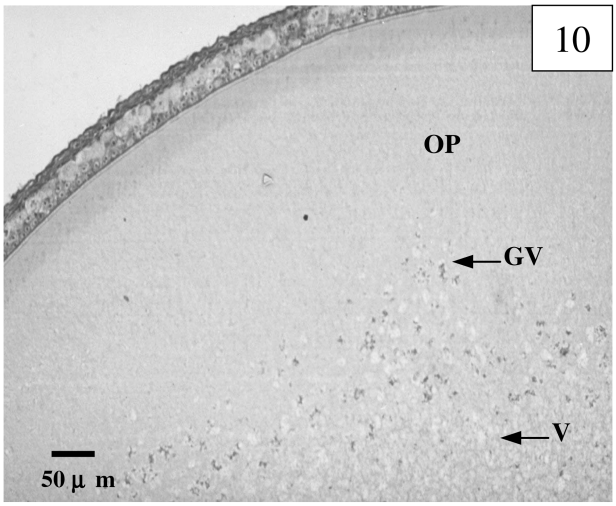
Fig. 5. – Ovocytes primaire dans le stroma ovarien. Notez la différenciation de la granulosa (G) et le début de formation de la zone pellucide (ZP). (OP : ooplasme).

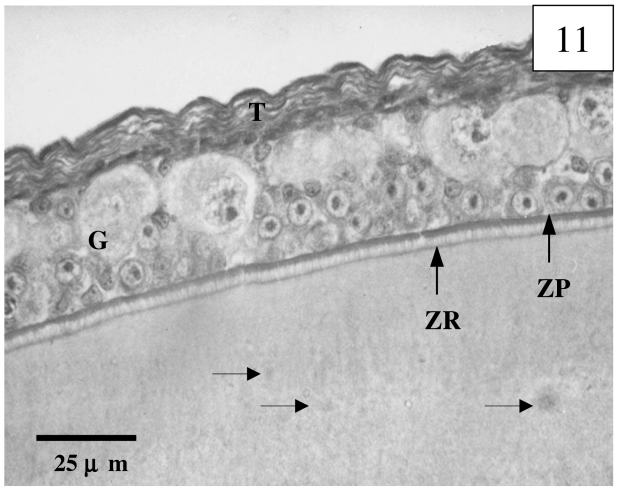
Fig. 6. – Ovocyte en croissance dans le stroma ovarien. Les flèches indiquent les granulations acidophiles. (OP : ooplasme ; G : granulosa).

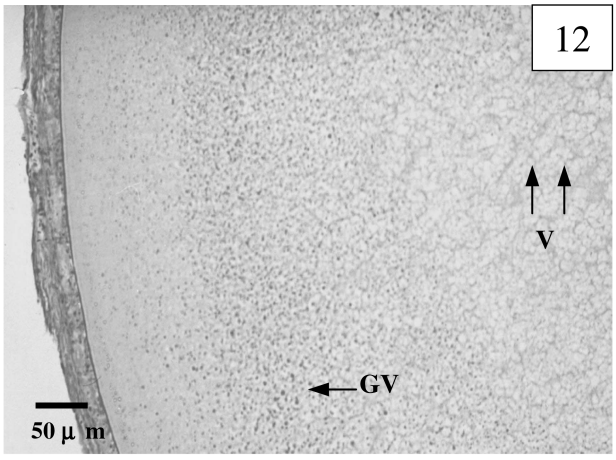
Fig. 7. – Vue d'ensemble de l'épithélium folliculaire d'un ovocyte au stade II. (CA : cellule apicale ; CB : cellule basale ; CI : cellule intermédiaire ; OP : ooplasme ; T : thèque ; ZP : zone pellucide).











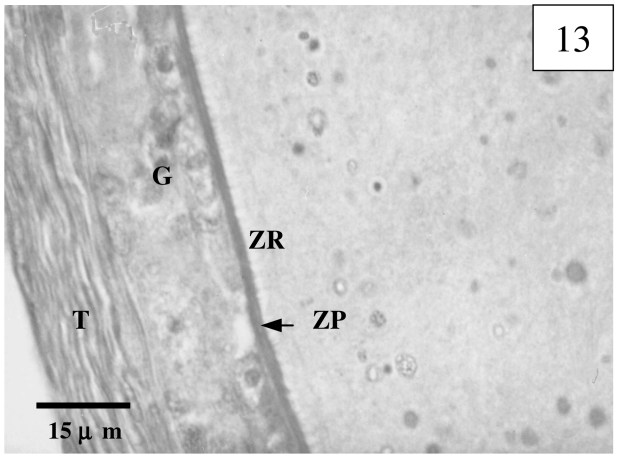


Fig. 8. – Thèque, granulosa et ooplasme d'un ovocyte au stade III. (G : granulosa ; OP : ooplasme ; T : thèque).

Fig. 9. – Détail de la figure précédente montrant l'apparition des cellules piriformes (CP) dans l'épithélium folliculaire. (CA : cellule apicale ; CB : cellule basale ; CI : cellule intermédiaire ; OP : ooplasme ; T : thèque ; ZP : zone pellucide ; ZR : zone radiata).

Fig. 10. – Follicule ovarien montrant les vacuoles (V) et granulations vitellines (GV) qui commencent à envahir la partie centrale du cytoplasme (OP).

Fig. 11. – Epithélium folliculaire et membrane vitelline d'un ovocyte au stade IV. Zone pellucide (ZP) et zone radiata (ZR) nettes. Les flèches indiquent les substances acidophiles. (G : granulosa ; T : thèque).

Fig. 12. – Follicule ovarien au stade V montrant une extension des vacuoles (V) et de granulations vitellins (GV) dans l'ooplasme.

Fig. 13. – Thèque (T), granulosa (G), zone pellucide (ZP), zone radiata (ZR) et ooplasme d'un ovocyte au stade V.

homogène et un début de formation de l'épithélium folliculaire (granulosa). Le noyau relativement arrondi et vésiculaire est placé de façon excentrique. La granulosa est constituée de cellules épithéliales qui commencent à se différencier. Ainsi, deux types de cellules peuvent être distinguées : les cellules intermédiaires qui sont grandes et les cellules basales de petite taille. L'épaisseur de la granulosa est en moyenne de 26 ± 1,0 mm. A ce jeune stade de l'ovocyte, la membrane ooplasmique est très fine et la théque non encore bien différenciée et constituée d'une très mince couche de fibroblastes aplatis. Pendant cette phase, l'ovocyte continue de s'accroître.

Stade II : Ovocyte de diamètre compris entre 0,3-0,5 mm (Fig. 6-7)

Les ovocytes sont entourés d'une granulosa épaisse. L'ooplasme faiblement coloré montre une apparence homogène avec un début de formation de granulations acidophiles. La granulosa est toujours formée de petites cellules basales et de grosses cellules intermédiaires qui sont plus abondantes qu'au stade précédent. Les grosses cellules sont caractérisées par un cytoplasme légèrement coloré et par des noyaux basophiles vésiculaires bien distincts. L'épithélium folliculaire est d'une épaisseur de 25 ± 4,1 mm. Ce stade se caractérise par l'épaississement de la membrane ooplasmique pour former une structure homogène, c'est la zone pellucide. La théque consiste en une mince couche de 7 ± 0,8 mm de fibroblastes non vascularisés. Les follicules de cette taille sont présents dans l'ovaire durant toute l'année.

Stade III : Ovocyte de diamètre compris entre 0,5-1 mm (Fig. 8-9)

L'ooplasme commence à se vacuoliser dans sa partie centrale. La membrane ooplasmique, toujours fine, commence à se dédoubler en deux feuillets par le début de la différenciation de la zone striée (zone radiata) selon une disposition radiaire au contact de l'ooplasme cortical. La zone pellucide est bien différenciée et est plus externe entre l'ooplasme et la granulosa. Leurs épaisseurs sont respectivement de 0,5 ± 0,1 mm et 1,5 ± 0,1 mm. La granulosa devient plus épaisse (48 ± 6,6 mm) avec l'apparition d'une nouvelle sorte de cellules. Elle se compose ainsi de trois types de cellules : grandes cellules piriformes, qui sont allongées avec un cytoplasme clair et un noyau bien coloré, cellules intermédiaires logées entre les parties effilées des cellules piriformes et contre la zone pellucide avec un diamètre de 15 mm environ, et enfin, les cellules de petite taille plaquées contre la zone pellucide (cellules apicales) ainsi que vers la partie externe de la granulosa (cellules basales). A ce stade, la théque est légèrement plus développée qu'au stade précèdent (7,8 ± 2,8 mm) et consiste en de fibroblastes arrangés de façon concentrique. Les follicules de ce type sont présents durant le cycle annuel.

Stade IV : Ovocyte de diamètre compris entre 1-1,5 mm (Fig. 10-11)

Les vacuoles envahissent l'ooplasme et plus particulièrement le centre de l'ovocyte alors que de minuscules sphères vitellines commencent à ce déposer dans sa partie périphérique et s'étendent à l'intérieur. Des inclusions supplémentaires acidophiles sont présents près de la périphérie de l'ovocyte. Les membranes vitellines sont maintenant très nettes autour de l'ovocyte avec le début d'apparition des striations sur la zone radiata. La zone pellucide se colore de façon homogène acidophile. La zone pellucide et la zone radiata deviennent plus épaisses et mesurent environ 3 mm chacune. L'épaisseur de la granulosa reste sensiblement le même qu'au stade précèdent avec une abondance de cellules basales et intermédiaires. En revanche, la théque est devenue plus épaisse (13,5 ± 4,5 mm) avec l'apparition de petits capillaires sanguins. Les follicules de cette taille sont présents dans l'ovaire le long de l'année.

Stade V : Ovocyte de diamètre compris entre 1,5-2 mm (Fig. 12-13)

L'augmentation du nombre et de la taille des vacuoles donne au cytoplasme une apparence alvéolaire avec une abondance importante de vitellus vers la partie périphérique. Des inclusions acidophiles apparaissent près de la zone radiata dont l'épaisseur ainsi que celle de la zone pellucide restent sensiblement identiques qu'au stade IV (2,5 ± 0,5 mm). On note une réduction de l'épaisseur de la granulosa (34 ± 7,4 mm), une diminution du nombre des cellules piriformes qui s'allongent parallèlement à la granulosa et une abondance des cellules basales. L'apparence de la théque est toujours indifférenciée et percée de vaisseaux sanguins qui sont plus fréquents près de la surface externe qu'ils traversent perpendiculairement. Son épaisseur subit une légère augmentation (16 ± 5,8 mm). Ce type d'ovocyte marque le début de la vitellogenèse.

Stade VI : Ovocyte de diamètre compris entre 2-3 mm (Fig. 14-15)

Les sphères vitellines deviennent plus nombreuses au sein du cytoplasme. Leur taille et leur abondance diminuent en allant de la zone périphérique vers la zone centrale du cytoplasme qui apparaît complètement vacuolisée. Une couche dense de sphères vitellines apparaît contre la face interne de la zone radiata. Comparée au stade précédent, l'épaisseur de cette dernière a augmenté (4,5 ± 0,6 mm) avec l'apparition d'une striation plus nette tandis que l'épaisseur de la zone pellucide est restée sensiblement la même et celle de la granulosa a considérablement diminué (15 ± 2,8 mm). La structure de la granulosa est toujours composée de cellules piriformes et de cellules basales qui sont plus fréquentes vers la limite externe alors que les cellules intermédiaires disparaissent.

La granulosa apparaît plus épaisse là où les cellules piriformes subsistent. La théque est devenue plus épaisse (18 ± 4,2 mm) avec une vascularisation plus importante dans sa partie externe. Les follicules de ce stade sont présents dans les ovaires durant la phase de la vitellogenèse.

Stade VII : Ovocyte de diamètre plus large que 3 mm (Fig. 16)

C'est le stade précédant la ponte ovulaire. Les follicules sont maintenant mûrs avec un dépôt massif de vitellus remplissant le cytoplasme d'une façon presque uniforme. La zone radiata est légèrement réduite en épaisseur (4,0 ± 1,6 mm) et les striations perpendiculaires deviennent plus nombreuses. La zone pellucide devient plus fine (1,8 ± 0,3 mm). La régression de la granulosa continue (3,8 ± 0,5 mm) et seules quelques cellules piriformes persistent ne formant plus qu'une seule assise plus ou moins discontinue de cellules squamosales aplaties. La théque garde a peu près la même épaisseur (18,5 ± 1,7 mm). Au moment de la ponte ovulaire, les enveloppes se rompent et l'ovule se détache de l'épithélium folliculaire et est recueilli par le pavillon de l'oviducte.

Follicules post-ovulatoires

Après l'ovulation, les enveloppes folliculaires restent attachées à l'ovaire sur un large secteur et forment des corpora lutea qui sont toujours placés dans la région la plus vascularisée. Les ovaires contiennent ainsi des corpora lutea et des follicules en croissance avec quelque fois des follicules atrésiques. Du fait que les femelles sacrifiées ne proviennent pas d'un élevage, l'évolution des corpora lutea est donc suivie sur des femelles fraîchement capturées dont la date de l'ovulation est inconnue ainsi que l'âge des corpora lutea.

Stade I (Fig. 17-18)

Juste après l'ovulation, l'ouverture par laquelle s'est échappé l'ovule est large. Les enveloppes folliculaires entourent un espace en forme de croissant. Les cellules de la granulosa subissent une hypertrophie rapide et commencent à remplir la cavité centrale. La théque devient plus épaisse que dans les follicules préovulatoires et comporte deux couches multicellulaires (externe et interne) facilement reconnaissables. La couche externe apparaît plus collagéneuse avec peu de fibroblastes qui ont tendance à avoir une disposition plutôt radiaire. Elle est parcourue par de gros vaisseaux parallèles à la surface. La couche interne est plus épaisse et est constituée de nombreuses cellules fibroblastiques et de fibres collagènes rangées de façon concentrique. La limite entre les deux couches est marquée par la présence des espaces capillaires contenant des hématies. La limite interne située près des cellules lutéales est marquée par la présence de fibroblastes basophiles.

Stade II (Fig. 19-20)

Le volume total du corpora lutea s'est réduit et manifeste une apparence plus arrondie que dans le stade précédent. Sa cavité centrale est entièrement comblée par les cellules lutéiniques. Ces dernières sont plus abondantes et contiennent des noyaux vésiculaires de taille variable. La théque est moins épaisse par rapport au stade précédent et les deux couches qui la composent, conservent les mêmes propriétés. Toutefois, elles montrent une plus forte vascularisation avec des vaisseaux sanguins de plus petite taille. La théque externe montre des signes de destruction et la plupart des capillaires se sont désintégrés. Le long de la bordure interne de la théque, les granulations pigmentaires sont particulièrement abondantes et fortement colorées. Ce type de corpora lutea se rencontre en présence de gros œufs oviductaires et de gros follicules en vitellogenèse qui correspondent au début du deuxième cycle vitellogénique.

Stage III (Fig. 21)

Les corpora lutea présentent des dimensions réduites et apparaissent pigmentés avec une disparition totale de la théque. Ils peuvent être observés simultanément à une seconde génération de gros follicules vitellogéniques. Les observations faites sur les différentes coupes n'ont pas mis en évidence la présence simultanée des corpora lutea résultant de la première et de la deuxième pontes. Ceci suggère une disparition relativement rapide des corpora lutea issus de la première ponte avant la mise en place de ceux correspondants à la seconde ponte. Par ailleurs, à la fin de la période de reproduction, on assiste à la dégénérescence totale des corpora lutea.

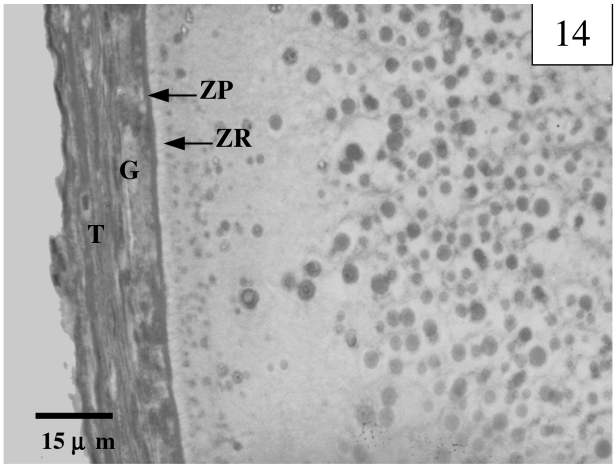
Les follicules atrésiques

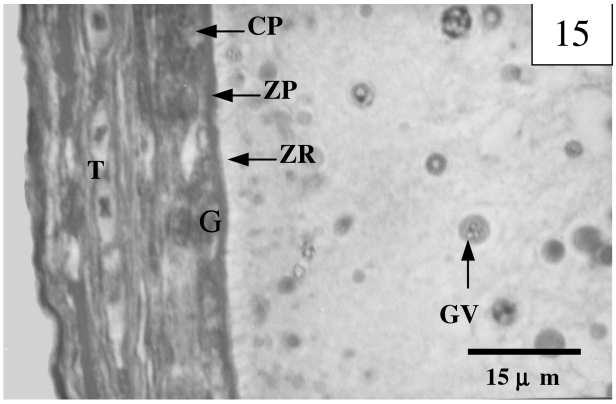
Les follicules atrésiques, non reconnaissables avec certitude que sur préparations histologiques, sont observés durant tous les stades du cycle reproductif. Cependant, l'atrésie frappe le plus souvent des follicules en vitellogenèse. Les follicules atrésiques se caractérisent par une hypertrophie des cellules de la granulosa et sont généralement de deux types : les follicules en phase d'hydratation et ceux en cours de la vitellogenèse.

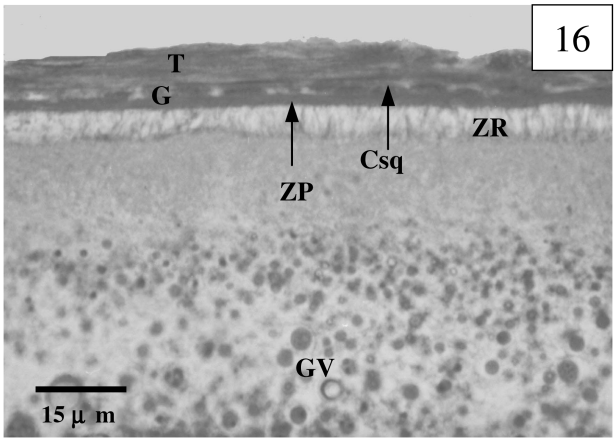
Sur la totalité des femelles examinées, l'atrésie folliculaire n'est rencontrée que chez 21% d'entre elles avec le plus souvent 1 à 2 follicules atrésiques par ovaire.

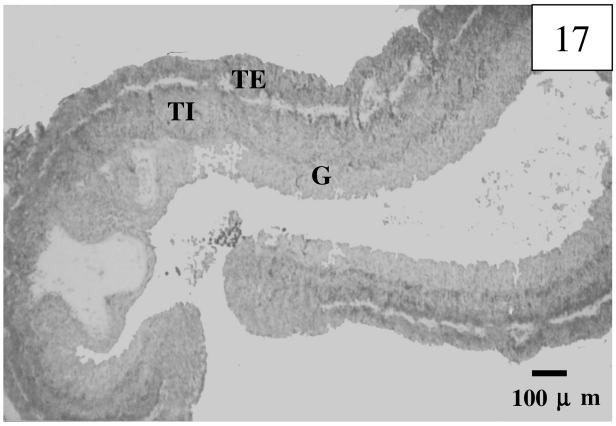
Atrésie en phase d'hydratation (Fig. 22-23)

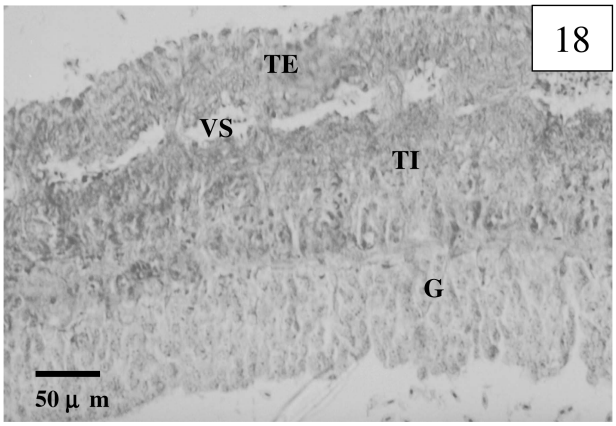
L'ooplasme présente une apparence granulaire avec des agrégations occasionnelles. Les macrophages amiboïdes sont abondants et présentent une activité phagocytaire évidente. Les membranes vitellines (zone pellucide et zone radiata) sont absentes de même que les cellules intermédiaires et piriformes de la granulosa. Cette dernière est











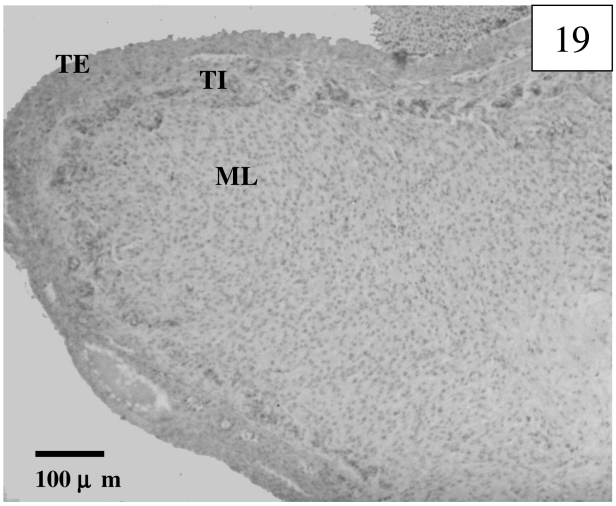


Fig. 14. – Follicule ovarien au stade VI. (G : granulosa ; T : thèque ; ZP : zone pellucide ; ZR : zone radiata).

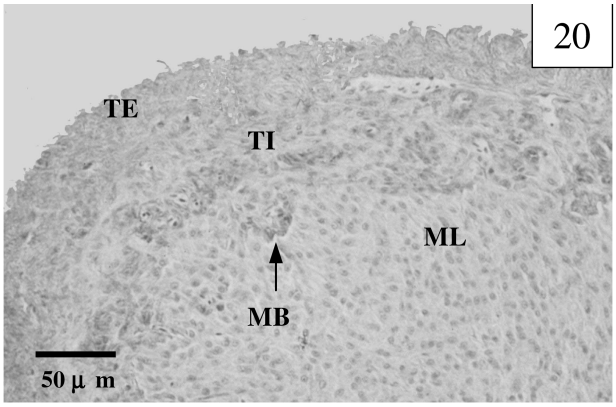
Fig. 15. – Détail de la figure précédente montrant la réduction du nombre et de la taille des cellules piriformes (CP). (G : granulosa ; GV : granulations vitellines ; T : thèque ; ZP : zone pellucide ; ZR : zone radiata).

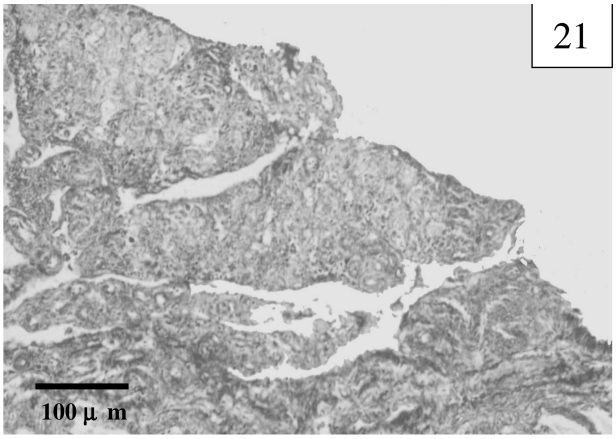
Fig. 16. – Follicule ovarien au stade VII (fin de la vitellogenèse) montrant les cellules squamosales (Csq) de la granulosa (G) et de large sphères vitellines (GV). (T : théque ; ZP : zone pellucide ; ZR : zone radiata).

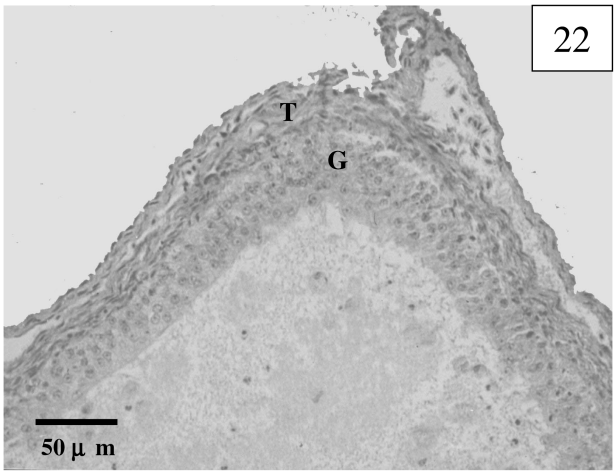
Fig. 17. – Follicule ovarien postovulatoire (corpora lutea) au stade I. (TE : thèque externe, TI : thèque interne, G : Granulosa).

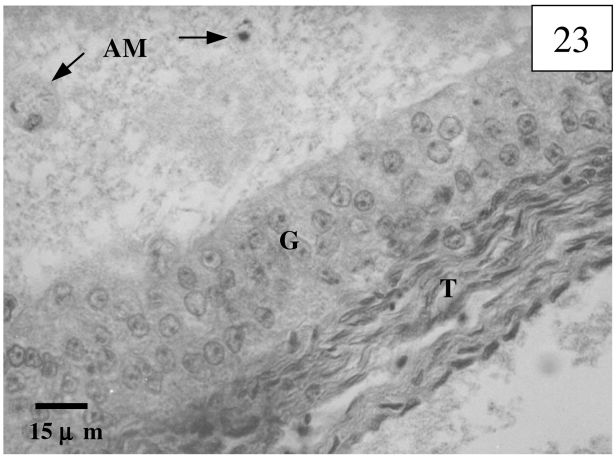
Fig. 18. – Détail de la figure précédente montrant trois couches distinctes : théque externe (TE), thèque interne (TI) et la granulosa (G). Les vaisseaux sanguins (VS) sont situés entre la thèque externe et la thèque interne.

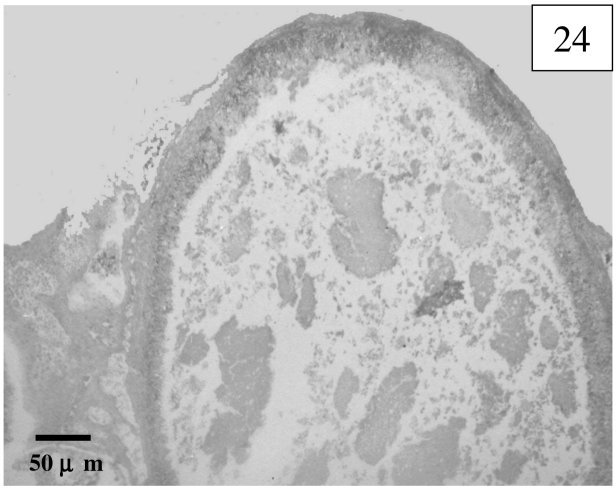
Fig. 19. – Corpora lutea au stade II postovulatoire. Les cellules lutéiniques ont complètement envahi la cavité centrale. (TE : thèque externe ; TI : thèque interne ; ML : masse lutéinique).











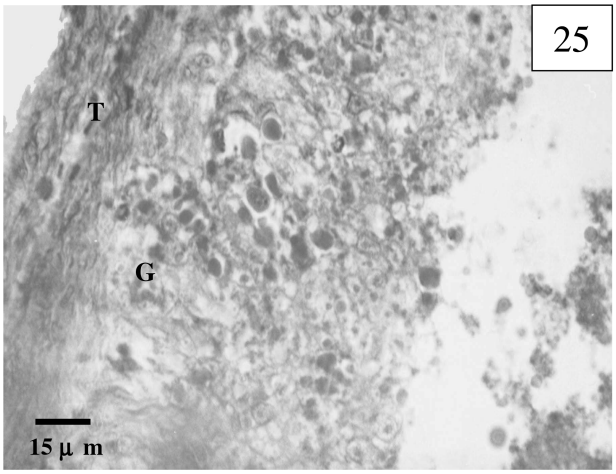


Fig. 20. – Détails de la figure précédente montrant les trois couches : thèque externe (TE), thèque interne (TI) et granulosa (= masse lutéinique, ML). (MB : membrane basale).

Fig. 21. – Dégénérescence des corpora lutea au stade III post-ovulatoire. On assiste à une disparition totale de la théque.

Fig. 22. – Follicule atrésique en phase d'hydratation. (T : thèque, G : Granulosa).

Fig. 23. – Détail d'un follicule atrésique en phase d'hydratation. Notez l'hypertrophie de la granulosa (G) et l'apparition des macrophages amiboïdes (MA) dans le cytoplasme. (T : Thèque).

Fig. 24. – Follicule atrésique en phase de vitellogénèse.

Fig. 25. – Follicule atrésique en phase de vitellogénèse. Notez l'hypertrophie de la granulosa (G) et la résorption de la majorité des granulations vitellines. (T : thèque).

constituée de cellules de forme variable avec des noyaux arrondis montrant des figures mitotiques fréquentes. La théque est hypertrophiée mais reste bien séparée de la granulosa. Sa couche externe est essentiellement constituée de tissus connectifs indifférenciés avec des vaisseaux sanguins. Les atrésies en phase d'hydratation n'ont été observées que durant la période de repos sexuel (octobre-novembre).

Atrésie en phase de vitellogenèse (Fig. 24-25)

Les cellules de la granulosa se sont hypertrophiées et envahissent la masse jaune. Les macrophages sont fréquents dans la granulosa. L'hypertrophie est aussi évidente au niveau de la théque. La zone pellucide est très difficilement discernable. De grosses sphères vitellines de forme variable apparaissent à l'intérieur des vacuoles intra-cytoplasmiques et une grande partie du vitellus est résorbée. Les atrésies en phase de vitellogenèse ne sont observées qu'au cours de la phase d'activité reproductrice.

DISCUSSION

Le cycle sexuel femelle d'*A. impalearis* se caractérise par une longue période de vitellogenèse qui débute en fin mars et se poursuit jusqu'à fin août (voir également Znari & El Mouden, 1997). De fait, cette espèce est l'une des plus précoces à se reproduire juste après son émergence de l'hivernage. Ceci est associé à une grande fécondité qui se traduit par la production de deux grosses pontes successives. En effet, les résultats histologiques obtenus chez la plupart des femelles examinées ont mis en évidence la présence simultanée de follicules en vitellogenèse et de corpora lutea ce qui confirme les données morphologiques de Bons (1968) et de Znari & El Mouden (1997) sur l'existence d'une double ponte chez cette espèce. Ce type de cycle vitellogénique est qualifié de prénuptial (Saint Girons, 1984) et a été décrit chez d'autres Agamidés (e.g., Chidress, 1970; Bons, 1972; Loumbourdis & Kattoulas, 1982a) ainsi que d'autres espèces de lézards (e.g. Saint Girons & Duguy, 1970; Filosa, 1973; Castilla & Bauwens, 1992). *A. impalearis* a un cycle très voisin de celui des lézards occupant les régions à climat tempéré avec une saison aride et qui présentent deux pontes annuelles (Bons, 1962; Childress, 1970; Pianka, 1970; Loumbourdis & Kattoulas, 1982a; voir Fitch, 1970 pour revue). La comparaison de nos données avec celles de Bons (1967) sur la longueur de la saison de reproduction avec une seule ponte chez la même espèce habitant l'extrême nord du Maroc, suppose l'existence d'une certaine variation géographique dans le cycle reproductif femelle. Le cycle reproductif apparaît ainsi très lié à la longueur de la saison de reproduction qui est déterminée par les conditions climatiques locales et plus particulièrement par la température ambiante (Nussbaum, 1981; Ferguson et al. 1990). Cette dernière exerce une influence directe sur la croissance folliculaire (Duvall et al., 1982). Dans d'autres cas, la photopériode peut être également un facteur important qui influence le cycle reproductif chez les reptiles des zones tempérées (e.g., Van wyk, 1983).

Chez les reptiles, les ovogonies sont localisés dans une ou plusieurs régions de l'ovaire connues sous le nom du lit germinatif. Contrairement aux mammifères dont le nombre d'ovogonies est fixé durant le développement embryonnaire, les ovogonies présents dans le lit germinatif des reptiles montrent une activité mitotique au début de chaque cycle reproductif. En général, les lézards ont un, deux ou plusieurs lits germinatifs par ovaire (Jones et al., 1982). Chez *A. impalearis*, un seul et unique lit germinatif a été mis en évidence. Des observations similaires ont été rapportées chez plusieurs autres lézards (Boyd, 1940; Varma, 1970; Jones et al., 1975a; 1975b; Van wyk, 1984a). Par contre, deux épithéliums germinatifs ont été mis en évidence chez *Xantusia vigilis* Baird, 1859 (Miller, 1948) et *Leiolopisma rhomboidalis* Peters, 1869 (Wilhoft, 1963). Cette variabilité du nombre de lits germinatifs par ovaire selon l'espèce considérée est probablement l'un des facteurs qui contrôlent la taille de la ponte (Jones et al., 1978).

Outre quelques différences dans la phénologie de la reproduction ainsi que la durée des différents événements reproductifs, le développement des structures ovariennes décrit chez *A. impalearis* est comparable à celui chez d'autres agamidés (Van wyk, 1984a; Varma, 1970) ainsi que chez d'autres groupes de lézards Boyd, 1940; Filosa, 1973; Klosterman, 1987). Selon les présentes investigations, la différenciation de la granulosa chez *A. impalearis* est caractérisée par la formation de trois catégories cellulaires (grandes cellules piriformes, cellules intermédiaires et petites cellules). Comme l'avait suggéré Van wyk (1984a), les cellules piriformes dériveraient des cellules intermédiaires qui dériveraient à leur tour des petites cellules. L'abondance relative de ces dernières indique que la formation de ces différents types de cellules est le résultat d'une différenciation plutôt qu'une activité mitotique (Hubert, 1971; 1973; Neaves, 1971; Lance & Callard, 1978; Laughran et al., 1981, Van wyk, 1984a). Le nombre de cellules piriformes augmente dans la granulosa simultanément à la croissance folliculaire initiale. Néanmoins, aucune activité mitotique n'apparaît au niveau des cellules intermédiaires et piriformes (Hubert, 1973) ou encore comme l'avait signalé Betz (1963), l'activité mitotique s'arrête juste après la différenciation des premières cellules piriformes. Ceci indique que ces dernières se différencient uniquement à partir des cellules de la granulosa déjà existantes. En revanche, plusieurs auteurs ont rapporté que les petites cellules de la granulosa continuent à se diviser jusqu'au début de la vitellogenèse (Blanc, 1971; Olma & Taddei, 1974; Lance & Lofts, 1978). Par ailleurs, Filosa et al. (1979) ont trouvé que le nombre des grosses cellules augmente durant la croissance ovocytaire simultanément à une aug-

mentation de celui des petites cellules avec un arrêt de la différenciation des grosses cellules mais une continuation de la division des petites. Cependant, des études autoradiographiques ont montré que les petites cellules basales de la granulosa prolifèrent et donnent naissance aux cellules piriformes et intermédiaires.

Les variations saisonnières de l'épaisseur de la granulosa ont été largement rapportées chez les squamates (Guraya, 1978). Comme c'est le cas chez *A. atra* (Van wyk, 1984a), ces variations sont en corrélation avec le développement folliculaire. L'épaississement de la granulosa durant la phase prévitellogénique est en relation avec l'augmentation du nombre des cellules piriformes alors que son amaincissement paraît être le résultat de la dégénérescence de ces cellules au début de la vitellogenèse. Le développement puis la régression de la granulosa impose l'idée de sa participation active dans les processus de la vitellogenèse chez les reptiles (Blanc, 1971). A cet égard, il a été suggéré que les cellules piriformes de la granulosa ont un rôle nutritif (Bons, 1972) et sont probablement impliquées dans la production de vitellus (Goldberg, 1970; Varma, 1970; Blanc, 1971, Neaves, 1971; Hubert, 1973; Lance & Callard, 1978, Van wyk, 1984a). Chez *A. atra*, Van wyk (1984a) signale l'existence de granulations APS positive dans le cytoplasme des cellules piriformes des follicules au stade prévitellogénique. Selon cet auteur, cette réaction pourrait être due à un échange de glycogène et de ribonucléoproteïne entre les cellules piriformes et l'ooplasme. Chez *Acanthodactylus erythrurus lineomaculatus* (Schinz, 1833), Bons (1972) a affirme que les cellules piriformes servent surtout de guide aux substances qui sont élaborées par les cellules basales. Après avoir suivi le contour des cellules piriformes, ces substances pénètrent dans l'ovocyte à travers les pores de la membrane ovocytaire qui coïncident avec l'emplacement des pôles apicaux des cellules piriformes. En revanche, des observations au microscope électronique chez *Anolis carolinensis* (Voigt, 1832) n'ont révélé aucune trace du dépôt vitellin dans le cytoplasme des cellules piriformes (Neaves, 1971). Notons qu'en dehors des squamates durant la prévitellogénèse à des stades très comparables à ceux observés chez les oiseaux (Callebaut, 1991) et les Elasmobranches (Andreuccetti et al., 1999), des cellules à aspect pyriforme ont été décrites dans la granulosa.

Comme il a été rapporté chez la plupart des vertébrés inférieurs, la zone pellucide est formée à l'interface ovocyte-épithélium folliculaire chez *A. impalearis*. Comme chez d'autres espèces de lézards (Varma, 1970; Jones et al., 1975a; Guraya & Varma, 1976; Van wyk, 1984a) durant les premiers stades de la croissance ovarienne, la zone pellucide forme tout d'abord une seule couche homogène puis au fur et à mesure de la différenciation folliculaire, elle se dédouble pour donner naissance à une autre couche striée (zone radiata). Chez *A. impalearis*, les petites sphères vitellines adjacentes à la limite de la zone radiata indiquent que l'accumulation du vitellus a probablement une origine extra-ovocytaire. Ceci est en accord avec les observations de Van wyk (1984a) chez *A. atra* chez laquelle il suppose que les petites sphères fusionnent entre elles pour former les grosses sphères qui occuperont tout le cytoplasme des follicules mûrs. D'autre part, la zone pellucide atteint son épaisseur maximale dans la phase vitellogénique chez *A. impalearis* comme c'est le cas chez d'autres agamidés en l'occurrence *C. versicolor* (Varma, 1970) et *A. atra* (Van wyk, 1984a). Cependant, si la zone pellucide représente l'accumulation des précurseurs du vitellus suite à l'endocytose au niveau de la zone périphérique de l'ooplasme, on pourrait s'attendre à ce que la zone pellucide atteigne son développement maximal durant les stades préovulatoires. Ceci n'est cependant pas le cas chez *A. impalearis* et la signification fonctionnelle de la zone pellucide reste ainsi inconnue.

Les corpora lutea ont été mis en évidence chez plusieurs espèces de reptiles y compris les lézards ovipares et vivipares (Fox, 1977). Chez *A. impalearis,* le développement des corpora lutea suit le type général décrit par plusieurs auteurs chez les reptiles (Goldberg, 1970; Varma, 1970; Lance & Lofts, 1978; Van Wyk, 1984a). De même, les cellules lutéiniques mises en évidence chez cette espèce correspondent à la description générale faite chez *C. versicolor* (Varma, 1970), *Sceloporus jarrovi* Cope, 1875 (Goldberg, 1970) et *A. atra* (Van wyk, 1984a). Comme l'ont suggéré Betz (1963) et Van wyk (1984), les cellules lutéiniques hypertrophiées dérivent de la granulosa aussi bien par prolifération que par différenciation. Toutefois, l'absence d'activité mitotique dans la granulosa suggère que la différenciation est le processus plutôt impliqué en accord avec les observations de Varma (1970) et de Van wyk (1984a). Certains auteurs supposent que les sécrétions des corpora lutea ont un rôle dans la rétention de l'œuf dans les oviductes (Panigel, 1956; Bons, 1972). Aussi, plusieurs études ont rapporté que les corpora lutea seraient les principales sources de progestérone chez les reptiles (Veith, 1974; Highfill & Mead, 1975; Arslan et al., 1978).

Le phénomène d'atrésie folliculaire est commun durant la croissance folliculaire et frappe plus particulièrement les grosses follicules en fin de vitellogenèse chez tous les vertébrés (Byskov, 1978). Chez les lézards, il a été observé chez les espèces aussi bien ovipares que vivipares (Goldberg, 1970; Goldberg, 1973; Jones, 1975; 1978; Guillette et al., 1981; Fox & Guillette, 1987; Guillette & Casas-Andreu, 1987; Méndez-de la Cruz et al., 1988). Cependant, contrairement aux mammifères (Ingram, 1972) et aux oiseaux (Erpino, 1973), la plupart des lézards étudiés présentent seulement deux types de follicules atrésiques (Goldberg, 1970; Varma, 1970; Eyeson, 1971; Lance & Callard, 1978; Van wyk, 1984a). Ceci est le cas chez *A. impalearis* dont l'analyse histologique a mis en évidence une atrésie en phase d'hydratation et une atrésie en phase de vitellogenèse. Plusieurs auteurs ont rapporté un accroissement du nombre des follicules atrésiques durant la phase postovulatoire

(Goldberg, 1970; Varma, 1970) alors que Jones et al., (1976) et Van wyk (1984a) ont trouvé, respectivement chez *A. carolinensis* et *A. atra*, que cet accroissement de l'atrésie folliculaire apparaît durant les stades prévitellogénique et vitellogénique. Ces dernières observations sont en accord avec celles faites chez *A. impalearis*.

L'atrésie folliculaire a été signalée dans la littérature écologique comme l'un des principaux facteurs qui déterminent la taille de la ponte chez plusieurs espèces de lézards. Ainsi, la taille de la ponte est réduite par atrésie folliculaire à 36% chez *X. vigilis* (Miller, 1948), 29% chez *Barisia imbricata* (Wiegmann, 1828) (Guillette & Casas-Andreu, 1987) et 52% chez *Sceloporus mucronatus* Cope, 1885 (Mendez-de la Cruz et al., 1988). Les résultats histologiques de la présente étude montre que l'atrésie folliculaire est peu fréquente chez *A. impalearis*. Sa fréquence serait associée au nombre de lits germinatifs par ovaire (Jones et al., 1982) et serait ainsi plus faible chez les espèces ayant un seul lit germinatif par ovaire comparativement aux espèces ayant plus d'un lit germinatif (Van wyk, 1984a).

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Geographic scaling and genetic differentiation in two highly mobile European saltmarsh beetles

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ABSTRACT. Genetic structure and diversity are studied in two European saltmarsh beetles, *Bembidion minimum* and *B. normannum*, on a regional as well as a Western European scale. Results are based on allozymes, studied at four polymorphic loci for more than 1600 individuals from all remaining saltmarshes in Belgium and from a selection of European reference sites. Average gene diversity is not related to habitat or population size, but is larger in the more common *B. minimum*, in comparison to Atlantic samples of *B. normannum*. One Mediterranean sample of the latter species reveals a much higher diversity and suggests this region as the evolutionary centre of origin and/or as a possible glacial refugium of the species. Significant overall genetic structure is observed in the complete data of both species, with 2 to 6 % of the total genetic variation explained by differentiation between populations. Genetic differentiation in both species is significant at different geographic scales, with higher values at a larger scale. A Mantel-test (isolation by distance) between geographic and genetic distance is significant in *B. normannum*. Our results indicate that habitat fragmentation has not yet resulted in genetic erosion, probably because of the large population sizes of both species, even in very small saltmarshes. The observed genetic differentiation suggests that metapopulations at a relatively large geographic scale are still functional in these highly mobile species. Re-establishment of even small saltmarshes is suggested as a positive conservation measure for long term survival of these specialised ground beetles.

KEY WORDS: Carabidae, European saltmarshes, *Bembidion minimum*, *Bembidion normannum*, population genetics, habitat fragmentation, genetic diversity, metapopulation structure, dispersal power, geographic scale.

INTRODUCTION

Insects prove useful models and indicators of geographic structure and genetic differentiation in relation to habitat fragmentation and isolation. Nowadays, populations of many terrestrial arthropods only survive in remnants of natural habitats, highly isolated from each other. This is certainly the case for many habitat types in Western Europe and in particular within the region of Flanders (Belgium). Fragmentation in general results in a reduced genetic diversity (e.g. ANDREN, 1994; AVISE & HAMRICK, 1996; FRANKHAM, 1996), but may also increase genetic differentiation between populations as a result of reduced gene flow (SLATKIN, 1994).

Population genetics studies the distribution and abundance of genotypes between and within natural populations. Geographic genetic structure and genetic diversity combine both demographic and genetic processes, such as extinction/recolonisation and metapopulation dynamics, gene flow, genetic drift and natural selection. Ground beetles (Coleoptera, Carabidae) appear to be ideal model organisms for such studies. In Western Europe, carabids belong to the most popular, diversified and best-studied invertebrates. They show a stable taxonomy and a pronounced habitat preference, and their large- and small-scale distribution is relatively well known. Abundant data on their occurrence are available for the last 150 years, especially in the Netherlands and Belgium (TURIN, 2000). The small size of most ground beetles, as well as their extreme diversity and high abundances, enable investigations on the effects of geographical scaling on genetic structure and diversity, while population genetic data can be relevant for conservation ecological purposes. Ground beetles also show wide variation in traits related to dispersal power. More importantly, potential gene flow can be directly or indirectly quantified by studying the morphology of the hind wings (macroptery, brachyptery or polymorphism) and flight muscles. Based on such data, model species with known but varying dispersal power

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and mobility can be compared in population genetic studies. Expected gene flow, as deduced from knowledge on dispersal power and commonness/rarity, can be confronted with gene flow estimates derived from genetic studies. Many carabid species even show a dispersal di- or polymorphism and such wing polymorphic species have enabled test and confirmation of the dispersal-gene flow hypothesis ('levels of gene flow among populations are correlated with dispersal power') (DESENDER & SERRANO, 1999; PETERSEN & DENNO, 1997).

In Western Europe and especially in Flanders, natural habitats have severely suffered from human impact and have decreased in size, number and quality. Therefore these habitats have become more and more fragmented. Habitats that have, in recent historical times, decreased dramatically in size are forests and saltmarshes (TACK et al., 1993; DIJKEMA et al., 1984). In a recently published 'Red list' of the ground beetles in Flanders the conclusion was reached that many typical saltmarsh species have become either rare, endangered and close to extinction, or probably even became extinct during recent decades (DESENDER et al. 1995). Similar patterns have been observed in other European countries (DESENDER & TURIN 1989). The main reasons for this general decrease are most probably habitat destruction and reduction, as well as pollution, which is known to be severe in estuaries and coastal ecosystems (DIJKEMA et al. 1984; WESTHOFF, 1985).

To study the impact of fragmentation, a regional, inter-institutional and conservation genetic study project was conducted in Flanders (for more details, see DESENDER et al., 1998). Within this project, case studies were performed on a large array of organisms, including many invertebrates (DE MEESTER et al., 2000). Insects were studied from either forest fragments (specialised forest dwelling beetles, cf. DESENDER et al., 1999) or isolated saltmarshes (halobiontic or halophilic ground beetles, cf. DESENDER et al., 1998).

In the present study, we investigate the genetic structure in two highly mobile saltmarsh beetle species of the genus *Bembidion*, *B. minimum* and *B. normannum*, on a regional as well as western European scale. We test whether effects of fragmentation are visible in species with high mobility, where we expect little or no differentiation. Results are based on allozyme electrophoresis, investigated by studying samples from all remaining saltmarshes in Belgium and some European reference sites at larger distances. Neither species has previously been studied with respect to its population genetics. They persist in at least some of the Flemish saltmarshes. In particular, we are interested in the effect of different geographic scaling on the observed genetic differentiation and diversity, with implications for metapopulation size estimation and conservation genetics. We will therefore relate the within-population genetic diversity and among-population genetic differentiation in both species to habitat size, population size and geographic distance between habitats.

MATERIAL AND METHODS

Study species

Bembidion (Emphanes) minimum Fabricius, 1792 is a small (total length about 2.7 mm), metallic-black halophilic ground beetle with a Palearctic distribution (Fig. 1 left). Its range extends from southern Scandinavia to southern Europe and eastwards as far as Siberia. The species is typically found in high densities (sometimes up to some 20 ind./m^2, cf. DESENDER & SEGERS, 1985 and DESENDER, unpublished data) in saltmarshes on marine clay soils, mainly in coastal estuaries. There are also inland observations from saline or brackish areas or from polder areas (DESENDER & MAELFAIT, 1999; TURIN, 2000). The species is active during daytime and reproduces in spring. *B. minimum* is constantly macropterous and, as far as studied, shows functional flight musculature. There are numerous flight observations (DESENDER, 1989) and the species is an excellent swimmer/floater (TURIN, 2000). In Flanders, it has recently been classified as being still at relatively low risk (not yet endangered) (DESENDER et al., 1995).

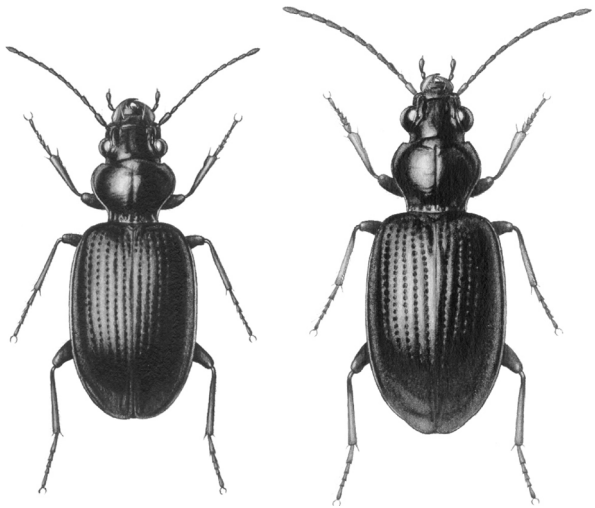


Fig. 1. – *Bembidion minimum* (left) and *B. normannum* (right): two sibling ground beetle species from saltmarshes, respectively about 2.7 and 3 mm (total length).

Bembidion normannum Dejean, 1831 is a somewhat larger (about 3 mm; Fig. 1 right), halobiontic sibling species of *B. minimum,* morphologically differentiated by its lighter leg colour, slightly different shape of pronotum and a more pronounced lighter apical spot on the elytra. This small carabid only occurs along the European coast, from southern Denmark to Italy. It is a typical inhabitant of marine saltmarshes and is also known from some inland high salinity sites (always in the vicinity of the coast). Although population densities can be locally quite high, *B. normannum* is much rarer than *B. minimum*.

Today, it survives in Flanders in a very restricted number of saltmarshes (see later). It has been categorised as 'vulnerable' in the Red data-book for Flanders (DESENDER et al., 1995). *B. normannum* is also active during daytime, reproduces during spring, is constantly macropterous and, as far as studied, always shows functional flight musculature (DESENDER, 1989; DESENDER & MAELFAIT, 1999).

Study sites and sampling

Saltmarsh ground beetles have been collected between 1992 and 1998 for genetic (electrophoretic) studies at different levels of spatial scale in populations with varying size and isolation. Fig. 2 shows the 24 sample locations of the two *Bembidion* species. Samples were taken in all remaining saltmarshes in Belgium and the adjacent southern part of the Netherlands (estuary of the river Schelde) (Fig. 2, sites 1-9) and from European reference sites at larger distances. In the Netherlands, population samples were taken from a saltmarsh area in Friesland (site 10). Other samples were collected in the UK: Morecambe Bay

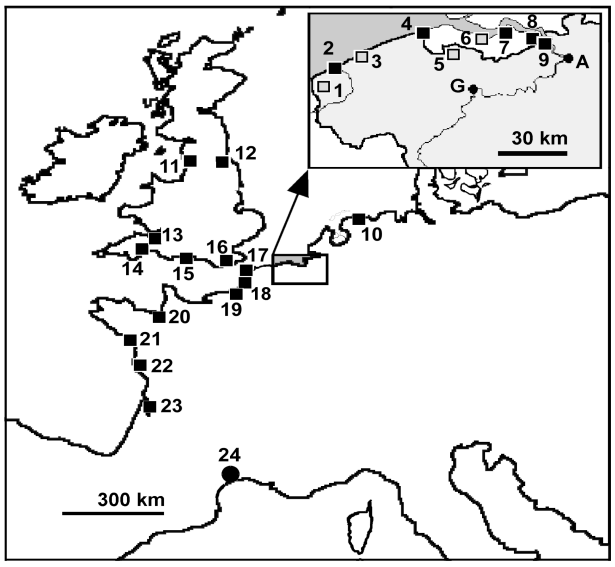


Fig. 2. – Location of the studied saltmarsh areas in Western Europe and in the region of Flanders and the southern part of the Netherlands (inset) (see Table 1 for details).

TABLE 1

Sampled saltmarshes (with information on habitat size and age) and populations of *Bembidion minimum* and *B. normannum* (with estimated total population sizes)

map code (cf. Fig. 2)	site/estuary	population code	saltmarsh area (ha)	saltmarsh age (y)	B. minimum	B. minimum number code	B. minimum population size	B. normannum	B. normannum number code	B. normannum population size
1	De Moeren	MOE	0.5	380	MOE	9	2000			
2	IJzer estuary	NIE	16	900	NIE	11	98133			
3	Oostende	OOS	0.1	250	OOS	12	1100			
4	Zwin	ZWC	120	700	ZWC	17	177143	ZWC	16	485714
"	"	ZWR	5	50	ZWR	18	50000	ZWR	17	485714
5	Molenkreek	MOL	0.5	300				MOL	10	1150
6	Braakman	BRA	1.5	300				BRA	2	1583
7	Ossenisse	OSS	50	700	OSS	13	200000			
8	Saeftinghe	SAE	300	700	SAE	14	251351			
9	Doel	DOE	51	80	DOE	3	53833			
10	Friesland	FER	400	200	FER	5	766667			
"	"	HOL	300	200	HOL	6	3133333	HOL	8	4433333
"	"	HOR	200	200	HOR	7	1300000			
11	Morecambe Bay	MOR	80	800	MOR	10	650667			
12	Humber estuary	HUM	200	?	HUM	8	1458333	HUM	9	1366667
13	Severn estuary	SEV	100	1000	SEV	15	350000			
14	Exe estuary	EXE	70	800	EXE	4	136111			
15	Thorney Island	THO	2	200				THO	15	3667
16	Rye estuary	RYE	10	700				RYE	14	50667
17	Authie estuary	AUT	250	400	AUT	1	1000000	AUT	1	375000
18	Canche estuary	CAN	200	1000	CAN	2	220000	CAN	3	380000
19	Somme estuary	SOM	200	2500	SOM	16	1966667			
20	Mont St Michel	MSB	500	7000				MSB	11	2500000
"	"	MSG	500	7000				MSG	12	1200000
21	la Guérande	GUA	2	400				GUA	6	4200000
"	"	GUB	20	1150				GUB	7	25400000
22	la Gachère	GAC	80	3500				GAC	4	313333
23	Gironde estuary	GIR	5	8000				GIR	5	13167
24	Rousillon	ROU	100	?				ROU	13	458333

(site 11), the Humber estuary (site 12), the Severn estuary (site 13), the Exe estuary (site 14), Thorney Island (site 15) and the Rye estuary (site 16). In France, samples were taken from the Bay of the Authie, Canche and Somme (sites 17-19), at the Mont St Michel (site 20), la Guérande (site 21 near the Loire estuary), La Gachère (site 22) and the Gironde estuary (site 23). Finally, for *B. normannum*, a sample from a Mediterranean saltmarsh at Bages in the Roussillon (France; site 24) was also studied.

Beetles were gathered by standardised hand collecting (per unit of time effort), transported alive to the lab, counted and identified under a binocular microscope. Subsequently, they were killed and stored in liquid nitrogen until electrophoresis. At some of the sampling sites absolute abundance estimates were made by means of a combined quadrat-flotation technique (DESENDER & SEGERS, 1985) in order to calibrate handcatches to densities.

Table 1 summarises information on the study sites, refers to their locations (as illustrated in Fig. 2), mentions population codes, as well as estimates of saltmarsh size (area) and age (when available). Number codes are also given for all populations studied for each species along with estimates of total population sizes. Such estimates are approximate because of the difficulty of estimating which areas of larger saltmarshes are actually inhabited by a given species. Nevertheless, we considered it better to incorporate a rough estimate of population density than to take only the area of a site into account. The age of saltmarshes was estimated using historical information, and is partly drawn from DESENDER (1985), GOETGHEBEUR (1976), HOFFMANN (1986) and HOUTHUYS et al. (1993). These estimates are maximum values of the historically documented existence of a particular site or area and are therefore less reliable for older sites. Varying ages or levels of spatial scale are included in our sampling design.

Allozyme electrophoresis

Samples were prepared for electrophoresis by homogenising the body (one elytrum was kept as morphological reference material) of individual beetles in 30 µl of distilled water on ice. Cellulose acetate electrophoresis (HEBERT & BEATON, 1989) permitted the examination of each individual for allelic variation. After a pilot study with 26 different enzymes, four polymorphic loci were selected in both *B. minimum* and *B. normannum*. These loci were chosen because they could be easily interpreted and scored, and because they were highly polymorphic (95%-criterion). For each gel, at least one reference individual was included for comparison. Continuous electrophoresis was carried out using standard methods (HEBERT & BEATON 1989. Two buffer systems were used: Tris-Glycine 10% (pH 8.5; HEBERT & BEATON 1989) and Tris-Maleate (pH 7.8; RICHARDSON et al. 1986). Samples from 18 and 17 populations were analysed for each species respectively (Table 1), yielding information on more than 800 individuals per species (at least 20 to 50 individuals from each population, if available).

The enzyme loci studied for *B. minimum* were Peptidase-D (dipeptide substrate: Phenylalanine Proline, PEPD, E.C. 3.4.-.-) and Mannose Phosphate Isomerase (MPI, E.C. 5.3.1.8) on a Tris-Maleate buffer and Glucose-6-phosphate Isomerase (GPI, E.C. 5.3.1.9) and Aldehyde Oxidase (AO, E.C. 1.2.3.1) on a Tris-Glycine buffer. Enzymes studied for *B. normannum* were AO and MPI on a Tris-Maleate buffer and GPI and Phosphoglucomutase (PGM, E.C. 2.7.5.1) on Tris-Glycine. We used slightly modified staining protocols from the ones outlined in HEBERT & BEATON (1989). In agreement with most studies on other animals (HEBERT & BEATON, 1989) and insects in particular (WARD et al. 1992), AO, GPI and PEPD showed a dimeric quaternary structure, while MPI and PGM were monomeric.

Analysis of enzyme allelic frequencies

Basic analyses were performed using BIOSYS-1 (SWOFFORD & SELANDER, 1981) and GENEPOP (v. 3; update from v. 1.2: RAYMOND & ROUSSET 1995a). Analyses were run with the four polymorphic enzymes (95%-criterion) in both species. Genetic diversity estimates were based on all scored loci. Allele frequency tables and basic genetic variability measures were produced with POP100GENE v1.03 (PIRY & BOUGET, 1999).

Genotype frequencies were first tested against Hardy-Weinberg expectations using an exact test procedure (ROUSSET & RAYMOND, 1995) and showed four significant deviations out of 72 tests for *B. minimum* and no significant deviations out of 56 tests for *B. normannum*. These results can be expected by chance alone and suggest that studied populations were all in Hardy-Weinberg equilibrium. The independence of the different markers used was investigated as described by RAYMOND & ROUSSET (1995b) in an exact probability test for genotypic linkage disequilibrium between each pair of loci for each population. Not a single significant linkage test-value was obtained in 108 tests for *B. minimum* and 70 tests for *B. normannum* (Bonferroni-corrected p-values). We can therefore safely conclude that no linkage was observed across all populations.

GENETIX v3.3 (BELKHIR et al., 1996-1998) was used to obtain, at different spatial scales, a variety of genetic differentiation estimates most widely used in recent population genetic studies. These included: F_{ST} (WEIR & COCKERHAM, 1984), G_{ST} (NEI, 1977) and G_{ST} -unbiased estimate (Nei & CHESSER, 1983). GENETIX also enables testing the significance of the F_{ST} -estimate (WEIR & COCKERHAM,1984), by means of a permutation procedure (estimate of the probability value of departure from the null hypothesis).

Within each species, genetic differentiation was tested between all pairs of populations with adjusted probability levels to avoid errors from multiple testing (sequential

Bonferroni method; RICE, 1989). Genetic distances between populations were visualised in dendrograms: Rogers' genetic distance (ROGERS, 1972) and Nei's unbiased genetic distance (NEI, 1978) were used to construct UPGMA-dendrograms for the different populations in both species. Bootstrap-values (1000 replicates) were estimated for each node of the dendrograms by means of TFPGA v1.3 (MILLER, 1997).

Isolation by distance was tested statistically by determining the significance of the correlation between (1) a matrix of Rogers' genetic distance estimates and (2) a matrix of Euclidian geographical distances. To this end, a Mantel-test was performed with p-values determined by a permutation procedure (as implemented in TFPGA and GENEPOP). The results of such a Mantel-test enable examination of the relative importance of gene flow as compared to drift and selection, and inspection for eventual geographical patterns in the data.

RESULTS

General population genetic analyses

Allele frequencies of the polymorphic loci, genetic variability measures and sample sizes are given for each population in Table 2 (*B. minimum*) and Table 3 (*B. nor-*

TABLE 2

Allele frequency table for four polymorphic enzymes studied in *Bembidion minimum,* along with calculated genetic variability estimates and sample sizes

LOCUS/POPULATION		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	TOTAL
Number of beetles (n)		36	22	19	33	22	43	22	53	30	56	138	93	44	30	47	59	43	14	804
A0	**Allele**																			**Means**
Gene Number (2n)		72	44	38	66	44	86	44	106	60	110	272	186	86	60	94	118	80	28	
Allele Number		3	4	2	2	2	2	2	2	3	2	3	3	4	2	2	4	3	2	2.611
	1	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.017	0.000	0.000	
	2	0.472	0.386	0.789	0.803	0.750	0.581	0.659	0.792	0.633	0.927	0.732	0.645	0.733	0.833	0.670	0.458	0.800	0.786	
	3	0.500	0.568	0.211	0.197	0.250	0.419	0.341	0.208	0.333	0.073	0.261	0.339	0.244	0.167	0.330	0.508	0.163	0.214	
	4	0.028	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.007	0.016	0.012	0.000	0.000	0.017	0.037	0.000	
Heterozygote Proportion (Hobs)		0.583	0.545	0.316	0.394	0.318	0.419	0.318	0.264	0.600	0.109	0.368	0.452	0.395	0.333	0.489	0.508	0.400	0.429	0.402
Gene Diversity (Hexp)		0.534	0.539	0.341	0.321	0.384	0.492	0.460	0.332	0.495	0.136	0.398	0.471	0.408	0.282	0.447	0.536	0.336	0.349	0.403
MPI	**Allele**																			**Means**
Gene Number		72	44	38	66	44	86	44	102	60	74	184	170	88	54	88	116	78	26	
Allele Number		2	2	3	2	2	2	2	2	2	2	3	2	2	2	3	2	2	2	2.167
	1	0.000	0.000	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.023	0.000	0.000	0.000	
	2	0.292	0.432	0.263	0.318	0.159	0.256	0.318	0.382	0.183	0.432	0.174	0.241	0.136	0.444	0.261	0.250	0.231	0.154	
	3	0.708	0.568	0.684	0.682	0.841	0.744	0.682	0.618	0.817	0.568	0.821	0.759	0.864	0.556	0.716	0.750	0.769	0.846	
Heterozygote Proportion		0.361	0.409	0.579	0.455	0.318	0.372	0.364	0.608	0.367	0.378	0.217	0.271	0.273	0.444	0.455	0.397	0.256	0.154	0.371
Gene Diversity		0.419	0.502	0.472	0.441	0.274	0.385	0.444	0.477	0.305	0.498	0.298	0.368	0.238	0.503	0.423	0.378	0.360	0.271	0.392
PEPD	**Allele**																			**Means**
Gene Number		72	44	38	66	44	82	44	106	60	112	274	186	88	60	94	118	84	28	
Allele Number		2	2	2	3	2	2	2	2	2	3	3	2	2	2	2	4	2	2	2.278
	1	0.181	0.045	0.079	0.091	0.205	0.037	0.023	0.057	0.100	0.071	0.084	0.054	0.068	0.100	0.064	0.186	0.060	0.179	
	2	0.819	0.955	0.921	0.894	0.795	0.963	0.977	0.943	0.900	0.911	0.909	0.946	0.932	0.900	0.936	0.797	0.940	0.821	
	3	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.018	0.007	0.000	0.000	0.000	0.000	0.008	0.000	0.000	
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	
Heterozygote Proportion		0.083	0.000	0.053	0.091	0.045	0.073	0.045	0.075	0.200	0.143	0.080	0.043	0.045	0.133	0.043	0.136	0.024	0.071	0.077
Gene Diversity		0.300	0.089	0.149	0.195	0.333	0.071	0.045	0.108	0.183	0.167	0.168	0.102	0.129	0.183	0.121	0.333	0.113	0.304	0.172
PGI	**Allele**																			**Means**
Gene Number		72	44	38	64	44	84	44	98	60	76	258	170	86	60	90	114	70	18	
Allele Number		4	3	3	3	3	4	3	3	3	3	3	3	4	4	3	4	3	3	3.278
	1	0.014	0.045	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	
	2	0.056	0.000	0.263	0.125	0.091	0.131	0.068	0.122	0.150	0.158	0.159	0.182	0.128	0.167	0.144	0.044	0.100	0.056	
	3	0.833	0.773	0.632	0.734	0.818	0.69	0.864	0.847	0.800	0.829	0.748	0.782	0.733	0.733	0.722	0.807	0.814	0.889	
	4	0.097	0.182	0.105	0.141	0.091	0.167	0.068	0.031	0.050	0.013	0.093	0.035	0.128	0.083	0.133	0.123	0.086	0.056	
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.026	0.000	0.000	
Heterozygote Proportion		0.333	0.455	0.474	0.469	0.364	0.524	0.273	0.224	0.267	0.237	0.411	0.329	0.372	0.433	0.311	0.316	0.200	0.222	0.345
Gene Diversity		0.297	0.376	0.535	0.432	0.321	0.484	0.251	0.270	0.341	0.292	0.408	0.356	0.436	0.434	0.445	0.334	0.324	0.216	0.364
ALL LOCI																				
Mean Allele Number		2.750	2.750	2.500	2.500	2.250	2.500	2.250	2.250	2.500	2.500	3.000	2.500	3.000	2.500	2.500	3.500	2.500	2.250	
Standard deviation		0.957	0.957	0.577	0.577	0.500	1.000	0.500	0.500	0.577	0.577	0.000	0.577	1.155	1.000	0.577	1.000	0.577	0.500	
Mean Heterozygote proportion		0.340	0.352	0.355	0.352	0.261	0.347	0.250	0.293	0.358	0.217	0.269	0.274	0.271	0.336	0.324	0.339	0.220	0.219	
Standard deviation		0.205	0.242	0.229	0.177	0.146	0.193	0.141	0.225	0.175	0.121	0.151	0.171	0.160	0.144	0.203	0.157	0.156	0.153	
Mean Gene Diversity		0.387	0.377	0.374	0.347	0.328	0.358	0.300	0.297	0.331	0.273	0.318	0.324	0.303	0.351	0.359	0.395	0.283	0.285	
Standard deviation		0.113	0.204	0.170	0.115	0.045	0.197	0.195	0.153	0.129	0.164	0.112	0.157	0.145	0.145	0.159	0.096	0.114	0.056	

TABLE 3

Allele frequency table for four polymorphic enzymes studied in *Bembidion normannum* along with calculated genetic variability estimates and sample sizes

LOCUS/POPULATION		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	TOTAL
Number of beetles (n)		45	18	38	46	63	41	50	75	35	62	46	44	45	55	43	58	57	821
AO	**Allele**																		**Means**
Gene Number (2n)		84	22	74	90	124	78	94	148	68	122	92	88	84	110	82	116	114	
Allele Number		3	2	3	3	3	3	3	3	2	3	2	2	3	3	3	2	2	2.647
	1	0.048	0.045	0.041	0.011	0.008	0.038	0.032	0.027	0.015	0.016	0.000	0.034	0.250	0.045	0.159	0.043	0.035	
	2	0.929	0.955	0.946	0.944	0.968	0.949	0.957	0.966	0.985	0.975	0.978	0.966	0.595	0.945	0.817	0.957	0.965	
	3	0.024	0.000	0.014	0.044	0.024	0.013	0.011	0.007	0.000	0.008	0.022	0.000	0.155	0.009	0.024	0.000	0.000	
Heterozygote Proportion (Hobs)		0.095	0.091	0.108	0.089	0.065	0.103	0.085	0.068	0.029	0.049	0.043	0.068	0.500	0.073	0.220	0.052	0.070	0.106
Gene Diversity (Hexp)		0.137	0.091	0.105	0.107	0.063	0.100	0.083	0.066	0.029	0.049	0.043	0.067	0.566	0.105	0.310	0.083	0.068	0.122
MPI	**Allele**																		**Means**
Gene Number		90	32	76	4	74	76	4	62	44	44	90	84	90	22	84	34	52	
Allele Number		2	2	2	1	1	1	1	2	1	1	2	3	4	2	2	1	1	1.706
	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.200	0.091	0.000	0.000	0.000	
	3	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.024	0.133	0.000	0.024	0.000	0.000	
	4	0.989	0.969	0.987	1.000	1.000	1.000	1.000	0.984	1.000	1.000	0.978	0.964	0.656	0.909	0.976	1.000	1.000	
	5	0.011	0.000	0.013	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Heterozygote Proportion		0.022	0.063	0.026	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.044	0.071	0.511	0.182	0.000	0.000	0.000	0.056
Gene Diversity		0.022	0.063	0.026	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.044	0.070	0.518	0.173	0.047	0.000	0.000	0.059
PGI	**Allele**																		**Means**
Gene Number		82	32	76	90	120	78	96	150	60	122	92	88	90	106	84	114	106	
Allele Number		3	3	3	2	2	3	2	2	3	2	2	2	2	3	2	2	2	2.353
	1	0.159	0.063	0.197	0.156	0.108	0.090	0.063	0.160	0.117	0.074	0.043	0.045	0.011	0.151	0.095	0.140	0.198	
	2	0.817	0.906	0.789	0.844	0.892	0.897	0.938	0.840	0.867	0.926	0.957	0.955	0.989	0.840	0.905	0.860	0.802	
	3	0.024	0.031	0.013	0.000	0.000	0.013	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	
Heterozygote Proportion		0.317	0.188	0.316	0.267	0.217	0.205	0.125	0.267	0.267	0.148	0.087	0.091	0.022	0.245	0.190	0.281	0.321	0.209
Gene Diversity		0.310	0.179	0.342	0.266	0.195	0.189	0.118	0.271	0.239	0.138	0.084	0.088	0.022	0.275	0.174	0.243	0.321	0.203
PGM	**Allele**																		**Means**
Gene Number		62	32	72	84	118	68	88	138	68	124	90	76	78	96	52	110	102	
Allele Number		2	2	2	2	3	2	3	2	2	3	3	2	5	2	2	3	3	2.529
	1	0.081	0.156	0.181	0.143	0.076	0.029	0.102	0.290	0.044	0.210	0.144	0.132	0.013	0.125	0.135	0.118	0.118	
	2	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.013	0.000	0.000	0.000	0.010	
	3	0.919	0.844	0.819	0.857	0.907	0.971	0.886	0.710	0.956	0.766	0.844	0.868	0.833	0.875	0.865	0.864	0.873	
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.011	0.000	0.128	0.000	0.000	0.018	0.000	
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	
Heterozygote Proportion		0.097	0.188	0.306	0.238	0.169	0.059	0.227	0.348	0.088	0.306	0.267	0.211	0.231	0.208	0.192	0.236	0.216	0.211
Gene Diversity		0.151	0.272	0.300	0.248	0.173	0.058	0.206	0.415	0.086	0.371	0.269	0.232	0.292	0.221	0.238	0.242	0.227	0.235
ALL LOCI																			
Mean Allele Number		2.500	2.250	2.500	2.000	2.250	2.250	2.250	2.250	2.000	2.250	2.250	2.250	3.500	2.500	2.250	2.000	2.000	
Standard deviation		0.577	0.500	0.577	0.816	0.957	0.957	0.957	0.500	0.816	0.957	0.500	0.500	1.291	0.577	0.500	0.816	0.816	
Mean Heterozygote proportion		0.133	0.132	0.189	0.148	0.113	0.092	0.109	0.179	0.096	0.126	0.110	0.110	0.316	0.177	0.151	0.142	0.152	
Standard deviation		0.128	0.065	0.145	0.126	0.098	0.087	0.094	0.153	0.119	0.135	0.106	0.068	0.235	0.074	0.101	0.137	0.144	
Mean Gene Diversity		0.155	0.151	0.193	0.155	0.108	0.087	0.102	0.196	0.088	0.139	0.110	0.114	0.350	0.193	0.192	0.142	0.154	
Standard deviation		0.119	0.095	0.152	0.125	0.092	0.079	0.085	0.180	0.106	0.165	0.108	0.079	0.249	0.072	0.112	0.121	0.146	

mannum). In general, *B. minimum* shows a distinctly larger genetic variation within populations than does *B. normannum* (based on all calculated genetic diversity measures; Table 2). Mean gene diversity ranges between 0.273 and 0.395 for *B. minimum* and only between 0.087 and 0.193 for *B. normannum* (Atlantic samples). The Mediterranean sample of the last-mentioned species in its turn shows a much higher genetic diversity (H_{exp}= 0.350) than Atlantic samples (Table 3).

F-statistics are summarised for both species in Table 4. Results of significant pairwise exact tests for genetic differentiation between populations are given in Table 5 (*B. minimum*) and Table 6 (*B. normannum*).

Overall genetic differentiation is highly significant for both species and for each polymorphic enzyme (Genepop-exact-tests, $p<0.001$). There is thus significant genetic structure in the complete dataset of both *B. minimum* and *B. normannum*.

TABLE 4

Genetic differentiation (F-statistics) in *Bembidion minimum* and *B. normannum*, at various geographic scales (A= regional, i.e. Flanders and southern part of the Netherlands, B= Atlantic, i.e. A + other Atlantic coastal regions, C= Atlantic + Mediterranean region); F_{ST} (WC)= according to WEIR & COCKERHAM; G_{ST} (N) according to NEI, G_{ST} (NC), according to NEI & CHESSER.

species	F_{ST} (WC)	p(F_{ST})	G_{ST} (N)	G_{ST} (NC)	geographic scale
B. minimum	0.0113	<0.01	0.0295	0.0131	A
B. minimum	0.0346	<0.001	0.0513	0.0353	B
B. normannum	0.0115	<0.01	0.0160	0.0039	A
B. normannum	0.0295	<0.001	0.0288	0.0152	B
B. normannum	0.0565	<0.001	0.0594	0.0453	C

TABLE 5

Significant pairwise exact tests on genetic differentiation between all pairs of populations in *Bembidion minimum*; all population pairs mentioned (pop 1 with each of the mentioned pop 2) are significantly different at p< 0.0033 (Bonferroni-corrected alpha-level); f.e., AUT is significantly different from HUM, MOR, NIE en ZWC for the AO locus; for abbreviations see Table 1.

pop1	pop2									locus
AUT			HUM	MOR	NIE				ZWC	*AO*
CAN	EXE	FER	HUM	MOR	NIE		OSS	SAE	ZWC	*AO*
CAN					NIE		OSS			*MPI*
CAN				MOR	NIE	OOS				*GPI*
SOM	EXE		HUM	MOR	NIE		OSS	SAE	ZWC	*AO*
SOM						OOS				*GPI*
SOM						OOS				*PEPD*
MOR			NIE		OSS					*MPI*
MOR	HOL	HOR	NIE	OOS	OSS	SEV				*AO*
HOL	ZWR									*AO*
HUM	NIE	OSS								*MPI*
SAE	NIE	OSS								*MPI*

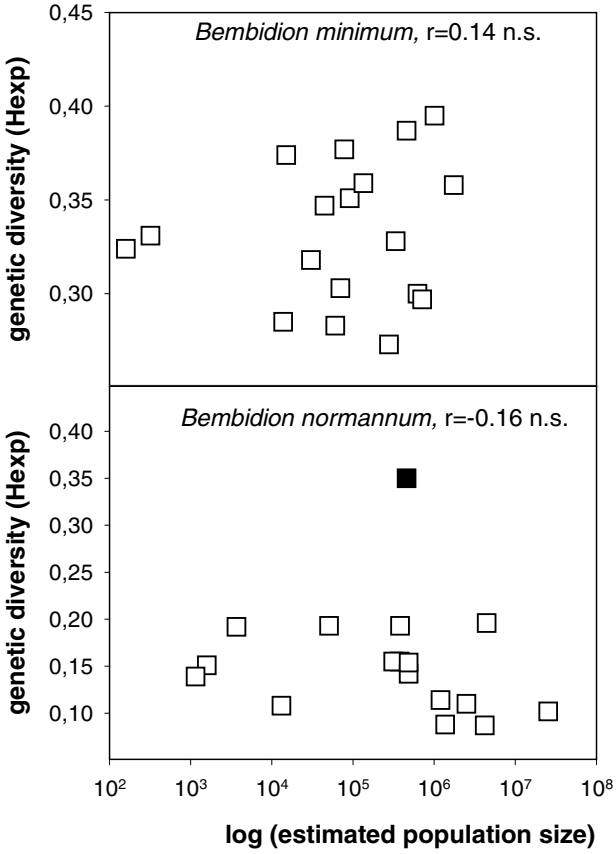
TABLE 6

Significant pairwise exact tests on genetic differentiation between all pairs of populations in *Bembidion normannum*; all population pairs mentioned (pop 1 and mentioned pop 2) are significantly different at p< 0.0036 (Bonferroni-corrected alpha-level); for abbreviations see Table 1.

pop1	pop2									locus
ROU		AUT	CAN	GAC	GIR	GUA	GUB	HOL	HUM	*AO*
ROU		AUT	CAN		GIR	GUA		HOL	HUM	*MPI*
ROU		AUT	CAN	GAC				HOL		*GPI*
ROU			CAN	GAC	GIR			HOL		*PGM*
ROU		MOL	MSB	MSG	RYE		ZWC	ZWR		*AO*
ROU		MOL	MSB	MSG		THO	ZWC	ZWR		*MPI*
ROU								ZWR		*GPI*
ROU		MOL	MSB	MSG	RYE	THO	ZWC	ZWR		*PGM*
THO	GIR	MOL	MSB	MSG						*AO*
HOL	GIR	GUA	HUM	ZWC						*PGM*

Geographic scaling and genetic diversity in relation to habitat and population size

Average gene diversities (expected heterozygosities) based on all loci are plotted for all populations of both species against the population size estimates (density x area) (Figs 3-4). Only a very weak and not significant relationship is observed in both species. No significant correlation is thus found between heterozygosities (H_{exp}) of either species and population size. Data on habitat size or saltmarsh age do not improve the significance of the relationships in multiple regressions. Fig. 4 shows that the distinctly larger value found in the Mediterranean sample cannot be explained as a consequence of a higher population size of the species in that particular saltmarsh. Inspection of the allele frequency table (Table 3) shows that a higher heterozygosity in the Mediterranean sample is especially visible at the AO and MPI loci. This higher variability is only to a very low degree caused by unique alleles but results especially from a higher mean number of (non-unique) alleles, occurring at more equal frequencies and thus increasing heterozygosity. In *B. minimum*, the three southernmost samples (Authie, Canche and Somme) also show higher genetic variability scores, especially resulting from slightly more elevated heterozygosities in AO and PEPD.



Figs. 3-4. – Genetic diversity (expected heterozygosity) in relation to population size estimates for *Bembidion minimum* and *B. normannum* (see text for further explanation; black square in Fig. 4 refers to the Mediterranean population).

Genetic differentiation and geographic distance

Both *Bembidion* species show significant genetic differentiation (F_{ST}) between populations (Table 4) at each of the tested geographic scales, even at the regional level. Overall, about 2 to 6 % of the total genetic variation is explained by differentiation between populations. Values derived from different F_{ST}-estimates yield comparable results, although G_{ST} (NEI)-estimates always are somewhat higher. More importantly, genetic differentiation estimates clearly increase at a larger geographic scale (Table 4). There is thus, to some extent, an increased genetic differentiation as a result of increased geographic scale (see later).

UPGMA-dendrograms based on Nei's genetic distance are shown in Fig. 5 for *B. minimum* and Fig. 6 for *B. normannum* (dendrograms based on Rogers' genetic distance yielded similar groupings and are therefore not shown). Detailed results on population differentiation (pairwise

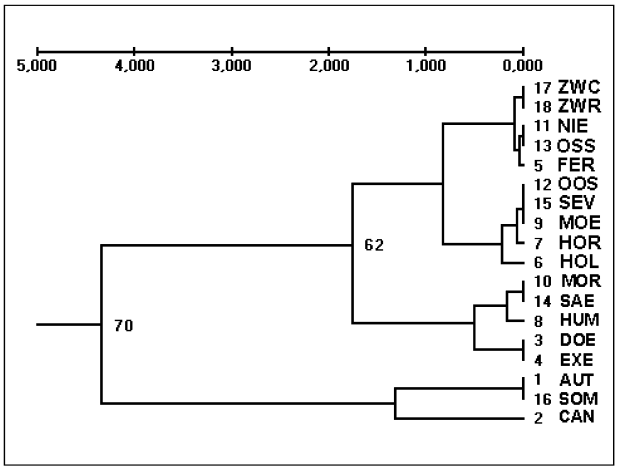


Fig. 5. – UPGMA-dendrogram based on Nei's genetic distance for all studied populations in *Bembidion minimum*; population number and letter codes added; bootstrap-values exceeding 50 added only (first node and second node).

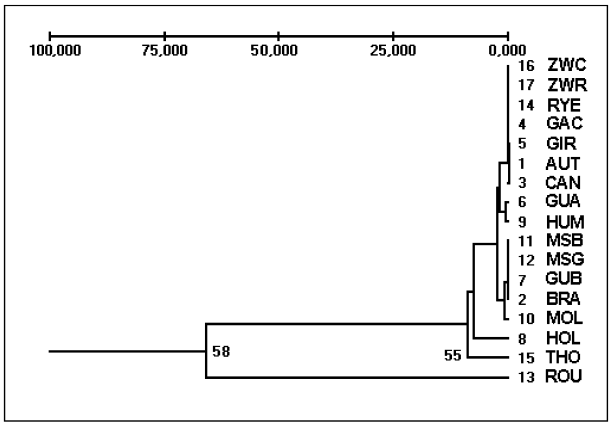
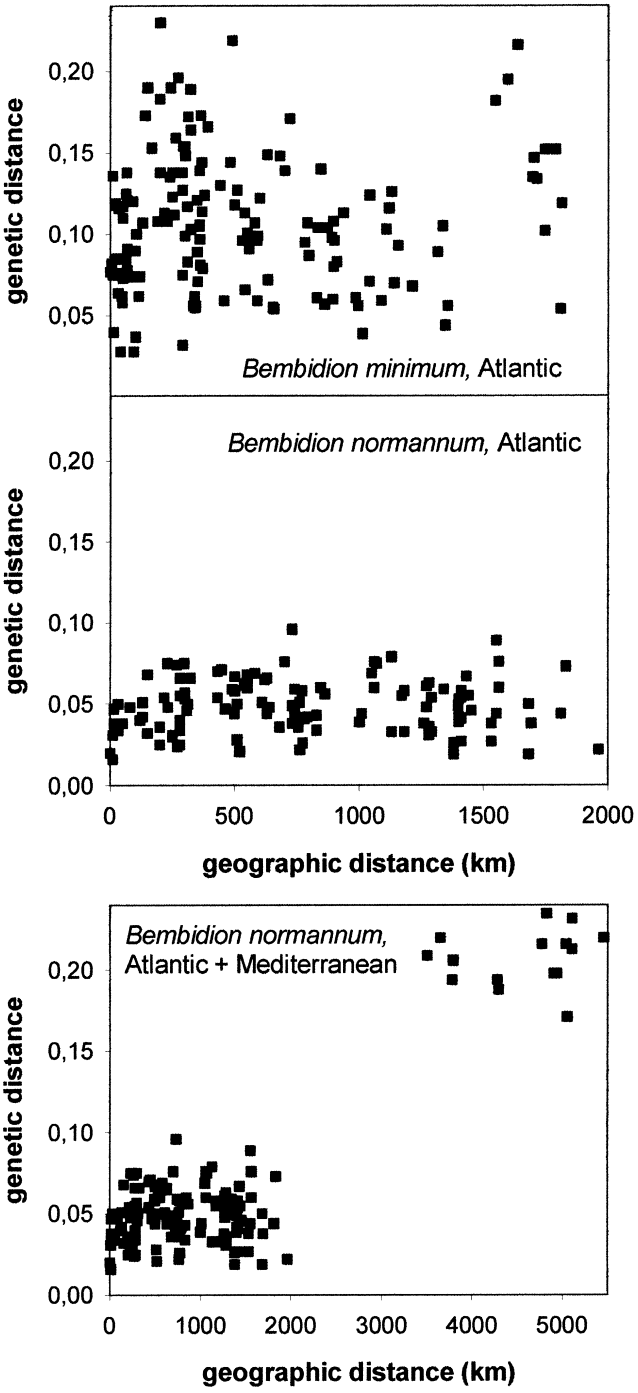


Fig. 6. – UPGMA-dendrogram based on Nei's genetic distance for all studied populations in *Bembidion normannum;* population number and letter codes added; bootstrap- values exceeding 50 added only (first node and second node).



Figs. 7-9. – Pairwise values for genetic distance in relation to geographic distance (km) for: *Bembidion minimum* (Atlantic data only, upper figure), *B. normannum* (Atlantic data only, middle figure) and *B. normannum* (Mediterranean and Atlantic data, lower figure).

exact tests, cf. Tables 5 and 6) show numerous highly significant differences, coinciding with the observed well-supported groups in the dendrograms (cf. relatively high bootstrap-values for basal nodes).

In *B. minimum*, beetles from the Authie, Canche and Somme estuaries are well differentiated from nearly all other marshes. These estuaries concern the three southernmost

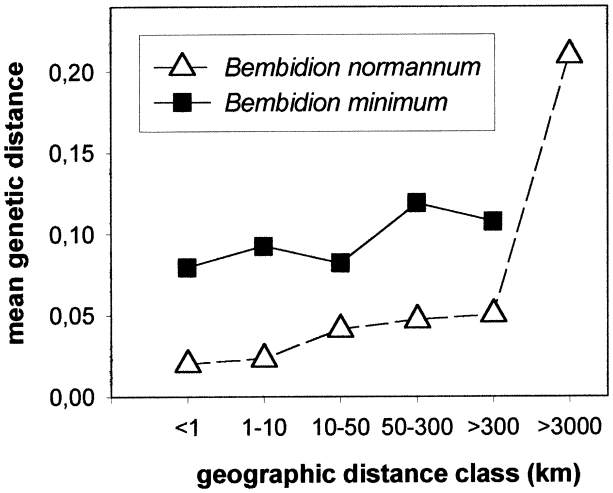


Fig. 10. – Mean genetic distance for various geographic distance classes compared between *Bembidion minimum* and *B. normannum*.

effect of isolation by distance at lower geographical scale. This holds true for both species, but again especially for *B. normannum.* At the same time, the comparison between Fig. 7 and 8, as well as the results for both species regrouped in Fig. 10, shows that for a similar geographic scale, genetic differentiation is always somewhat higher for Atlantic *B. minimum* populations than for Atlantic *B. normannum*. This coincides with much lower genetic variability estimates for Atlantic *B. normannum* (Figs 3-4), a much rarer species and more discontinuously distributed in Western Europe than *B. minimum*.

DISCUSSION

Mean gene diversity is larger in the Atlantic populations of *B. minimum* than in the much more discontinuously distributed Atlantic populations of *B. normannum*. For the latter species, the single Mediterranean sample yields a distinctly higher value than do all Atlantic samples. This coincides with a much higher incidence of *B. normannum* in Mediterranean saltmarshes. Indeed, in that area *B. normannum* is one of the most common halobiontic ground beetles occurring in high densities at numerous sites (GAUTIER, 1979; VERDIER & QUÉZEL, 1951). *B. minimum*, on the other hand, appears to be much rarer and less abundant in Mediterranean saltmarshes. Although our result is derived from a relatively low number of loci only, it gives a strong indication that the evolutionary origin of *B. normannum* lies in the Mediterranean area and/or that this area has served as glacial refugium. It is not possible to suggest such a possible centre of origin for *B. minimum* based on our data. We intend to study this question further, if possible by means of additional and more powerful genetic markers, such as microsatellite markers. In an earlier study on two other saltmarsh beetles we concluded that age and size of European saltmarshes, although diffi-

cult to study independently, appeared to be important for the genetic structure of halobiontic beetles (DESENDER et al., 1998). There was, however, not a clearcut linear increase in genetic diversity with population size.

Several European ground beetles, including these *Bembidion* species, are highly specialised halobionts, limited in their occurrence to one or several saltmarsh microhabitats, where relatively high densities (occasionally up to 10-20 individuals per m^2) can be reached (THIELE 1977). Effects of genetic erosion in fragmented populations cannot be observed in the studied populations of *B. minimum* and *B. normannum*. The absence of a relation between population or habitat size and genetic diversity indicates that effective population sizes in our study sites still are sufficiently high. Indeed, even in the smallest saltmarshes studied, population estimates of *Bembidion* always exceeded 1000 individuals. It is not excluded that genetic erosion could be observed in other sites where these species occur in lower numbers (e.g. *Bembidion minimum* in Mediterranean populations?). On the other hand, both species possess a high dispersal power. Therefore some gene flow probably still occurs regularly between most populations (at least in *B. minimum* and at a regional scale), counteracting possible temporary losses of genetic variability in small populations.

Nevertheless, we observe for both species significant genetic substructuring (differentiation), indicating at least some influence of geographic scaling and position between at least some of the sites and/or suggesting isolation by geographical distance. Among-population genetic differentiation in two other halobiontic ground beetles in a previous study was highly significant (DESENDER et al., 1998). Especially in the wing polymorphic *Pogonus chalceus* nearly all populations studied appeared to be genetically distinct, based on both allozyme and wing polymorphism data. Even the constantly winged *Dicheirotrichus gustavii* showed numerous statistically significant differences in allele frequencies between pairs of populations (DESENDER et al., 1998). Conserving a maximal genetic diversity for saltmarsh beetles therefore requires the protection of as much of the few remaining sites as possible. Significant genetic substructuring (allozymes) has been reported already for many insects, including beetles (HSIAO, 1989; KING, 1987, KNOLL et al., 1996)

Estimates of genetic differentiation in the present study are lower than the mean values (F_{ST} = 0.103) obtained for 30 other beetle species (HSIAO, 1989), which are known to be among the highest recorded for insects (WARD et al., 1992). Theories that relate variation in F_{ST} to variation in rates of gene flow indeed predict that species with a high dispersal power should show less population structuring (WAPLES, 1998; WARD et al., 1992). Empirical results for two ground beetle species with a supposedly low dispersal potential and occurring in heathland fragments did not follow this prediction (DE VRIES, 1996). Only low levels of population substructuring were observed and gene flow between populations appeared to be difficult to estimate in a fragmented landscape without additional data on dispersal. Differences between both *Bembidion* species (differentiation at a similar geographical scaling is somewhat higher in *B. minimum* as in *B. normannum*) might also be due to unequal dispersal power or flight behaviour. In the future we will study the morphology of flight muscles in time-series of both species in order to look for possible differences in the seasonal occurrence of functional flight musculature in conjunction with reproduction. A hypothesis to be tested is that less flight activity (gene flow) would be expected to occur in *B. minimum* than in *B. normannum.*

Although *B. minimum* and *B. normannum* are considered highly mobile, they are readily affected by the current state of isolation. At the moment, effects of isolation between *B. minimum* and *B. normannum* populations only appear at a relatively large geographical scale. Mediterranean *B. normannum* are highly significantly differentiated from Atlantic populations. Atlantic beetles of another saltmarsh species, *Pogonus chalceus,* were also genetically distinct from Mediterranean populations (DESENDER & SERRANO, 1999), while genetic diversity was not distinctly higher in the Mediterranean area. With further disappearance of saltmarshes or further decrease of saltmarsh habitat quality in the future, isolation between extant *Bembidion* populations is expected to increase as gene flow could become more limited, especially for the rarer *B. normannum*. As size and age of saltmarshes does not seem to be of major importance for the genetic constitution of these species, the maintenance of small and even young salt marshes could already be a good choice for maintaining sufficient genetic variation and stable populations of both species. Creation of new, even small, saltmarshes is expected to be positive for the protection of these *Bembidion* species. However, we have to consider that such nature development actions may be positive for both *Bembidion* species, but not sufficient for other, less mobile, saltmarsh beetles. An example is the halobiontic *Pogonus chalceus,* which showed brachyptery and a low dispersal power in old and isolated saltmarshes (DESENDER & SERRANO, 1999). DESENDER et al. (1998) already came to the conclusion that small and recent saltmarshes are nevertheless very important in the long term survival of *Pogonus chalceus* populations too, but for a different reason. Indeed, such sites appeared to be the only ones left with populations of macropterous individuals (capable of dispersal by flight and thus of (re)colonisation). Overall, such (recently established) populations thus are expected to contribute substantially in a well-functioning metapopulation structure. In this way, long term survival of the species would be much increased (HASTINGS & HARRISON, 1994). Genetic results, as obtained in our study, suggest that metapopulations probably function at a relatively large and at least regional scale in both *Bembidion minimum* and *B. normannum.* More genetic data, especially with more and powerful markers, are needed to confirm this hypothesis.

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Checklist of isopods (Crustacea: Peracarida: Isopoda) from the Eastern Tropical Pacific

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ABSTRACT. Literature dealing with isopods from the east Pacific is reviewed. Marine and brackish water species reported at least once in the Eastern Tropical Pacific zoogeographic subregion, which extends from Magdalena Bay, on the west coast of Southern Baja California, Mexico, to Paita, in northern Peru, are listed, and their ranges along the Pacific coast of America are provided. Unpublished records, based on material kept in the collections of the authors, were also considered, to modify previously published distribution ranges within the study area. A total of 124 species, belonging to 68 genera, is included in the checklist and new records or new distribution data are provided for 19 species. A list of names of species and subspecies currently recognized as invalid for the area is also included.

KEY WORDS: Isopoda, Eastern Tropical Pacific, Checklist, Distribution.

INTRODUCTION

Isopods belong to the superorder Peracarida, which represents approximately 50% of all described crustaceans. The order Isopoda contains about 9500 species that inhabit marine, fresh water and terrestrial ecosystems (BRUSCA, pers. comm., April 2000). As in other ecosystems where they occur, marine isopods play an important role in the food web, in particular in removing decaying material from natural or altered environments. Isopods also represent an important factor of economic unbalance. Some species (e.g., *Limnoria lignorum* Rathke, 1799) are wood-burrowing species which damage wooden marine structures (e.g., docks, ships, piers, etc.), although they also contribute to the initial breakdown of woody detritus in natural shallow waters ecosystems (SCHMITT, 1965; SCHULTZ, 1969; BRUSCA, 1980). Species of *Sphaeroma*, known to affect red mangrove roots (e.g., *S. peruvianum* Richardson, 1910; see PERRY & BRUSCA, 1989) have also been observed boring into sandstone and chalk (SCHMITT, 1965). Other species are parasites on commercially important fishes and crustaceans (BRUSCA, 1981; MARKHAM, 1985) and can cause damage to gills (e.g., males of species of *Livoneca*), skin (e.g., species of *Rocinela* and *Nerocila*), or within the mouth (e.g., females of species of *Cymothoa* and *Lironeca*) (BRUSCA, 1980; 1981; BRUSCA & IVERSON, 1985).

The high diversity and ecological success of this group are reflected by their presence in a great variety of marine and brackish water habitats. They are virtually found everywhere in the seas and oceans, from the supralittoral zone (e.g., *Ligia* species) to abyssal depths (e.g., species of the suborder Asellota). They live in a wide variety of habitats, including mangrove roots (RIBI, 1981; ELLISON & FARNSWORTH, 1990), mixed and soft bottoms (TAYLOR & MOORE, 1995), seagrasses (KANG & YUN, 1988), coral reefs (DELANEY, 1984) and rocky and sandy beaches (SCHULTZ, 1961; DEXTER, 1972; 1974; 1976).

As in other tropical marine regions of the world, the isopods of the Eastern Tropical Pacific (ETP) include a wide variety of forms, contained in many families from different habitats and sites. Although generally rather small, Sphaeromatidae are among the most obvious marine isopods as they live in intertidal or shallow waters, hence they are easy to observe or collect. They occur under rocks but also in high numbers among intertidal algae, within the chambers of sponges and with other encrusting animals such as bryozoans. Aegidae and Cymothoidae are temporary and obligatory parasites in fishes, sometimes with a very low host specificity; *Nerocila acuminata* Schiödte & Meinert, 1881, for instance, has been observed on as many as 40 species of fishes (families Engraulidae, Atherinidae, Serranidae,

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Mugilidae and Embiotocidae). Predaceous species of the family Cirolanidae are found in many habitats (e.g., in algae and mussel beds, under rocks and among intertidal animals, on sandy beaches) of the intertidal zone and shallow waters. Anthuridae are often small and usually do not occur in large numbers. Hence, anthurids are relatively rare in collections although they share a wide variety of intertidal and shallow water habitats with more common species. Anthurids usually build burrows or are associated with encrusting organisms, which might be the major reason for their scarcity in collections. Idoteidae are usually solitary animals with a clear preference for seaweed, although they occupy other habitats as well (e.g., mussel beds, muddy substrates). Due to their mimetic capacity, idoteids are difficult to spot while they grasp to algal leaves and stems. Corallanidae include free-living and temporary parasitic species, mostly on fishes, turtles and shrimps. They are wide-spread marine, brackish water and fresh water organisms, found from the intertidal zone to the upper shelf fringe in virtually every type of habitat (Brusca, 1980; 1981; Brusca & Iverson, 1985; Delaney, 1989; Schultz, 1961).

Other families are represented in the ETP by only one or two species. Oniscidae are found on rocky and sandy beaches, generally along the upper tidal limit where the substrate remains humid and detritus offers food and shelter. Members of the family Serolidae are rarely found in the Gulf of California. Tylidae contains one genus only, with terrestrial habits. Found at the upper limit of the tides on sandy beaches, they feed on all kinds of organic detritus, especially seaweeds. The families Microcerberidae and Munnidae (with at least one undescribed species for the area) are poorly known and only a few records are available. Gnathiidae are known from all depths. Gnathiids are a poorly known group, with the taxonomy almost entirely based on the morphology of males due to the similarity among female specimens. Arcturidae are among the most bizarre isopods species. Often associated with seagrass and crinoids, they feature striking adaptations of pereopods related to their clinging habits. Jaeropsidae, Janiridae, Paramunnidae, and Gnathostenetroididae are represented in the area by a single species each; maximum size is usually of the order of 2 mm or less, making these animals difficult to spot and collect; there might be numerous undescribed species present along the ETP coast. Paramunnidae are usually found on muddy subtrates in both shallow and deep water. Species of Scyphacidae are also of small size and occur in the littoral zone, on sandy substrate covered with detritus or among rocks and pebbles (Brusca, 1980; Kensley & Schotte, 1989).

All species of Epicaridea are ectoparasites of other crustaceans. They have been reported on a wide variety of crustaceans groups, from Copepoda to Decapoda. Bopyridae represent by far the most widespread family of Epicaridea; Eastern Tropical Pacific species have been reported on ghost shrimps, squad lobsters, hermit-crabs, porcellain crabs and caridean shrimps (e.g., *Upogebia dawsoni* Williams, 1986; *Upogebia macginitieorum* Williams, 1986; *Munida refulgens* Faxon, 1893; *Pleuroncodes planipes* Stimpson, 1860; *Parapagurodes laurentae* McLaughlin & Haig, 1973; *Parapagurodes makarovi* McLaughlin & Haig, 1973; *Petrolisthes hians* Nobili, 1901; *Glyphocrangon spinulosa* Faxon, 1893; *Hyppolyte californiensis* Holmes, 1895; *Thor algicola* Wicksten, 1987) (Hansen, 1897; Nierstrasz & Brender à Brandis, 1929; Markham, 1975; Campos-González & Campoy-Favela, 1987; Salazar-Vallejo & Leija-Tristán, 1989). The other epicaridean family known in the area, Dajidae, is represented in the ETP by one species only, an ectoparasite of euphausiacean shrimps (Shields & Gómez-Gutiérrez, 1996).

The earliest works specifically related to isopods of the east Pacific were published at the end of last century (Hansen, 1890, 1897). A few years later Harriet Richardson presented her Monograph of the North America Isopods (Richardson, 1905), which still represents to date the most complete monograph on isopods from the American continent. Many papers have been produced since then, most by American scientists (see Menzies, 1962; Schultz, 1969; Bowman, 1977; Nunomura, 1978). In the mid 70's a very important series of taxonomic, ecological and zoogeographic works were presented by a group of Californian scientists under the leadership of Richard C. Brusca. Their most relevant works were a review of the species known from the Gulf of California (Brusca, 1980), a review of the families Cymothoidae (Brusca 1978a, 1978b, 1981), Aegidae (Brusca, 1983) and Idoteidae (Brusca & Wallerstein, 1979a; 1979b), a monograph on isopods of the Pacific coast of Costa Rica (Brusca & Iverson, 1985) and a review of the Cirolanidae of the Eastern Tropical Pacific (ETP) (Brusca et al., 1995). For more complete lists of recent literature on marine isopods of the ETP see papers by Brusca (1980), Delaney (1984) and Brusca et al. (1995).

METHODS

Records in the checklist were derived from a review of the literature dealing with Eastern Tropical Pacific isopods, combined with unpublished data obtained during recent surveys of the fauna of the Pacific coast of Mexico. Unpublished information was also obtained while reviewing museum collections and through the generosity of colleagues. As in previous works dealing with species of Crustacea ocurring in the ETP (see Hendrickx, 1995; Hendrickx & Harvey, 1999), this area is herein defined as extending from Magdalena Bay, on the west coast of Baja California Sur, to the area of Paita, Peru, including the entire Gulf of California and all oceanic islands that lie within the latitudinally-defined tropical fringe.

Occurrence of species in other geographic regions of the world has been indicated by the following abbrevia-

tions: I-PAC, Indo-Pacific; I-WPAC, Indo-West Pacific; W-ATL, West Atlantic; ATL, West and East Atlantic; N-PAC, North Pacific; MED, Mediterranean. Records on offshore islands or rocks are cited separately, as they often correspond to southern or northern distribution limits markedly different from those known along the continent. As a rule, we considered only oceanic islands (Clarion and Socorro, Revillagigedo, Mexico; Clipperton, France; Coco, Costa Rica; Malpelo, Colombia; Galapagos, Ecuador; Juan Fernandez, Chile) or rocks (Alijos, Mexico) to be "offshore" records. For the sake of clarity, all records for "Isla del Coco", Costa Rica (such as "Coco Island", "Cocos Island", "isla Cocos" and [rarely] "isla del Coco") are referred to as "Coco Island". Records on close-to-continent islands, such as Gorgona Island, the Tres Marias Islands, and those in the Gulf of California and the Gulf of Panama, are included in the general continental range of the species.

The taxonomic sequence of suborders follows BRUSCA & WILSON (1991). Families, genera and species are listed alphabetically. If the range was given in the original description and has not been modified since, the source is the original citation. Author citations are given at the end of the range. An appendix, at the end of the list, provides names of non-valid species or subspecies cited for the Eastern Tropical Pacific. The correct, currently recognized valid name is provided in parenthesis. Invalid names cited for other zoogeographic regions are not included, as they are not relevant to this study.

RESULTS

The present paper provides an updated taxonomic list of all species of Isopoda known from the area: 124 species, belonging to 68 genera. Those include eight undescribed species of isopods known to us in the study area: two species of the suborder Epicarida and six species of the suborder Flabellifera (genera *Rocinela*, *Eurydice*, *Alcirona*, *Limnoria*, *Exosphaeroma* and *Paracerceis*).

Although this list is not a systematic paper, the following comments are in order together with some considerations on species distribution or occurrence in the area. *Coxicerberus mexicanus* (Pennak, 1958) is known only from the type locality (Acapulco, Guerrero, Mexico); this diminutive species has never been reported since its original description by PENNAK (1958). *Cirolana diminuta* Menzies, 1962 had previously been synonymized with *C. parva* Hansen, 1890 (BRUCE & BOWMAN, 1982), but BRUSCA et al. (1995) have presented numerous taxonomic arguments that lead them to the conclusion that both species should be considered valid. According to R.C. BRUSCA (pers. comm., April 2000), Californian records of *C. parva* are likely to correspond to *C. diminuta*. *Alcirona insularis* Hansen, 1890, *A. hirsuta* Moore, 1902 and *A. malvadensis* Stebbing, 1904 are synonyms of *A. krebsii* Hansen, 1890, but according to DELANEY (1989) *A. krebsii* might well represent a species complex in need of a thorough revision. *Idusa carinata* Richardson, 1904, has been considered an incertae sedis species (Bruce, 1990) but is reported here to account for records of this species in the area.

Records of *Paracerceis sculpta* (Holmes, 1904) from the Mediterranean (RODRIGUEZ et al., 1992) and from the West Atlantic (PIRES, 1980; 1981) are questioned by R.C. BRUSCA (pers. comm., April 2000); although *P. sculpta* is without any doubt one of the most commonly reported species of isopods along the west coast of Mexico and occurs in a wide variety of habitats (from the rocky intertidal to 69 m; among algae, in sponges and on mangrove aerial roots), such a wide distribution pattern is indeed rather surprising. *Munna (Uromunna) ubiquita* Menzies, 1952 is cited herein as *Uromunna ubiquita*, following the proposal of POORE (1984b) who elevated *Uromunna* to the category of genus. The genus *Calafia*, described by Carvacho (1983) to accommodate a new species of Asellota from western Mexico, is a junior synonym of *Maresiella* Fresi & Scipione, 1980 (G. WILSON, pers. comm., Oct. 2000).

Colidotea findleyi Brusca & Wallerstein, 1977, known from both sides of the Baja California Peninsula (upper Gulf of California and southern California to northwestern Baja California), appears to be a warm-temperate disjunct species. *Idotea fewkesi* (Richardson, 1904) and *I. montereyensis* Maloney, 1933, clearly belong to the temperate fauna of the East Pacific; in addition to records from Alaska to northern California for the first species and from British Colombia to northern California for the second, each species has been recorded once in Baja California (AUSTIN, 1985) without further precision as to the exact locality. It is unlikely, however, that they occur south of Magdalena Bay and they have therefore not been included in the list. *Idotea wosnesenskii* Brandt, 1851, a clearly temperate species recorded from Alaska to California has been registered only once in the SE end of the Gulf of California (La Paz, Southern Baja California; "Albatross" sample) by BRUSCA & WALLERSTEIN (1977) who questioned the validity of this record, which most likely has resulted from an erroneous label rather than represents a distribution anomaly; indeed, La Paz is not in an upwelling zone. The records of the supralittoral species *Ligia exotica* Roux, 1828 for California by RICHARDSON (1899; 1901; 1905), might be in error; R.C. BRUSCA's opinion (pers. comm., April 2000) is that *L. exotica* is rather a strictly tropical species and species found along the Californian littoral are either *L. occidentalis* Dana, 1853 or *L. pallasii* Brandt, 1833. In spite of its singular taxonomic characteristics *Alloniscus thalassophilus* Rioja, 1964 has not been registered since its original description by RIOJA (1963). *Alloniscus perconvexus* Dana, 1855 and *Armadilloniscus holmesi* Arcangeli, 1933, are temperate species known to Magdalena Bay, the northernmost limit of the study area considered herein; however, due to the fact that these genera have been occasionally recorded for subtropical regions, both species were included in the checklist as they might also occur south of Magdalena Bay.

Some interesting results related to species distribution were also obtained during this study (Table 1) and this information has been added to the distribution ranges, referring to these records as "unpublished data" in the text.

TABLE 1

List of species of isopods occurring in the Eastern Tropical Pacific and for which new records or new distribution data were made available during this study.

Species	New record or distribution range
Cortezura penascoensis	Coast of Oaxaca, Mexico.
Mesanthura occidentalis	Guaymas, Sonora; Tenacatita Bay, Jalisco, Mexico.
Paranthura elegans	Guaymas, Sonora; Banderas Bay, Nayarit, Mexico.
Rocinela belliceps	Mazatlan, Sinaloa, Mexico.
Anopsilana oaxaca	Off Chamela River, Jalisco, Mexico.
Cirolana nielbrucei	Banco Gordo, Baja California Sur, Mexico.
Excorallana truncata	San Marcos Island, west coast of Gulf of California, Mexico.
Ceratothoa gaudichaudii	Topolobampo Bay, Sinaloa, Mexico.
Elthusa menziesi	Tastiota estuary, Sonora, Mexico.
Elthusa vulgaris	Off Puerto Madero, Chiapas, Mexico.
Paradella dianae	San Juan de Alima, Michoacan, Mexico.
Dynoides crenulatus	Raza Point, Nayarit, Mexico.
Dynoides saldanai	Raza Point, Nayarit, Mexico.
Exosphaeroma sp.	Los Arcos, Jalisco, Mexico.
Paracerceis sculpta	San Juan de Alima, Michoacan, Mexico.
Neastacilla californica	Topolobampo Bay, Sinaloa, Mexico.
Synisoma wetzerae	Guaymas, Sonora, Mexico.
Tylos punctatus punctatus	La Paz, Baja California Sur; Mazatlan, Sinaloa, Mexico.
Ligia occidentalis	Chamela Bay, Jalisco, Mexico.

SYSTEMATIC ACCOUNT

ISOPODA Latreille, 1817
ANTHURIDEA Leach, 1814

Anthuridae Leach, 1814

1. *Cortezura penascoensis* Schultz, 1977
   Puerto Peñasco, east coast of the Gulf of California, Mexico, to Gulf of Tehuantepec, Oaxaca, Mexico (Schultz, 1977; unpublished data).
2. *Cyathura guaroensis* Brusca & Iverson, 1985
   Known only from Costa Rica (Brusca & Iverson, 1985; Wetzer et al., 1991).
3. *Cyathura munda* Menzies, 1951
   Marine County, California, USA, to Mexican border. Gulf of California, Mexico (Menzies, 1951; Wetzer & Brusca, 1997).
4. *Mesanthura nubifera* Wägele, 1984
   Known only from Tiburon Island, Sonora, Mexico (Negoescu & Wägele, 1984).
5. *Mesanthura occidentalis* Menzies & Barnard, 1959
   Point Conception, California, USA, to Gulf of Nicoya, Costa Rica, including the east coast of the Gulf of California, north to Puerto Peñasco, Sonora, Mexico (Brusca, 1980; Vargas et al., 1985; Wetzer et al., 1991; unpublished data).

Paranthuridae Menzies & Glynn, 1968

6. *Califanthura squamosissima* (Menzies, 1951)
   Marine County, California, USA, to Tangola-Tangola Bay, Oaxaca, Mexico, including the east coast of the Gulf of California, probably to Puerto Peñasco, Sonora (Nunomura, 1978; Hendrickx & van der Heiden, 1983; Poore, 1984a; Wetzer et al., 1991; Calderón & Campoy, 1993).
7. *Colanthura bruscai* Poore, 1984
   Off San Clemente (33º22.9'N, 117º35.8'W), California, USA, to Salinas Bay, Costa Rica, including the east coast of the Gulf of California, Mexico (Poore, 1984a; Wetzer et al., 1991).
8. *Paranthura californiae* Nunomura, 1978
   Known only from Magdalena Bay, west coast of Baja California, Mexico (Nunomura, 1978; Negoescu & Wägele, 1984).
9. *Paranthura elegans* Menzies, 1951
   Tomales Point, Marine County, California, USA, to San Quintin Bay, Baja California, Mexico; including the east coast of the Gulf of California, north to Guaymas, Sonora, Mexico (Menzies, 1951; Wetzer & Brusca, 1997; unpublished data)
10. *Paranthura longitelson* Wägele, 1984
    Gulf of California, Estanque, Angel de la Guarda and Tiburon Islands to Guaymas, Sonora and La Paz, Southern Baja California, Mexico (Wägele, 1984).

MICROCERBERIDEA Lang, 1961

Microcerberidae Karaman, 1933

11. *Coxicerberus mexicanus* (Pennak, 1958)
    Known only from Acapulco, Guerrero, Mexico (Pennak, 1958).

FLABELLIFERA Sars, 1882

Aegidae Dana, 1853

12. *Aega acuminata* Hansen, 1897
    Off Blanco Cape, Costa Rica. Galapagos Islands (Hansen, 1897; Brusca, 1983).

13. *Aega deshaysiana* (H. Milne-Edwards, 1840)
Coco Island (Brusca, 1983). HAW. I-PAC. ATL.

14. *Aega maxima* Hansen, 1897
A single record off Coco Island (5º56'N, 85º10'30"W) (Hansen, 1897; Brusca, 1983).

15. *Aega longicornis* Hansen, 1897
Galapagos Islands (Hansen, 1897; Brusca, 1983).

16. *Aega plebeia* Hansen, 1987
Aleutian Islands, Alaska, USA south to Tierra del Fuego, Argentina (not in the Gulf of California). Coco Island (Hansen, 1897; Richardson, 1909; Brusca, 1983; pers. comm., April 2000). I-PAC.

17. *Rocinela belliceps* (Stimpson, 1864)
Aleutian Islands, Alaska, to Channel Islands, California, USA. Gulf of California, Angel de la Guarda Island (29º19.9'N, 113º10.4'W) and Mazatlan, Sinaloa, Mexico. Clarion Island (Brusca & France, 1992; unpublished data).

18. *Rocinela laticauda* Hansen, 1897
Piedras Blancas, California, USA, and from Guaymas, Sonora, to Acapulco, Guerrero, Mexico (Richardson, 1905; Brusca & France, 1992; Calderón & Campoy, 1993).

19. *Rocinela modesta* Hansen, 1897
Known only from Gulf of Panama (7º31'30"N, 79º14'W) (Hansen, 1897; Brusca et al., 1995).

20. *Rocinela murilloi* Brusca & Iverson, 1985
Point Sur, California, USA, south to Valparaiso, Chile, including the southeastern Gulf of California, Mexico to San Blas, Nayarit (Brusca & France, 1992).

21. *Rocinela signata* Schioedte & Meinert, 1879
From Newport Bay, California, USA, to Gulf of Guayaquil, Ecuador, including the whole Gulf of California, Mexico. Galapagos Islands (Bowman, 1977; Brusca & France, 1992). W-ATL.

22. *Rocinela tuberculosa* Richardson, 1898
Gulf of California, from San Marcos Island to Gorda Bank (west coast), Mexico (Brusca, 1980; Brusca & France, 1992).

23. *Rocinela* sp.
Northern Gulf of California, Mexico (Brusca, 1980).

Ancinidae Tattersall, 1905

24. *Ancinus granulatus* Holmes & Gay, 1909
Southern California, USA, to Cedros Island, west coast of Baja California, Mexico. Gulf of California, San Felipe, Baja California (northwest coast) and Mazatlan, Sinaloa (southeast coast) (Glynn & Glynn, 1974; Wallerstein, 1980; van der Heiden & Hendrickx, 1982).

25. *Ancinus panamensis* Glynn & Glynn, 1974
From Santa Rosa National Park, Costa Rica to Malaga Bay, Colombia (Glynn & Glynn, 1974; Brusca & Iverson, 1985).

Cirolanidae Dana, 1853

26. *Anopsilana aleci* Brusca, Wetzer & France, 1995.
Miraflores Locks, Panama Channel, Panama (Brusca et al., 1995).

27. *Anopsilana browni* (Van Name, 1936)
Gulf of Nicoya, Costa Rica (Bruce, 1986a; Brusca et al., 1995). W- ATL.

28. *Anopsilana oaxaca* Carvacho & Haasmann, 1984
From Chamela Bay, Jalisco to Manialtepec Lagoon, Oaxaca, Mexico. Clipperton Island (Brusca et al., 1995; unpublished data).

29. *Cirolana diminuta* Menzies, 1962
Point Conception, California, USA, to San Ignacio Lagoon, west coast of Baja California, Mexico. A single record at Espiritu Santo Island, southern Gulf of California, Mexico. Galapagos Islands (Brusca et al., 1995; Brusca, pers. comm., April 2000).

30. *Cirolana harfordi* (Lockington, 1877)
Vancouver Island to Magdalena Bay, west coast of Baja California, Mexico. A single record at La Paz, southwestern tip of the Gulf of California (Brusca et al., 1995). I-PAC.

31. *Cirolana namelessensis* Brusca, Wetzer & France, 1995
Utria, Colombia. Malpelo and Galapagos Islands (Brusca et al., 1995).

32. *Cirolana nielbrucei* Brusca, Wetzer & France, 1995
Gulf of California, from Tiburon Island to Arboleda Point, Sonora, and from Chivato Point to Gorda Bank, Baja California (east coast), Mexico (Brusca et al., 1995; unpublished data).

33. *Cirolana parva* Hansen, 1890
Gulf of California, at Tortuga Island (west coast), and from Mazatlan, Sinaloa to Punta Mita, Nayarit (east coast), Mexico, and to Santa Elena Point, Ecuador. Coco and Galapagos Islands (Brusca et al., 1995). W-ATL.

34. *Conilera bullisi* Brusca, Wetzer & France, 1995
From San Miguel Cape, west coast of Gulf of California, Mexico, south to Gulf of Guayaquil, Ecuador (Brusca et al., 1995; Espinosa-Pérez & Hendrickx, 1997).

35. *Eurydice caudata* Richardson, 1899
From San Diego, California, USA, to La Libertad, Ecuador, including the Gulf of California, Mexico. Guadalupe, Revillagigedo, Coco and Galapagos Islands (Bowman, 1977; Wallerstein, 1980; Brusca et al., 1995).

36. *Excirolana braziliensis* Richardson, 1912
From San Felipe and Puerto Peñasco, Gulf of California, Mexico, south to Concepcion, Chile (Ríos & Ramos, 1990; Brusca et al., 1995). ATL.

37. *Excirolana chamensis* Brusca & Weinberg, 1987
Punta Chame Bay and Perlas Island, Panama (Brusca & Weinberg, 1987; Brusca et al., 1995).

38. *Excirolana mayana* (Ives, 1891)
Ojo de Liebre (Scammon's) Lagoon, west coast of Baja California, Mexico. From Rio Colorado Delta, Gulf of California, Mexico, to Playa Blanca Island, Colombia (Brusca et al., 1995). ATL.

39. *Metacirolana calypso* Brusca, Wetzer & France, 1995.
Galapagos Islands (Brusca et al., 1995).

40. *Metacirolana costaricensis* Brusca & Iverson, 1985
From Guaymas, Sonora, east coast of the Gulf of California, Mexico to Panama Bay, Panama. Galapagos

Islands (Brusca et al., 1995; Espinosa-Pérez & Hendrickx, 1997).

41. *Natatolana californiensis* (Schultz, 1966)
From southern California, USA, to Cedros Island, west coast of Baja California, Mexico. In the Gulf of California, at Angel de la Guarda Island and off La Paz, South Baja California, Mexico (Brusca & Ninos, 1978; Brusca et al., 1995). A single record in Costa Rica (Brusca, pers. comm., April 2000) and another in the Peru-Chile Trench (7º7.9'S, 80º37'W) (Menzies & George, 1972).

42. *Natatolana carlenae* Brusca, Wetzer & France, 1995
From Cedros Island, west coast of Baja California, Mexico, to Secas Island, Panama, including the whole Gulf of California, Mexico (Brusca et al., 1995).

43. *Oncilorpheus jerrybarnardi* Brusca, Wetzer & France, 1995.
From Playas Blancas, Costa Rica, to Honda Bay, Panama (Brusca et al., 1995).

Corallanidae Hansen, 1890

44. *Alcirona krebsii* Hansen, 1890
From off San Lucas Cape, Southern Baja California, Gulf of California, Mexico, to Panama Bay, Panama (Menzies & Kruczynski, 1983; Delaney, 1989). I-PAC. W-ATL.

45. *Alcirona* sp.
Puerto Peñasco, Sonora, northern Gulf of California, Mexico (Delaney, 1989).

46. *Excorallana bruscai* Delaney, 1984
From Puerto Peñasco, Sonora, and Concepcion Bay, Baja California, Gulf of California, south to Boca de San Francisco, Oaxaca, Mexico (Delaney, 1989; Wetzer et al., 1991; Hendrickx & Espinosa-Pérez, 1998b).

47. *Excorallana conabioae* Hendrickx & Espinosa-Pérez, 1998
From San Miguel Cape to San Marcial Point, west coast of the Gulf of California, Mexico (Hendrickx & Espinosa-Pérez, 1998b).

48. *Excorallana houstoni* Delaney, 1984
From San Francisco Island and San Lucas Cape, Southern Baja California, Gulf of California, to Manzanillo, Colima, Mexico. Galapagos Islands (Delaney, 1989).

49. *Excorallana tricornis occidentalis* Richardson, 1905
From Santa Catalina Island, California, USA, south to Panama, including the whole Gulf of California, Mexico (Brusca, pers. comm., April 2000; Delaney, 1984; 1989; 1993; Guzman et al., 1988).

50. *Excorallana truncata* (Richardson, 1899)
Point Conception, California, USA, to Panama, including Puerto Peñasco, Sonora, and San Marcos Island, Gulf of California, Mexico. Galapagos Islands (Delaney, 1989; unpublished data).

Cymothoidae Leach, 1818

51. *Anilocra laticauda* H. Milne-Edwards, 1840
Off Acapulco, Guerrero, Mexico to Peru (Coventry, 1944; Miers, 1877; Brusca, 1981). ATL.

52. *Anilocra meridionalis* Richardson, 1914
Pacific Ocean (10º0'N, 142º50'W). Northwest of the Galapagos Islands (9º31'N, 106º30'W) (Brusca, 1981; Brusca & Iverson, 1985).

53. *Ceratothoa gaudichaudii* (H. Milne-Edwards, 1840)
From southern California, USA, south to Cape Horn, Chile, including the whole Gulf of California, Mexico. Galapagos Islands (Brusca, 1981; Molina & Manrique. 1996; unpublished data). I-PAC.

54. *Ceratothoa gilberti* (Richardson, 1904)
From southern California, USA, to Punta Banda, west coast of Baja California; Tortugas Bay, Southern Baja California, and Mazatlan, Sinaloa, Gulf of California, Mexico (Brusca, 1981).

55. *Cymothoa exigua* Schioedte & Meinert, 1884
From San Juanico Bay, west coast of South Baja California, Mexico, south to Panama, including the whole Gulf of California. Galapagos Islands (Brusca, 1977; 1981; Alvarez & Flores, 1997). ATL.

56. *Elthusa menziesi* (Brusca, 1981)
Todos Santos and San Quintin Bays, west coast of Baja California, and Gulf of California, Mexico. Alijos Rocks. Guadalupe Island (Campos et al., 1986; Wetzer et al., 1991; unpublished data).

57. *Elthusa vulgaris* (Stimpson, 1857)
From Washington, USA, south to off Puerto Madero, Chiapas, including the whole Gulf of California, Mexico. Near Malpelo Island (Brusca, 1981; Austin, 1985; unpublished data).

58. *Enispa convexa* (Richardson, 1905)
San Diego, California, USA, to Gulf of Guayaquil, Ecuador. A single record at Playa Novilleros, southern Gulf of California, Mexico (Brusca, 1981; Brusca & Iverson, 1985; Wetzer et al., 1991).

59. *Idusa carinata* Richardson, 1904
From Gulf of Panama, Panama to Gulf of Guayaquil, Ecuador (Richardson, 1904; Brusca, 1981).

60. *Livoneca bowmani* Brusca, 1981
Gulf of California, from off Colorado River and Santa Cruz Island (west coast), to Nayarit (20º40'N, 105º20'W), Mexico. Near Malpelo Island (3º24'N, 80º45'W) (Brusca, 1981; Wetzer et al., 1991).

61. *Mothocya gilli* Bruce, 1986
From Asuncion Bay to Almeja Bay, west coast of Baja California, and from Guaymas, Sonora, Gulf of California, to Manzanillo, Colima, Mexico (Bruce, 1986b; Wetzer et al., 1991).

62. *Nerocila acuminata* Schioedte & Meinert, 1881
From Long Beach, California, USA, south to Peru, including the whole Gulf of California, Mexico. Galapagos Islands (Brusca, 1981). HAWAII. W-ATL.

63. *Nerocila excisa* (Richardson, 1914)
Galapagos and Coco Islands (Richardson, 1914; Brusca, 1981). PAC.

64. *Renocila thresherorum* Williams & Williams, 1980
A single record at Corona del Mar, California, USA. Magdalena Bay, west coast of Baja California, and Gulf of

California, from Loreto to San Jose del Cabo, Mexico (Brusca, 1981).

Limnoriidae Harger, 1880

65. *Limnoria tripunctata* Menzies, 1951
From San Francisco Bay, California, USA south to Mazatlan, Sinaloa, including the whole Gulf of California, Mexico (Menzies, 1951; Brusca & Iverson, 1985). ATL.

66. *Limnoria* sp.
Known only from the coast of Sonora, Gulf of California, Mexico (Brusca, 1980).

Serolidae Dana, 1853

67. *Heteroserolis carinata* (Lockington, 1877)
From Santa Monica Bay, California, USA, to San Quintin Bay, Baja California, Mexico. Gulf of California, from Angel de la Guarda Island to San Miguel Cape and Tiburon Island to Lobos Bay (Menzies & Barnard, 1959; Calderón & Campoy, 1993; Espinosa-Pérez & Hendrickx, 1997; Wetzer & Brusca, 1997).

68. *Heteroserolis tropica* (Glynn, 1976)
A single record from Panama Bay, Panama (Glynn, 1976).

Sphaeromatidae H. Milne-Edwards, 1840

69. *Cassidinidea mexicana* Hendrickx & Espinosa-Pérez, 1998
Estero el Verde (23º09.03'N, 106º19.00'W) and Mazatlan, Sinaloa, Mexico (Hendrickx & Espinosa-Pérez, 1998a).

70. *Paradella dianae* (Menzies, 1962)
Ventura County, California, USA, to San Juan de Alima, Michoacan, Mexico. West coast of the Gulf of California, from Guaymas, Sonora to Sayulita, Nayarit, Mexico (Menzies, 1962; Iverson, 1974; unpublished data). I-PAC. ATL. MED.

71. *Paradella setosa* (Glynn, 1968)
Tortola and Naos Islands, Panama (Glynn, 1968).

72. *Paraleptosphaeroma glynni* Buss & Iverson, 1981
From Tarcoles Beach, Costa Rica to Paitilla Point, Panama (Buss & Iverson, 1981; Brusca & Iverson, 1985).

73. *Dynamenella josephi* Glynn, 1968
From Tarcoles, Costa Rica to Tortola, Perico and Naos Islands, Panama (Glynn, 1968; Brusca & Iverson, 1985).

74. *Dynoides crenulatus* Carvacho & Haasmann, 1984
From Raza Point, Nayarit (21º02.6'N, 105º19.4'W) to Puerto Escondido, Oaxaca, Mexico (Carvacho & Haasmann, 1984; unpublished data)

75. *Dynoides saldanai* Carvacho & Haasmann, 1984
From Raza Point, Nayarit (21º02.6'N, 105º19.4'W) to Puerto Escondido, Oaxaca, Mexico (Carvacho & Haasmann, 1984; unpublished data)

76. *Exosphaeroma* sp.
From Guaymas, Sonora and La Paz, Southern Baja California, Gulf of California, Mexico, south to Malaga Bay, Colombia (Brusca & Iverson, 1985; Ríos & Ramos, 1990; unpublished data).

77. *Paracerceis richardsonae* Lombardo, 1988
Magdalena Bay, west coast of Baja California; Guaymas, Sonora (Gulf of California), Mexico (Lombardo, 1988).

78. *Paracerceis sculpta* (Holmes, 1904)
From San Clemente Island, California, USA, south to San Juan de Alima, Michoacan, Mexico, including the whole Gulf of California, Mexico (Richardson, 1905; Brusca, 1980; unpublished data). ATL. MED.

79. *Paracerceis* sp.
Gulf of California, from San Esteban and San Pedro Islands to Guaymas, Sonora (east coast), and San Jose Island, east coast of Baja California, Mexico (Brusca, 1980).

80. *Sphaeroma peruvianum* Richardson, 1910
From Gulf of Nicoya, Costa Rica to Matapalo, Peru (Richardson, 1910; Brusca & Iverson, 1985; Perry & Brusca, 1989).

81. *Striella balani* Glynn, 1968
From Santa Cruz Bay, Oaxaca, Mexico, to Naos Island, Panama (Glynn, 1968; Carvacho & Haasmann, 1984).

ASELLOTA Latreille, 1803

Janiridae Sars, 1899

82. *Carpias villalobosi* (Carvacho, 1983)
Gulf of California, Concepcion Bay, Mexico (Carvacho, 1983).

Joeropsididae Nordenstam, 1933

83. *Joeropsis dubia* Menzies, 1951
Tomales Point, Marine County, California, USA, to San Quintin Bay, west coast of Baja California, Mexico. Gulf of California, Percebu Lagoon and Concepcion Bay, Mexico (Menzies, 1962; Carvacho, 1983; Wetzer et al., 1991).

Munnidae Sars, 1897

84. *Uromunna ubiquita* (Menzies, 1952)
San Juan Archipelago, Washington, USA, to San Quintin Bay, west coast of Baja California, Mexico. Northern Gulf of California, Guaymas, Sonora and Percebu Lagoon, Mexico (Menzies, 1962; George & Stromberg, 1968; Carvacho, 1983; Wetzer et al., 1991; Calderón & Campoy, 1993).

85. *Uromunna* sp.
Estero de Urias, Sinaloa, Mexico (García-Guerrero, unpubl. masterthesis, 1999).

Gnathostenetroididae Kussakin, 1967

86. *Maresiella brevicornis* (Carvacho, 1983)
Known only from Concepcion Bay, Gulf of California, Mexico (Carvacho, 1983).

VALVIFERA Sars, 1882

Arcturidae Sars, 1899

87. *Neastacilla californica* (Boone, 1918)
Southern California, USA. Consag Rocks and Topolobampo Bay, Gulf of California, Mexico (Menzies & Barnard, 1959; unpublished data).

Hognathidae Thomson, 1904

88. *Cleantioides occidentalis* (Richardson, 1899)
From the southern coast of California, USA south to Ecuador, including the east coast of the Gulf of California, from Puerto Peñasco, Sonora, to Mazatlan, Sinaloa, Mexico. Galapagos Islands (Kensley & Kaufman, 1978; Brusca & Iverson, 1985; Brusca, pers. comm., April 2000).

89. *Cleantioides planicauda* (Richardson, 1899)
Oaxaca, Mexico (Brusca & Wallerstein, 1979a). ATL.

90. *Cleantioides vonprahli* Ríos & Ramos, 1990
Known only from Malaga Bay, Colombia (Ríos & Ramos, 1990).

Idoteidae H. Milne-Edwards, 1840

91. *Colidotea findleyi* Brusca & Wallerstein, 1977
From San Diego, California, USA to San Eugenio Point, west coast of Baja California, Mexico. Gulf of California, from San Felipe, Baja California and Puerto Peñasco to Lobos Point, Sonora. Guadalupe Island (Wetzer et al., 1991; Brusca, pers. comm., April 2000).

92. *Edotia sublittoralis* Menzies & Barnard, 1959
Conception Point, California, USA, to Mexican border (Wallerstein, 1980; Wetzer et al, 1991). A single record at Gulf of Nicoya, Costa Rica (Vargas et al., 1985).

93. *Erichsonella cortezi* Brusca & Wallerstein, 1977
Known only from Puerto Peñasco, northern Gulf of California, Mexico (Brusca & Wallerstein, 1977).

94. *Eusymmerus antennatus* Richardson, 1899
From San Eugenio Point, west coast of Baja California, south to Gulf of Nicoya, Costa Rica, including the east coast of the Gulf of California (Brusca & Wallerstein, 1977; Vargas et al., 1985; Calderón & Campoy, 1993).

95. *Idotea urotoma* Stimpson, 1864
From Alaska, USA, to the west coast of Baja California, Mexico. Guaymas, Sonora and la Paz, South Baja California, Gulf of California (Brusca & Wallerstein, 1977; Austin, 1985; Calderón & Campoy, 1993; Brusca, pers. comm., April 2000).

96. *Parasymmerus annamaryae* Brusca & Wallerstein, 1979
From Mazatlan, Sinaloa, southeast coast of the Gulf of California, south to Puerto Angel, Oaxaca, Mexico (Carvacho & Hassmann, 1984; Wetzer et al., 1991).

97. *Pentidotea aculeata* (Stafford, 1913)
British Columbia, Canada, to Cedros Island, west coast of Baja California, Mexico. Gulf of California, Mexico, Guaymas, Sonora and La Paz, South Baja California (Menzies, 1950; Brusca & Wallerstein, 1977; Austin, 1985; Calderón & Campoy, 1993; Brusca, pers. comm., April 2000).

98. *Pentidotea resecata* (Stimpson, 1857)
From Karta Bay, Alaska, USA, south to Tortola Bay, west coast of Baja California, Mexico; San Lucas Cape and La Paz, South Baja California, Gulf of California. Alijos Rocks (Brusca & Wallerstein, 1977; Austin, 1985; Brusca & Wetzer, pers. comm.).

99. *Pentidotea stenops* (Benedict, 1898)
From Alaska, USA, to San Eugenio Point, west coast of Baja California, Mexico, and from San Telmo Point to La Paz, South Baja California, Mexico (Brusca & Wallerstein, 1977; Austin, 1985; Brusca & Wetzer, pers. comm.).

100. *Pentidotea wosnesenskii* (Brandt, 1851)
Aleutian Islands, Alaska, to southern California, USA. A single record at La Paz, South Baja California, Gulf of California, Mexico (Brusca & Wallerstein, 1977; Brusca, 1980; Austin, 1985). I-PAC.

101. *Synidotea francesae* Brusca, 1983
Known only from Gulf of Santa Clara, Sonora, east coast of the Gulf of California, Mexico (Wetzer et al., 1991).

102. *Synidotea harfordi* Benedict, 1897
Southern California, USA, to the the Gulf of Nicoya, Costa Rica, including whole Gulf of California, Mexico (Brusca & Wallerstein, 1979a; Wallerstein, 1980; Vargas et al., 1985). I-PAC.

103. *Synisoma wetzerae* Ormsby, 1991
Santa Catalina Island, California, USA, and Guaymas, Sonora, Gulf of California, Mexico (Ormsby, 1991; unpublished data).

EPICARIDEA Latreille, 1831

Bopyridae Rafinesque, 1815

104. *Aporobopyrus trilobata* (Nierstrasz & Brender à Brandis, 1925)
Zihuatanejo, Guerrero, Mexico (Salazar-Vallejo & Leija-Tristán, 1989). W-ATL.

105. *Bathygyge grandis* Hansen, 1897
From coast of Nayarit to off Acapulco, Guerrero, Mexico (Hansen, 1897; Salazar-Vallejo & Leija-Tristán, 1989).

106. *Cryptione elongata* Hansen, 1897
A single record from off Acapulco, Guerrero, Mexico (Richardson, 1905; Schultz, 1969).

107. *Parageia ornata* Hansen, 1897
Off Acapulco, Guerrero, Mexico (Richardson, 1905; Salazar-Vallejo & Leija-Tristán, 1989).

108. *Probopyrus markhami* Román-Contreras, 1996
From off Piaxtla, Sinaloa to off Coyuca, Guerrero, Mexico (Román-Contreras, 1996).

109. *Probopyrus pacificensis* Román-Contreras, 1993
Coast of Guerrero, Mexico south to El Salvador (Holthuis, 1954; Román-Contreras, 1993).

110. *Probopyrus pandalicola* (Packard, 1879)
From Puertecitos and Coloradito, west coast of Gulf of California, Mexico south to Panama (Campos & Campos, 1989). W-ATL.

111. *Progebiophilus bruscai* Salazar-Vallejo & Leija-Tristán, 1989
West coast of Baja California, Tortugas and Todos Santos Bays, and on the west coast of the Gulf of California, from San Felipe, Baja California, to La Paz, South Baja California, Mexico (Salazar-Vallejo & Leija-Tristán, 1989).

112. *Pseudione galacanthae* Hansen, 1897
Coast of Canada and into the Gulf of California, Mexico (Brusca, 1980; Austin, 1985; Salazar-Vallejo & Leija-Tristán, 1989).

113. *Pseudione* sp. 1
Puerto Libertad, Sonora, Gulf of California, Mexico (Brusca, 1980).

114. *Pseudione* sp. 2
Gulf of California, Mexico (Salazar-Vallejo & Leija-Tristán, 1989).

115. *Schizobopyrina bruscai* Campos & Campos, 1990
Only known from Concepcion Bay, west coast of Gulf of California, Mexico (Campos & Campos, 1990).

116. *Schizobopyrina striata* Nierstrasz & Brender à Brandis, 1929
Northern California, USA and Puertecitos, Baja California, Gulf of California, Mexico (Campos & Campos, 1990).

Dajidae Sars, 1882

117. *Oculophryxus bicaulis* Shields & Gómez-Gutiérrez, 1996
West coast of Baja California (20-29°N – 112-118°W), Mexico. W-PAC. W-ATL.

ONISCIDEA Latreille, 1803

Tylidae H. Milne-Edwards, 1840

118. *Tylos punctatus punctatus* Holmes & Gay, 1909
San Diego, California, USA, to Ensenada, west coast of Baja California, Mexico. Gulf of California, Mexico, Puerto Peñasco, Sonora to Mazatlan, Sinaloa (east coast) and la Paz, South Baja California (west coast) (Schultz, 1970; Austin, 1985; unpublished data).

Ligiidae Brandt, 1883

119. *Ligia baudiniana* H. Milne-Edwards, 1840.
Raza Island, Gulf of California, Mexico, and Malaga Bay, Colombia (Mulaik, 1960; Ríos & Ramos, 1990). ATL.

120. *Ligia exotica* Roux, 1828
From California, USA (dubious records), south to Chile, including the Gulf of California, Mexico. Guadalupe and Clipperton Islands (Richardson, 1905; Mulaik, 1960). HAWAII. I-PAC. ATL.

121. *Ligia occidentalis* Dana, 1853
From Oregon, USA, south to Chamela Bay, Jalisco, Mexico, including the whole Gulf of California, Mexico (Richardson, 1905; Austin, 1985; Bowman, 1977; unpublished data).

Scyphacidae Dana, 1852

122. *Alloniscus perconvexus* Dana, 1856
From San Juan Archipelago, Washington, USA, south to Magdalena Bay, west coast of Baja California, Mexico (Mulaik, 1960; George & Strömberg, 1968).

123. *Alloniscus thalassophilus* Rioja, 1964
Known only from Zihuatanejo, Guerrero, Mexico (Rioja, 1963 [1964]).

Scyphacidae Dana, 1853

124. *Armadilloniscus holmesi* Arcangeli, 1933
From British Columbia, Canada, to Magdalena Bay, west coast of Baja California, Mexico (Mulaik, 1960; Bowman, 1977; Wallerstein, 1980; Austin, 1985).

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APPENDIX

List of invalid names (with the original orthograph) cited for the Eastern Tropical Pacific. The valid name to which they are currently synonymized appears in parenthesis. Invalid names cited only from other zoogeographic regions where ETP species occur (e.g., Western Atlantic, Indo-West Pacific) have not been included in the list.

FLABELLIFERA Sars, 1882

Aegidae Dana, 1853
Aega excisa Richardson, 1910 (=*Aega deshaysiana*)
Aega antillensis Schioedte & Meinert, 1879 (=*Aega deshaysiana*)
Aega Schioedteana Bovallius, 1885 (=*Aega deshaysiana*)
Aega magnoculis Richardson, 1909 (=*Aega plebeia*)
Aega alaskensis Lockington, 1877 (=*Rocinela belliceps*)

Rocinela aries Schioedte & Meinert, 1879 (=*Rocinela signata*)

Cirolanidae Dana, 1853
Cirolana californica Hansen, 1890 (=*Cirolana harfordi*)
Cirolana harfordi japonica Theilemann, 1910 (=*Cirolana harfordi*)
Cirolana theilemanni Kussakin, 1979 (=*Cirolana harfordi*)
Cirolana toyamaensis Nunomura, 1982 (=*Cirolana harfordi*)
Eurydice branchuropus Menzies & Barnard, 1959 (=*Eurydice caudata*)
Cirolana koepckei Bott, 1954 (=*Excirolana braziliensis*)
Cirolana salvadorensis Schuster, 1954 (=*Excirolana braziliensis*)

Corallanidae Hansen, 1890
Alcinora insularis Hansen, 1890 (=*Alcinora krebsii*)
Alcinora hirsuta Moore, 1902 (=*Alcinora krebsii*)
Alcinora maldivensis Stebbing, 1904 (=*Alcinora krebsii*)
Excorallana kathyae Menzies, 1962 (=*Excorallana truncata*)

Cymothoidae Leach, 1818
Anilocra mexicana de Saussure, 1857 (=*Anilocra laticauda*)
Anilocra leachii Schioedte, 1866 (=*Anilocra laticauda*)
Anilocra laevis Miers, 1877 (=*Anilocra laticauda*)
Ceratothoa rapax Haller, 1865 (=*Ceratothoa gaudichaudii*)
Livoneca panamensis Schioedte & Meinert, 1884 (=*Elthusa vulgaris*)
Anilocra occidentalis Richardson, 1899 (=*Elthusa vulgaris*)
Nerocila californica Schiödte & Meinert, 1881 (=*Nerocila acuminata*)
Pterisopodus bartschi Boone, 1918 (=*Nerocila acuminata*)

Sphaeromatidae H. Milne-Edwards, 1840
Sergiella angra Pires, 1980 (=*Paracerceis sculpta*)

VALVIFERA Sars, 1882

Idoteidae H. Milne-Edwards, 1840
Idotea rectilinea Lockington, 1877 (=*Idotea urotoma*)
Cleantis heathii Richardson, 1900 (=*Idotea urotoma*)
Idotea hirtipes Dana, 1852 (=*Idotea wosnesenskii*)
Idotea media Dana, 1854 (=*Idotea wosnesenskii*)
Idotea oregonensis Dana, 1854 (=*Idotea wosnesenskii*)

ONISCIDEA Latreille, 1803

Ligiidae Brandt, 1883
Ligia exotica var. *hirtitarsis* Dollfus, 1890 (=*Ligia baudiniana*)

Scyphacidae Dana, 1853
Actoniscus tuberculatus Holmes & Gay, 1909 (=*Armadilloniscus holmesi*)

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Constitutive expression of FGF4 disrupts the development of the eye and the anterior CNS during mouse embryogenesis, but does not influence the expression of *shh* in these areas

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ABSTRACT. The biological consequences of constitutive *fibroblast growth factor-4 (fgf4)* expression have been analysed during anterior CNS development of mouse chimeric embryos. Severe mutant embryos exhibit exencephaly, absence of eye development and anomalous differentiation of the neuroepithelium. These embryos also show ectopic limb buds resembling the early phases of limb development. Because our results show that anterior CNS in those chimeric embryos does not express *shh* ectopically, we suggest that malformations may be due to interference between the ectopic expression of *fgf4* in the cephalic area and the receptors for the members of the FGF family that regulate brain and eye development, namely *fgf8*. If this is correct, the results indirectly support the crucial role of *fgf8* in patterning the anterior CNS.

KEY WORDS: Fibroblast Growth Factors, CNS development, eye development, mouse chimeric embryos, FGF4 gain of function.

INTRODUCTION

Inductive interactions are fundamental to the development of multicellular organisms. Among the different types of molecules involved in these interactions, the Fibroblast Growth Factor family (FGFs) has been shown to have multiple roles during embryogenesis, including the formation of skeletal structures and patterning of the limbs (reviewed by TABIN, 1995; and WILKIE et al., 1995) and brain (CROSSLEY et al., 1996; MEYERS et al., 1998; SHAMIM et al., 1999; LIU et al., 1999). The *fgf* gene family encodes a group of ligands that promote the growth and differentiation of many mesoderm and ectoderm cell types by binding to specific receptors (*fgfrs*) with a broad range of affinities (reviewed by BASILICO &MOSCATELLI, 1992; BAIRD, 1994; WILKIE et al., 1995).

Mutations and anomalous expression of both ligands and receptors in humans have been related to different syndromes that affect different structures including the head. The evidence for the distinct roles of FGFs in regulating the growth and patterning of the vertebrate embryo comes from their distinct patterns of expression during embryogenesis and from the generation of null mutations of several *fgfs* and their receptors in mice (MANSOUR et al., 1993; WERNER et al., 1993, 1994; HÉBERT et al., 1994; PETERS et al., 1994; FELDMAN et al., 1995; MEYERS et al., 1998) and experimental manipulations of chick limb buds (NISWANDER et al., 1993, 1994; COHN et al., 1995) and cephalic vesicles (CROSSLEY et al., 1996; LEE et al., 1997; SHAMIN et al., 1999).

An alternative approach to understanding *fgf* functions during mammalian development is to prepare chimeras between wild-type embryos and pluripotential embryonic stem cells (ES cells) that harbour regulatory mutations in the FGF system. One of the members of the *fgf* family with key roles in embryonic patterning is *fgf4*. The expression of *fgf4* and its putative receptors has been described during mouse embryogenesis (NISWANDER & MARTIN, 1992; ORR-URTREGER et al.,

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1991; YAMAGUCHI et al., 1992; ORR-URTREGER et al., 1993). For example, null mutations affecting *fgf4* are lethal in homozygotes just after implantation (FELDMAN et al., 1995), indicating the crucial role of this molecule during the first steps of mammalian development. On the other hand, ABUD et al. (1996) obtained chimeric mouse embryos constitutively expressing *fgf4* (gain of function). The most dramatic consequences of *fgf4* gain of function occur in the development of the limbs and anterior Central Nervous System (CNS). While ABUD et al. (1996) analysed the development of the limbs in these *fgf4* mouse chimeric embryos, the purpose of the experiments reported here was to describe the development of the CNS and to explain the malformations induced by *fgf4* constitutive expression.

METHODS

Generation of chimeras

The generation of the mutant chimeric mouse embryos constitutively expressing *fgf4* used in this work has been described elsewhere (ABUD et al., 1996). Briefly, they were generated using a ROSAβ-geo11 ES cell line (with constitutive expression of β-galactosidase; FRIEDERICH & SORIANO, 1991) transformed by electroporation with a PGKFGF4 expression construct, that contains a genomic DNA fragment including the entire mouse *fgf4* coding region under the control of mouse PGK-1 promoter and the SV40 small T antigen and SV40 polyadenylation sequences from PGK-o-term. PGKFGF4-ROSAβ-geo11 ES cells were injected into the blastocoel cavity of C57BL6/J blastocysts at day 3.5 post coitum (d.p.c.), and transferred to the uteri of random bred MF1 (purchased from Olac, U.K.) pseudopregnant recipients at day 2.5 p.c. The age of the embryos was calculated according to the date of mating of the mother (either pregnant or pseudopregnant foster mothers).

β-galactosidase staining

Chimerism was determined by staining for β-galactosidase activity as described by WHITING et al. (1991). Embryos dissected free of maternal decidual tissue were fixed for 1 h in 0.2% glutaraldehyde in a buffer containing 0.1 M potassium phosphate, 5 mM EGTA (Ethylene Glycol-bis(b-aminoethyl Ether) N,N,N',N'-Tetraacetic Acid; Sigma) and 2 mM $MgCl_2$ (pH 7.4). They were then washed for 3 x 20 min in the same buffer supplemented with 0.01% Na deoxycholate and 0.02% Nonidet P-40. The staining reaction was carried out overnight at 37ºC in the same buffer supplemented with 10 mM $K_3Fe(CN)_6$, 10 mM $K_4Fe(CN)_6$, and 0.5 mg/ml X-gal (Boehringer-Mannheim) dissolved in dimethylformamide. In cases where the embryo was to be used for *in situ* hybridisation, chimerism was determined by staining the yolk sac for β-galactosidase activity.

Haematoxylin-eosin staining

The embryos were fixed in Bouin fixative, dehydrated and embedded in Paraplast according to standard protocols. 5-7μm sections were stained with regular haematoxylin-eosin staining.

RNA in situ hybridisation

Double RNA in situ hybridisation was performed as described by BUENO et al. (1996a,b) using single stranded digoxigenin- and fluorescein-UTP labelled (Boehringer Mannheim) antisense RNA probes, detected sequentially with anti-digoxigenin and anti-fluorescein antibodies coupled to alkaline phosphatase. The first probe was detected by NBT/BCIP staining and visualised using transmitted light, and the second probe by ELF (Enzyme Labelled Fluorescence mRNA *in situ* hybridisation kit, Molecular Probes) substrate and UV light respectively. The *shh* (*sonic hedgehog*) probe was a 0.6 kb transcript generated by *Hin*dIII linearisation and T3 transcription (ECHELARD et al, 1993). The *fgf4* probe was a 0.6 kb transcript containing the full-length coding sequence and was generated by *Bam*HI linearisation and T3 transcription (HÉBERT et al., 1990).

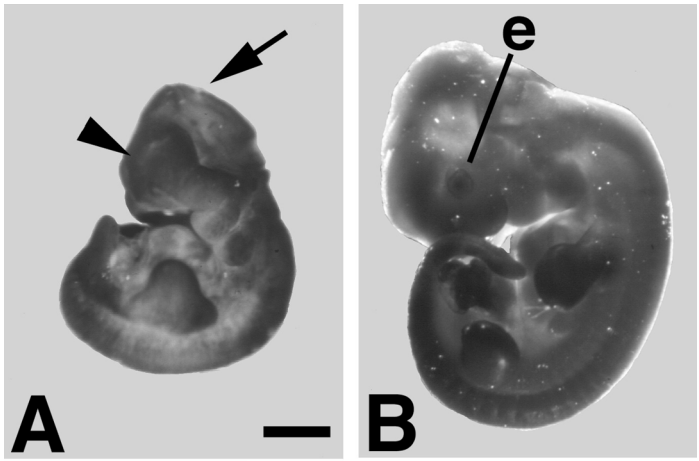
Photography

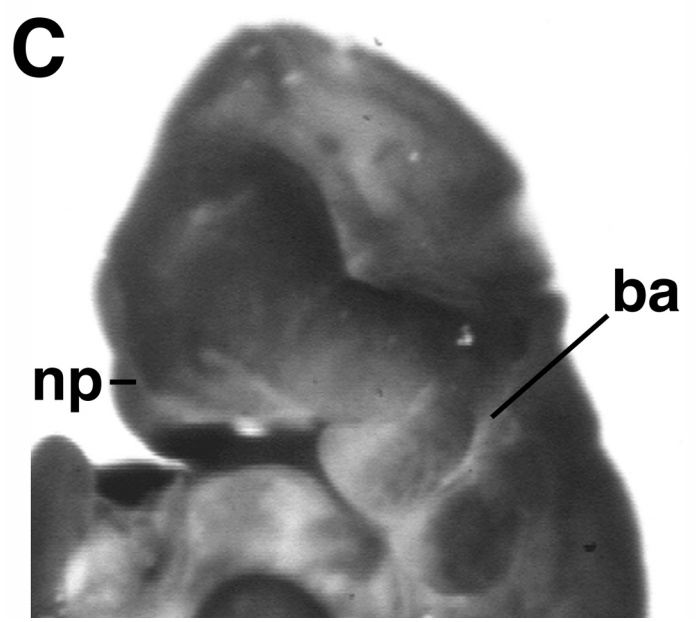
Photomicrographs of whole-mount embryos stained for b-galactosidase activity were taken on a Wild M8 photomicroscope on Agfa 64T film. Photomicrographs of sectioned embryos were taken using a Zeiss Axiophot microscope on Kodak 160T ASA film (NBT/BCIP or haematoxylin-eosin stained sections) or on Kodak 400 ASA film (ELF stained sections).

RESULTS AND DISCUSSION

All chimeras that, on the basis of whole-mount staining for β-galactosidase, contained a substantial ES cell contribution (>50%) from PGKFGF4-ROSAβ-geo11 ES cells (33 chimeras analysed), showed anomalous development of the head regions upon external inspection (Fig. 1A, C). No phenotypic defects were detected in homozygous control embryos prepared with wild type ROSAβ-geo11 ES cells (Fig. 1B). Embryos younger than 9.5 d.p.c. did not show phenotypic defects in the anterior CNS upon external inspection (not shown), and embryos older than 14-14.5 d.p.c. with phenotypic defects were never recovered, and may have been reabsorbed.

Most embryos showed absence of eye development at 10.5-11.5 d.p.c. The embryos with the most severe phenotype also showed failure in neural tube closure in the midbrain area, exhibited exencephaly and a reduction of the diencephalon (Fig. 1A, C). The craniofacial area (including the brachial arches, the nasal prominence, the nasal pit and the region overlaying the telencephalic vesicle), which derives from the neural crest, appeared normal (Fig. 1C).





The internal structure of the head of some of the chimeras was examined histologically on series of sections at 10.5-12.5 d.p.c (Figs 2A, B). The neural tube of the mutant shown (Fig. 2A) is closed and, despite the angle of sectioning, the head is obviously asymmetrical. The hindbrain and telencephalon are visible but the structure of the diencephalon and midbrain is distorted (Figs 1A, 2A, and data not shown). Eye development is severely impaired in the mutant. The optic cups are visible in the centre of the head rather than contacting the surface ectoderm. These eyes consist of an optic cup but lack both optic stalk and lens structure (Figs 2C, D). The structure of the neuro-epithelium throughout the brain is distorted, resulting in a thinner neuro-epithelial layer. The central area of the head in the more severe mutants consists of a thin layer of neuro-epithelium, which had no differentiated internal structures (data not shown).

It has been reported that anterior structures, including eyes, are lost as a result of the ectopic expression of the homologue of *fgf4* in *Xenopus* embryos (e*fgf*; Isaacs et al., 1994). Our results suggest that eye development and CNS formation are disrupted by the activation of FGF-dependent signalling processes. The development of eyes and their lenses involves a series of complex interactions, and the failure of lens formation in the mutant chimeras may have resulted from the fail-

Fig. 1 (upper left). – Control and mutant embryos showing the malformations produced by FGF4 gain of function. (A) 11.5 d.p.c. mouse chimeric embryo prepared with PGKFGF4 ROSAβ-geo11 ES cells. Note the absence of an eye (arrowhead) and the exencephaly (arrow). Also note the malformations in the lateral ridge and limb buds compared with a control embryo. (B) Homozygous control embryo prepared with wild type ROSAβ-geo11 ES cells. (C) Magnification of the head region of (A). Note that the brachial arches and the nasal prominence are normal. Both embryos were stained for β-galactosidase activity to show the presence and extent of ROSAβ-geo11 ES cells derivatives. Bar: 1 mm (A, B); 0.25mm (C). Abbreviations: e, eye; ba, brachial arches; np; nasal prominence.

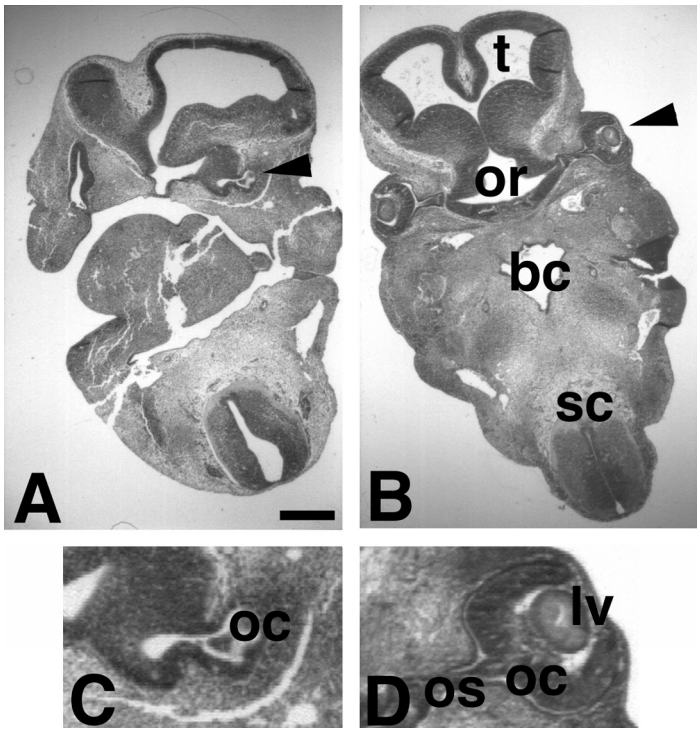


Fig. 2 (lower left). – Coronal sections through the head of chimera and control embryos at 11.5 d.p.c. stained with haematoxylin- eosin. (A) FGF4 mutant chimera. Note the position of the right eye structure (arrowhead). The opposite eye structure is in a contiguous serial section. (B) Control embryo. The position of the eyes is indicated by an arrowhead. (C) Defective eye of (A). (D) Right eye of (B). Sections are of 5 μm thick. Bar: 0.1 mm (A, B); 0.025 mm (C, D). Abbreviations: bc, buccal cavity; lv, lens vesicle; oc, optic cup, or; optic recess; os, optic stalk; sc, spinal chord; t, telencephalic vesicle.

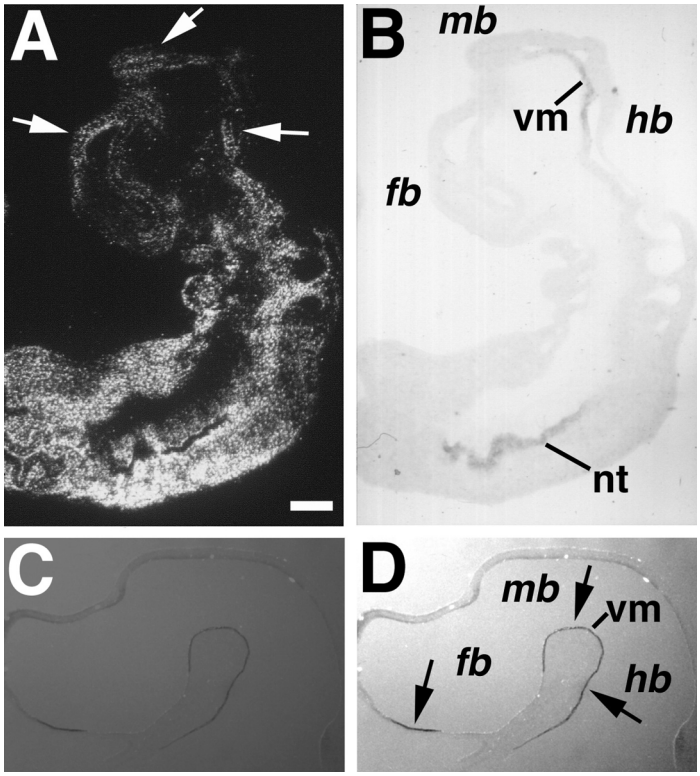


Fig. 3. – Sagittal sections of chimera and control embryos at 11.5 d.p.c. hybridised simultaneously to an antisense *fgf4* probe (DIG-labelled and ELF stained [in green]) and *shh* probe (FITC-labelled and NBT/BCIP stained [in blue]). (A) FGF4 mutant chimera showing ectopic expression of *fgf4*. The arrows indicate sites of *fgf4* ectopic expression in the developing brain. (B) Same section as (A) showing *shh* expression in the notochord (nt) and the ventral midline of the neuroectoderm (vm). The apparent absence of notochord in some areas of this section is due to the overall malformations of the embryo, which make it impossible to obtain a histological section that contains the entire notochord. (C) Sagittal section through the head of a control embryo at an equivalent level to (A) and (B) hybridised with *fgf4* antisense riboprobe. Note that *fgf4* expression is not detected. (D) Same section as (C) showing *shh* expression in the ventral midline of the neuroectoderm. Note that *shh* expression is detected at the ventral midline of the neuroectoderm (arrows) as in the mutant chimera. Sections are of 10 μm thick. Bar: 0.25 mm. Abbreviations: fb, forebrain; hb, hindbrain; mb, midbrain; nt, notochord; vm, ventral midline of neuroectoderm.

ure of the optic vesicle to reach the ectoderm (Saha et al., 1989). Normally, the optic vesicles develop from the diencephalon, and when they come into contact with the head ectoderm, the ectoderm thickens and initiates lens formation (reviewed by Saha et al., 1992).

At a molecular level, it has been previously shown that several members of the *fgf* family, namely *fgf2* and *fgf8*, function in eye development (Pittack et al., 1997; Desire et al., 1998; Picker et al., 1999; Heisenberg et al., 1999). Moreover, several FGFRs can bind FGF4 and other members of the FGF family, e.g. FGF8, with a similar specificity (MacArthur et al., 1995). It is tempting to speculate that the malformations in eye development observed here are due to interference between the ectopic expression of *fgf4* and the receptors for FGF8 and/or FGF2, that affects their binding and/or ectopically activates their receptors. However, as the eyes develop from the diencephalon (which is also severely affected in the chimeras) it is impossible to conclude from the current evidence whether the defects observed in eye development were the result of the *fgf4* ectopic expression or a secondary result due to disruption of the development of the anterior CNS.

The causes of the exencephaly, reduction of the diencephalon and anomalous differentiation of the neuroepithelium also remain unclear. It has been suggested that *shh* might play a role in the CNS phenotype of these mutant chimeras (Abud et al., 1996). This hypothesis is based on the fact that *shh* expression has been detected in areas adjacent to FGF expression domains in a number of anatomical sites during development, including the node, the floor plate, and the developing limb (Bueno et al., 1996b). Moreover, functional relationships between *fgf4* and *shh* have been described for the developing limb (Laufer et al., 1994; Niswander et al., 1994; Cohn et al., 1995; Yang & Niswander, 1995), and the expression of *shh* in ectopic limb buds has been reported in parallel mouse chimeric embryos (Abud et al., 1996).

In order to explore this possibility, we used double *in situ* hybridisation to analyse the expression of *fgf4* and *shh* in the cephalic region of mice embryos with severe mutant cephalic phenotypes. *Fgf4* gene expression was detected, at 10.5-11.5 d.p.c., in most tissues of the mutant embryos, including those of the cephalic area (Fig. 3A). In wild type embryos of this stage, the *fgf4* transcript was not detected in the tissues forming the cephalic vesicles (Fig. 3C). However, in the mutant embryos *shh* was detected only in the areas where it was expressed in wild type embryos (in the cephalic area, *shh* was expressed in the ventral midline of the neuroectoderm, Figs. 3B, D). Ectopic expression of *shh* was not detected either in the cephalic area or in the internal organs of the trunk or tail. Its absence may be due to the absence of the correct FGF4 receptors and signal transduction pathways in these regions that are necessary to activate *shh* expression (Laufer et al., 1994).

The absence of ectopic expression of *shh* in the heads of mutant mice indicates that this molecule may not be responsible for anterior CNS malformations. These malformations could be explained in other ways. Several FGFRs can bind FGF4 and other members of the FGF family (e.g. FGF8) with a similar specificity (MacArthur et al., 1995). Moreover, Crossley et al. (1996) identified FGF8 as an important signalling molecule for midbrain development, because it is expressed at discrete sites in the cephalic neuroectoderm during development (Crossley & Martin, 1995; Bueno et al.,

1996b). It is tempting to speculate that the reported malformations in the anterior CNS are due to interference between FGF4 ectopic expression and the receptors for FGF8. This would be consistent with recent reports (MEYERS et al., 1998; SHAMIN et al., 1999) showing that hypomorphic alleles, null mutants and ectopic expression of FGF8 in the midbrain and caudal forebrain cause exencephaly in mouse and chick embryos respectively, and showing that exogenous FGF4 can suppress anterior development in the mouse embryo during neurulation and early organogenesis (DAVIDSON et al., 2000). It would also explain the lack of malformations in the craniofacial area neural crest-derivatives. These exhibit a different set of FGFRs. If the hypothesis of FGF4 interference is correct, the results presented here would indirectly support a crucial role of *fgf*8 in patterning the anterior CNS and show once again the complexity of the FGF family in patterning embryonic structures.

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Study of the development of the caudal endoskeleton of the turbot *Scophthalmus maximus* L., 1758 (Pleuronectiformes: Scophthalmidae).

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ABSTRACT. The development of the caudal endoskeleton of the turbot (*Scophthalmus maximus* L.) is described and compared to the setting-up of the caudal endoskeleton in other flatfishes. In the turbot, caudal bony elements develop very early, before the eye migration. The same pattern occurs in the flatfishes of the bothoid group, while in the Soleidae the supporting caudal fin elements develop once the eye has migrated. This discrepancy is discussed; it points out that more work on the ontogeny of flatfishes is needed.

KEY WORDS: Pleuronectiformes, turbot, caudal endoskeleton, development.

INTRODUCTION

Several authors have shown the importance of studying the caudal endoskeleton in order to define major events in the phylogeny of the pleuronectiform fishes (HENSLEY & AHLSTROM, 1984; CHAPLEAU, 1993; COOPER & CHAPLEAU, 1998). In this supporting caudal fin structure, different features can be identified as unique, or apomorphic, for several flatfish groups. However, the sister group of the Pleuronectiformes within the Percomorpha being still uncertain (JOHNSON, 1993; CHAPLEAU, 1993), it is not always easy to decide the character states through outgroup comparison and some problems of homology remain. Thus, HENSLEY (1997) insisted that phylogenetic works associated with the study of the ontogeny were very badly needed. In that perspective, we present here data concerning the development of the caudal endoskele-

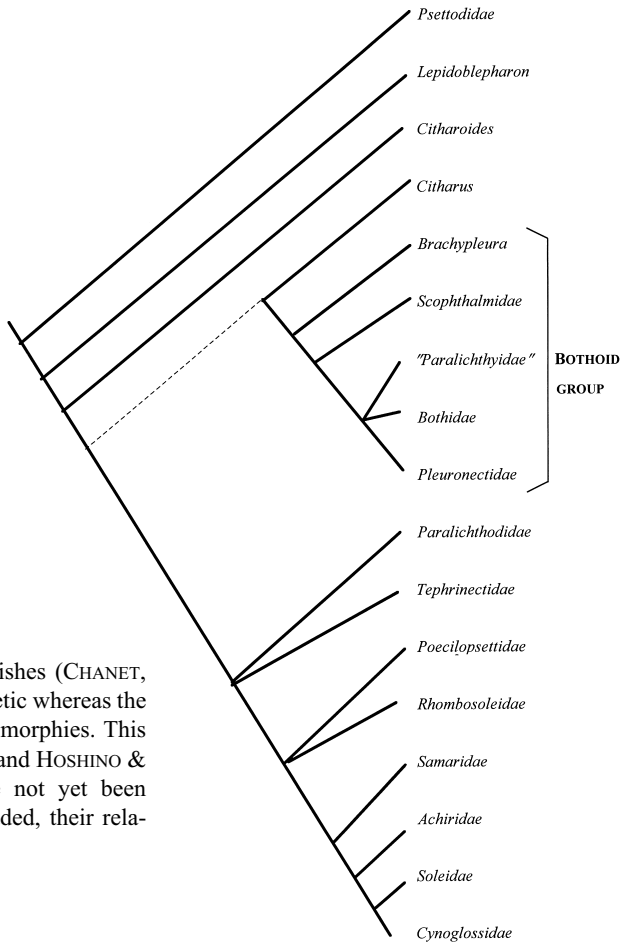

Fig. 1. – Tree of relationships between the major groups of flatfishes (CHANET, 1999). The inverted commas (") indicate that the group is paraphyletic whereas the dotted line shows a clade that is not well corroborated by synapomorphies. This tree is reconstructed from the data of COOPER & CHAPLEAU (1998) and HOSHINO & AMAOKA (1998). Because Pleuronectiformes relationships have not yet been analysed with both the Paralichthodidae and Tephrinectidae included, their relationships with other taxa are indicated by a polytomy.

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ton in the turbot. The turbot (*Scophthalmus maximus* L. 1758) is a well-known flatfish species belonging to the family Scophthalmidae, within the bothoid group in the pleuronectiform order (Fig. 1). We have already noted anomalies in the development of the caudal endoskeleton of the turbot and proposed a mechanism to explain it (CHANET & WAGEMANS, 1997), but here we intend to: i) present the ontogenetic data, ii) compare our observations to previous descriptions of the development of the caudal endoskeleton in other flatfish species.

MATERIAL AND METHODS

Scophthalmus maximus fry were raised in the aquaculture-station of France Turbot-NATA (Noirmoutiers, France) at 15 °C. Samples of 30 fry were sampled on days 0 to 61 post-hatching. The fry were fixed in a $CaCO_3$ buffered 10% formalin solution and were cleared with trypsin. Some of them were stained with alcian blue to reveal the cartilage and others with alizarine, to stain the calcified bones, according to TAYLOR & VAN DYKE's method (1985). It was possible to stain the oldest stages simultaneously with alizarine and alcian blue. Finally, the fry were stored in glycerin. A 6 month-old specimen was cleared with trypsin, stained with alizarine and stored in glycerin according to TAYLOR and VAN DYKE's method. The specimens -270 larval and juvenile turbots- have been studied with a binocular Wild M10 Leica dissecting microscope at 8x magnification, and a drawing tube. The length from the tip of the snout to the posterior margin of hypural elements (standard length - SL) was measured for each specimen.

RESULTS

The first discernible caudal fin elements appear at day 13: the notochord is already slightly flexed and its ventral edge shows a slender cartilaginous ribbon with a ventrally protrusive expansion (Fig. 2A). At day 14, this ventral ribbon is dissociated into three ventral elements (Fig. 2B). The anterior-most one – the future parhypural – is never in contact with the notochord, the following one – the future hypural 1 and 2 plate – is a broad plate, the posterior one – the future hypural 3 and 4 plate – is smaller and has an irregular dorsal edge. These latter two elements are in contact dorsally with the notochord and ventrally support the first caudal fin rays. Anteriorly, the vertebral cartilaginous structures develop with neural spines dorsally and haemal spines ventrally. At day 22 (Fig. 3A), the notochord is strongly curved with an almost vertically directed distal part. Ventrally, the parhypural and the two hypural plates are larger and their median parts begin to ossify, while their proximal and distal parts remain cartilaginous. A little hypural 5 is visible, two cartilaginous epurals are present dorsally. At day 26 (Fig. 3B), as the hypural elements are well developed, the notochord regresses, hypural 5 is close to its distal tip. The posterior-most epural is smaller and more ventrally placed than its serial homologue. The haemal and neural spines are more ossified. At day 29 (Fig. 4A), the vertebral centra are fully ossified and fused with their haemal and neural spines. In some specimens (55%), the second preural centrum (PU2) shows two neural and haemal spines (Fig. 4A-B). We showed (CHANET & WAGEMANS, 1997) that this anomaly was the result of a

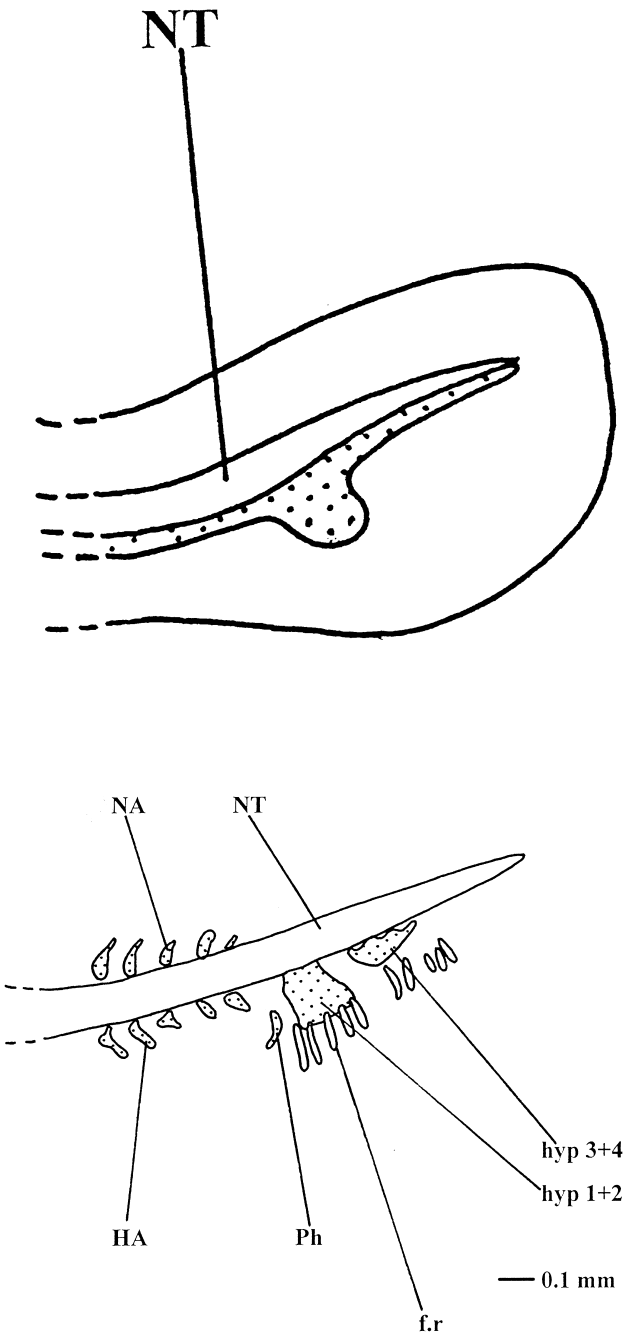

Fig. 2. – A. Caudal endoskeleton of a young turbot at day 13 (LS= 5 mm). B. Caudal endoskeleton of a young turbot at day 14 (LS= 6.5 mm). The stippled areas are cartilaginous regions. f.r: fin ray, HA: haemapophyis, hyp: hypural, NA: neurapophysis, NT notochord, Ph: parhypural.

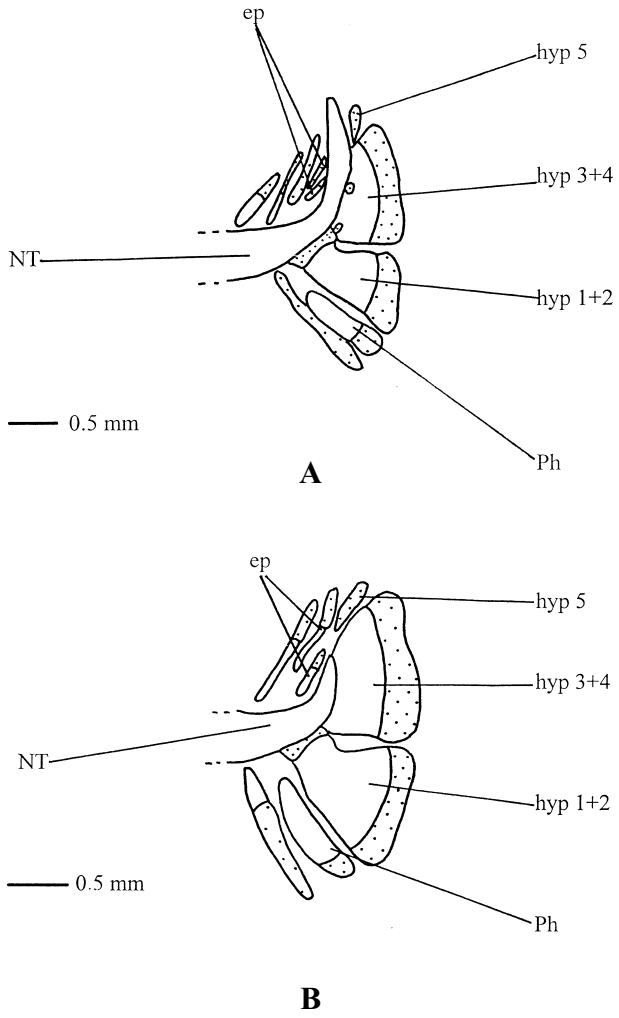

Fig. 3. – A. Caudal endoskeleton of a young turbot at day 22 (LS= 9 mm). B. Caudal endoskeleton of a young turbot at day 26 (LS= 15 mm). The stippled areas are cartilaginous regions. ep: epural.

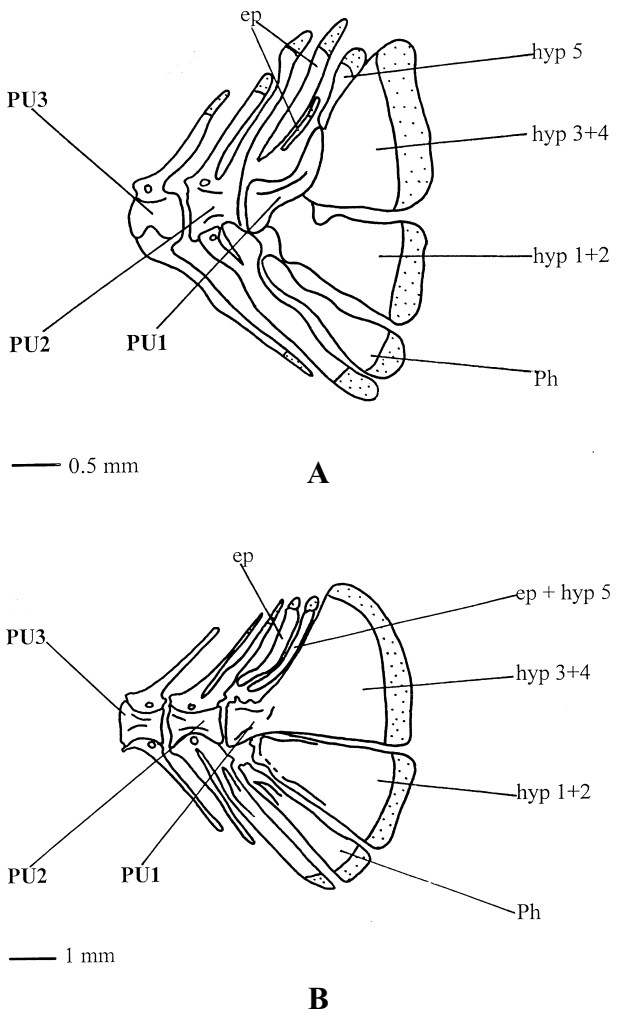

Fig. 4. – A. Caudal endoskeleton of a young turbot at day 29 (LS= 16 mm). B. Caudal endoskeleton of a young turbot at day 61 (LS= 28 mm). PU; preural centrum.

fusion between the third preural centrum and the second preural centrum. At this stage, the hypural plates are large and in contact with the ventral edge of the first preural centrum (PU1), the latter being dorsally curved. At this stage, the posterior epural is still independent, slender and in contact with a large anterior epural. However, at day 61 (Fig. 4B), the posterior epural is fused with the hypural 5 it (Chanet & Wagemans, 1997). No uroneural is discernible.

DISCUSSION

Different information can be deduced from this description: first, on the development of the endoskeleton among the flatfishes, second, on the setting up of this structure compared to the metamorphosis.

One of the first points to note is that the set-up of the different elements of the caudal skeleton in the turbot is not different to what has been described in three other bothoid species: one pleuronectid, *Pleuronectes platessa* L., 1758 (Barrington, 1937; Cole & Johnstone, 1902), and two bothids: *Trichopsetta ventralis* (Goode & Bean, 1885) (Futch, 1977) and *Engyophrys senta* Ginsburg, 1933 (Hensley, 1977). The same bony elements develop in the same pattern and in the same order. Thus, it corroborates previous observations. Moreover, the works of several authors (Hensley & Ahlstrom, 1984; Chapleau, 1993; Cooper & Chapleau, 1998) showed that the type of caudal endoskeleton present in the turbot (with hypurals 3 and 4 fused together and to PU1 centrum and hypurals 1 and 2 fused in a plate possessing a ball and socket articulation with the ventral edge of PU1 centrum) is unique among the percomorphs and is characteristic of the flatfishes belonging to the Scophthalmidae, Bothidae, Paralichthyidae, Pleuronectidae and *Brachypleura novaezeelandiae* Günther, 1862 previously referred to "Citharidae" a family recognised now as paraphyletic (Chapleau, 1993). On the basis of this peculiar caudal

endoskeleton, these flatfishes were regrouped in a bothoid group (Fig.1) (HENSLEY & AHLSTROM, 1984; COOPER & CHAPLEAU, 1998). The present description provides data that corroborate the homologies between the different caudal structures, and thus provides elements to confirm the monophyly of the bothoid group.

In the turbot, the eye migration – one the main features of flatfish metamorphosis – occurs between the 23rd and the 60th day of development (WAGEMANS et al., 1998). Then, the caudal endoskeleton is fully developed before the metamorphosis in this species. If we compare this result to what has been described in other bothoids, we can notice that, in each case, the caudal endoskeleton is formed before the eye begins to migrate. FUTCH (1977) noted that, in *Trichopsetta ventralis* (Bothidae), the hypurals were fused when the larva measured 6 mm (SL), whereas the ocular migration occurred when the larva was 28.5-35.7 mm long (SL). In *Engyophrys senta* (Bothidae), the migration begins on 18.9 mm long (SL) larvae and the hypurals are already fused at 4.6 mm (SL) (HENSLEY, 1977). In *Pleuronectes platessa* (Pleuronectidae), the hypurals are already well fused on 15-17 mm long (TL : Total Length) larvae, while the beginning of the migration occurs when the larva is 15 mm long (TL) (COLE & JOHNSTONE, 1902). SCHNAKENBECK (1928) described the morphology of some bothoid larvae, but his data are hardly useful. Nevertheless, in *Glyptocephalus cynoglossus* L. 1758 (Pleuronectidae) caudal elements seem to take place when the larva is 10 mm long and the metamorphosis occurs at 20-26 mm length (SCHNAKENBECK, 1928). In the scophthalmid *Phrynorhombus norvegicus* (GÜNTHER) 1862, the caudal endoskeleton is already well developed when the larva is 7.5 mm long whereas the eye migration takes place between 6 and 10 mm in total length (SCHNAKENBECK, 1928). On the other hand, in Soleidae, the hypurals fuse with the PU1 centrum after metamorphosis. In the sole (*Solea solea* L., 1758), the fusion occurring between hypurals and PU1 appears when the larva is between 18 and 470 mm long (TL) (HENSLEY & AHLSTROM, 1984), while the metamorphosis occurs on 8 mm long (TL) larvae – 18 days after hatching- (WAGEMANS & VANDEWALLE, 1999). In *Dicologlossa cuneata* (Moreau, 1881), the caudal complex develops at 11-22 mm on larvae that have already the two eyes on the right side (LAGARDÈRE & ABOUSSOUAN, 1981).

Pending new evidence, we can only say that the caudal endoskeleton of Pleuronectiformes develops before or after eye migration according to species. Is the relative shift in developmental timing a synapomorphy of the bothoids? Does it mean that heterochronic events occur during the development of some flatfishes? Such hypotheses are yet premature and must be confirmed through the study of many flatfish species -bothoids and non-bothoids. More work on other flatfish species is needed to confirm these ideas.

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Arthropods in nests of the red-backed shrike (*Lanius collurio*) in Poland

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ABSTRACT. Beetles, moths, spiders and mites collected from the nests of the red-backed shrike (*Lanius collurio*) in the years 1997-98 in a study area near Leszno in W Poland were analysed. The total number of nests checked was 28, and arthropods were found in 75 % of them. Only one nest was inhabited by a parasitic arthropod species (the blowfly *Protocalliphora azurea*). Altogether two species of moths, five of beetles, two of spiders, and 44 species of mites (21 spp. Oribatida and 23 spp. Gamasida) were identified. One species (*Typhlodromus wichmanni*, Acari, Gamasida) was found for the first time, and one (*Paragarmania dentriticus*, Acari, Gamasida) for the second time in Poland.

KEY WORDS: nesting biology, nests, spiders, beetles, fleas, mites, moths, Lanius collurio.

INTRODUCTION

With the recent upsurge of interest in host-parasite interactions, the influence of mites, ticks, lice, fleas and bugs living on birds has been studied with regard to antagonistic relationships with harmful effect on the fitness of an avian host, as implied in the definition of parasitism (review in MØLLER et al., 1990; LOYE & ZUK, 1991). Birds and their nests have been rarely studied as the resident sites of non-parasitic arthropods. However, the fauna of bird nests have been described only for a few species of birds, as e.g. penduline tit *Remiz pendulinus* (Linnaeus, 1758) – KRISTOFÍK et al. (1993, 1995), MASÁN & KRISTOFÍK (1995), KRISTOFÍK & MASÁN (1996), the sand martin *Riparia riparia* Linnaeus, 1758 – MASÁN & KRISTOFÍK (1993), KRISTOFÍK et al. (1994), the bee-eater *Merops apiaster* Linnaeus, 1758 – KRISTOFÍK et al. (1996), sparrows *Passer domesticus* Linnaeus, 1758 and *Passer montanus* Linnaeus, 1758 – WASYLIK (1971), CYPRICH et al. (1997), DRABER-MONKO (1997), FENA & PINOWSKI (1997). These species however, either build very characteristic closed nests as does the penduline tit, or stay in burrows as do the sand martin and the bee-eater or in holes as do the two species of sparrow. Most European bird species build open nests (NEWTON, 1998), which have been rarely studied by entomologists and acarologists. One of the exceptions is an old study by NORDBERG (1936), and earlier papers referring to spiders and gamasid mites found in bird nests in Slovakia (GAJDOS et al., 1991; AMBROS et al., 1992). In these studies, based on the same material, the nests of the red-backed shrike *Lanius collurio* (Linnaeus, 1758) were analysed. However, no ecological relationship between the nest size and the number of inhabiting spiders and mites was sought. Nest size can be a key feature affecting clutch size or, more generally, parental investment of birds (SLAGSVOLD, 1989; SOLER et al., 1998; TRYJANOWSKI, 1999), and also, indirectly, the presence of parasites (POIANI, 1993). Nest size is also a measure of the quantity of material used for its construction, which influences the probability of the presence of arthropods (HICKS, 1959; WASYLIK, 1971).

The study reported here aimed to: (1) recognise the arthropod fauna living in the red-backed shrike nests; (2) check if the number of arthropods living in a nest

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depends on its size; (3) find a possible correlation between the number of different insect species and mites, (4) establish a population structure of chosen species (gamasid mites).

MATERIAL AND METHODS

The study was conducted over a study area near Leszno in south-western Poland (51º51'N, 16º35'E). A detailed description of this study area has been presented elsewhere (KU{NIAK, 1991). The area was surveyed for shrike nests. The nests were visited at several-day intervals throughout the period from mid-May to July in 1997-1998. Each nest was visited two to seven times. The minimum number of two visits occurred when a nest was found in the stage of laying eggs and visited for the second time near the end of the nestling stage to ascertain the number of fledglings and to determine the breeding success. A higher number of visits was made to nests found during incubation and to the nests in which selected elements of breeding biology were more thoroughly examined (*cf.* KU{NIAK, 1991; TRYJANOWSKI & KU{NIAK, 1999). Nests were picked up a few days after the end of breeding, and transported to a laboratory, where arthropods were startled in Berlese – type funnels. Altogether 28 nests were collected but seven of them were kept in foil bags for more than 10 days before extracting the arthropods. The difference between the nests put directly (2-3 days) into the Berlese - type funnels and those kept longer in foil bags was statistically significant (c^2 with Yates correction = 4.25; df = 1; $P = 0.04$). In view of these results, only the data from the 21 nests were used in further analysis. After the arthropods were startled, some nests (n = 15) were weighted to an accuracy of 1 g and the relationships examined between the weight of the nest and the number of inhabiting arthropods. As a large number of nests were inhabited by only one arthropod species, resulting in a large number of "null" samples, we abandoned the search for any correlation between individual species and nest size. Only correlations between nest weight and total number of individuals and number of species of all arthropods, gamasid mites and oribatid mites were examined. There was a significant difference between the distribution observed in our study and a normal distribution (Kolomogorov-Smirnov test), so the Spearman rank correlation was applied. The material of gamasid and oribatid mites was analysed considering the following indices: constancy of occurrence (percentage of nests in which the species occurred) and dominance (ratio of the number of individuals of a given species to the number of all the individuals of the taxon under study, in percent).

TABLE 1

Oribatid mites (Oribatida) found in the red-backed shrike nests

No.	Species	No. of ind.	Constancy (%)	Dominance (%)
1	*Phthiracarus crinitus* (Koch, 1841)	1	4.8	0.11
2	*Hoplophthiracarus vanderhammeni* Niedbala, 1991	1	4.8	0.11
3	*Nothrus pratensis* Sellnick, 1928	2	4.8	0.22
4	*Camisia spinifer* (Koch, 1835)	4	19.0	0.43
5	*Heminothrus peltifer* (Koch, 1839)	7	14.3	0.76
6	*Liacarus coracinus* (Koch, 1841)	2	4.8	0.22
7	*Ceratoppia quadridentata* Haller, 1882	65	38.1	7.07
8	*Carabodes areolatus* Berlese, 1916	1	4.8	0.11
9	*Carabodes labyrinthicus* (Michael, 1879)	1	4.8	0.11
10	*Tectocepheus velatus* (Michael, 1880)	22	23.8	2.39
11	*Micreremus brevipes* (Michael, 1888)	1	4.8	0.11
12	*Scheloribates laevigatus* (Koch, 1836)	1	4.8	0.11
13	*Zygoribatula exilis* (Nicolet, 1855)	241	28.6	26.20
14	*Protoribates variabilis* Rajski, 1958	56	47.6	6.09
15	*Chamobates voigtsi* (Oudemans, 1902)	263	28.6	28.59
16	*Ceratozetella minima* (Sellnick, 1929)	2	4.8	0.22
17	*Trichoribates novus* (Sellnick, 1928)	83	61.2	9.02
18	*Eupelops torulosus* (Koch, 1840)	4	9.5	0.43
19	*Oribatella berlesei* (Michael, 1898)	67	4.8	7.28
20	*Achipteria coleoptrata* (Linnaeus, 1758)	12	4.8	1.30
21	*Pergalumna nervosa* (Berlese, 1914)	7	14.3	0.76
22	*Damaeidae*	30	14.3	3.26
23	*Belbidae*	24	14.3	2.61
24	*Oppiidae*	6	9.5	0.65
25	juveniles indet.	17	23.8	1.85
	Total	**920**	**95.2**	**100**

RESULTS

Spiders

In eight nests, (38.1 %) a total of 35 individuals (mean ± SD = 4.4 ± 6.0) of the *Oxyptila trux* (Blackwall, 1846) were found. A single female of *Troxochrus scabriculus* (Westring, 1851) was identified. These two species are common in the fauna of Poland, occurring mainly in grassy habitats, both dry and wet, where they live at ground level. These species have not been noted in the nests of red-backed shrike in Slovakia (GAJDOS et al., 1991).

Oribatid mites

A total of 920 oribatid mites belonging to at least 21 species were found (Table 1). Representatives of the three species: *Trichoribates novus* (in 61.2% of nests), *Protoribates variabilis* (47.6%) and *Ceratoppia quadridentata* (38.1%) were noted in the greatest number of nests. Representatives of the other species were met in individual nests, although sometimes in a large numbers, e.g. *Chamobates voigtsi* (max. 167 individuals in one nest). The majority of them are saprophages and fungivores. All the species encountered are widespread, mainly in forest habitats in Poland (OLSZANOWSKI et al., 1996). The number of oribatid mite species found in the nest was correlated with the number of these mites in the nest (r_s = 0.77, n = 21, $P < 0.001$).

Gamasid mites

The total number of gamasid mite individuals found in all nests was 1303, representing at least 23 species (Table 2). Two species in particular are of interest. One,

TABLE 2

Gamasid mites (Gamasida) found in the red-backed shrike nests

Explanations: P - protonymphs, D - deutonymphs, F - females, M - males

No.	Species	P	D	F	M	Total	Constancy	Dominance
1	*Alliphis siculus* (Oudemans, 1905)		13	74	35	122	47.62	9.36
2	*Amblyseius* sp.			9	2	11	4.76	0.84
3	*Typhlodromus* (*Anthoseius*) sp.			27	15	42	28.57	3.22
4	*Arctoseius semiscissus* (Berlese, 1892)			15		15	4.76	1.15
5	*Asca bicornis* (Canestrini et Fanzago, 1887)			1		1	4.76	0.08
6	*Blattisocius tarsalis* (Berlese, 1918)			1		1	4.76	0.08
7	*Celaenopsis badius* Koch, 1836				1	1	4.76	0.08
8	*Cornigamasus lunaris* (Berlese, 1882)		1			1	4.76	0.08
9	*Discourella modesta* (Leonardi, 1899)			1		1	4.76	0.08
10	*Gamasellodes bicolor* (Berlese, 1918)		7	83	8	98	47.62	7.52
11	*Gamasodes spiniger* (Trägardh, 1910)		21		1	22	19.05	1.69
12	*Holoparasitus calcaratus* (Koch, 1839)			4	2	6	9.52	0.46
13	*Hypoaspis (Cosmolaelaps) vacua* (Michael, 1891)			1		1	4.76	0.08
14	*Lasioseius ometes* (Oudemans, 1903)			6	1	7	14.29	0.54
15	*Lasioseius* sp.			10	5	15	14.29	1.15
16	*Macrocheles glaber* (Müller, 1860)	3	4	60	20	87	23.81	6.68
17	*Macrocheles rotundiscutis* Bregetova et Koroleva, 1960			35	28	63	4.76	4.83
18	*Paragamasus (Anidogamasus) vagabundus* (Karg, 1968)			6	1	7	4.76	0.54
19	*Paragarmania dentriticus* (Berlese, 1918)		9	65	14	88	38.10	6.75
20	*Parasitus fimetorum* (Berlese, 1904)	15	133	14	10	172	14.29	13.20
21	*Pergamasus (Pergamasus)* sp.		1			1	4.76	0.08
22	*Pergamasus (Thenargamasus)* sp.		4			4	14.29	0.31
23	*Proctolaelaps pygmaeus* (Müller, 1860)		6	307	3	316	28.57	24.25
24	*Proctolaelaps* sp.			5		5	9.52	0.38
25	*Trichouropoda ovalis* (Koch, 1839)	1	61	52	27	141	14.29	10.82
26	*Trichouropoda* sp.		1			1	4.76	0.08
27	*Typhlodromus* (*Anthoseius*) wichmanni Hirschmann, 1962			7		7	14.29	0.54
28	*Uroobovella* (group *flagelliger*) sp.			1		1	4.76	0.08
29	*Uropoda orbicularis* (Müller, 1776)		41	10	1	52	4.76	3.99
30	*Uroseius infirmus* (Berlese, 1887)			10	3	13	4.76	1.00
31	*Zercon peltatus* Koch, 1836			1		1	4.76	0.08
	Total	**19**	**302**	**805**	**177**	**1303**		**100**

Typhlodromus (*Anthoseius*) *wichmanni* – present in three nests – has not previously been noted in Poland and this is the first record establishing its site of occurrence. The other *Paragarmania dentriticus* – found in eight nests – has to date been reported only from the vicinity of Poznan, *ca*. 60 km N of the area of study (BLASZAK, 1976). Thus, this work reports the second site of occurrence of this species in Poland. The other species finds favourable conditions in the red-backed shrike nests. Considering the frequency of occurrence in the nests and the presence of juveniles, two other species also find suitable conditions in the nests of the red-backed shrike: *Parasitus fimetorum* and *Trichouropoda ovalis*. A correlation was found between the number of species of gamasid mites in the nest and their abundance ($r_s = 0.74$, n = 24, $P < 0.0001$). None of the gamasid mite species found in the nests studied has been noted in the nests of the same bird species in Slovakia (AMBROS et al., 1992).

Coleoptera

We found as few as six beetles in only three (14.3 %) nests. Two individuals of *Tachyporus hypnorum* (Fabricius, 1775) (Staphylinidae) were found in one nest. The other species were represented by single individuals in the nest: *Phyllodrepa nigra* (Gravenhorst, 1806) (Staphylinidae), *Meligethes aeneus* (Fabricius, 1775) (Nitidulidae), *Corticaria elongata* Gyllenhal, 1827 and *Cortinicara gibbosa* (Herbst, 1793) (Latridiidae). All the species found are eurytypic and common, and their presence in the nests was accidental, although both lathridid species, as typical mycetophages, are often inhabitants of bird nests (KRISTOFÍK et al., 1995, 1996).

Moths and other insects

The presence of tineid moths *Tinea trinotella* (Thunberg, 1794) (Lepidoptera, Tineidae) was evidenced in 17 nests (81%). The total number of adult individuals was 103 (64 males and 39 females, max 14 ind. and 35 ind. in one nest, respectively) and 30 larvae. This species, like other tineid moths, is often met in bird nests as they provide favourable conditions for reproduction and – as far as larvae are concerned – a good source of nutrition (HICKS, 1959; HANNEMANN, 1977; BUSZKO, 1996). Moreover, one imago *Coleophora milvipennis* Zeller, 1839 (Lepidoptera, Coleophoridae) and one imago *Nomophila noctuella* (Denis et Schifferm^ller, 1775) (Lepidoptera, Pyralidae) were found. Their presence was accidental since the developmental stages of neither are related to bird nests. Also a single larva of *Raphidia notata* (Fabricius, 1781) (Raphidoptera, Raphidiidae) was found, which probably searched there for larvae of other insects. In one nest we found a large number (ca. 35) of larvae of the blowfly *Protocalliphora azurea* (Fallen, 1817) (Diptera, Calliphoridae), which is a well known parasite of nestlings of different species of birds (LINDNER, 1956).

DISCUSSION

Ecological relationships

Typical parasites of birds were found only in one nest. These were the blowfly larvae in an unsuccessful nest, destroyed by a predator. The other arthropods were either brought into the nest by accident together with the construction material or sought a suitable site for reproduction as did the tineid moths. It is generally known that many elements of bird nest fauna are of accidental origin (NORDBERG, 1936; HICKS, 1959; GAJDOS et al., 1991; AMBROS et al., 1992; KRISTOFÍK et al., 1993, 1996; NEUBIG & SMALLWOOD, 1999) and their presence is not related to the host of the nest. For many of these species, in contrast to parasites, the most important factor is the size and construction of the nest (NORDBERG, 1936; HICKS, 1959; WASYLIK, 1971; BUSZKO, 1996). The mean weight of a dried nest of red-backed shrike (from our data in the area of study) was 43.2 (± 8.5; range 28-59) g. The nest mass was significantly positively correlated with the abundance and number of species of gamasid mites (r_s = 0.51 and r_s = 0.54, respectively; n = 21 and $P < 0.05$ in both cases). For the other groups of arthropods this relationship was statistically insignificant ($P > 0.3$ in all cases). Analysis of possible correlations between different groups of arthropods living in the nests revealed only one significant correlation, between the abundance of gamasid mites and larvae of tineid moths ($r_s = 0.49$; $P < 0.05$). This relationship is most probably related to the common way of feeding of these two groups of arthropods, which live on dead organic matter (HANNEMANN, 1977).

Concluding remarks

In 28 studied nests of red-backed shrike, representatives of arthropods were found in 21 (75 %). The total number of individuals was 2436 and they represented at least 54 species. The most abundant groups were gamasid mites (1303 indiv. and at least 23 spp.) and oribatid mites (920 indiv. and at least 21 spp.). The representatives of mites made up 91% of the number of arthropod individuals and their species constituted 81% of all arthropod species found. Among all arthropods evidenced in the nests only one species, the blowfly *Protocalliphora azurea,* is known as a bird parasite. The other species are sapro-, fungi-,phytophagous and predators such as moths, beetles, and some mites, which found themselves in the nests when looking for sites rich in dead organic matter either as sources of nutrition or as suitable sites for reproduction. A large number of the representatives of these arthropods could have been brought into the nests together with the nest constructing material or with food for the nestlings.

An interesting observation is the lack of species of spiders and gamasid mites in the nests of red-backed shrike which would be common in the nests studied in Poland (this study) and Slovakia (GAJDOS et al., 1991; AMBROS et

al., 1992) and in fact, that generally arthropod numbers in shrike nests in Poland were surprisingly low. The differences may be due to the geographical distribution of the species or, more probably, to the differences in the time of nest collection (WASYLIK, 1971; KRISTOFÍK et al., 1995; KRISTOFÍK & MASÁN, 1996). In general, the number of evidenced representatives of gamasid mites is close to that found in the nests of birds of other species, while the number of representatives of spiders and beetles is lower than the corresponding data for other birds' species (GAJDOS et al., 1991; AMBROS et al., 1992; KRISTOFÍK et al., 1993, 1996; MASÁN & KRISTOFÍK, 1993, 1995; FENA & PINOWSKI, 1997). The results reported in this work cannot be reliably compared with those obtained by other authors because of differences in the time of nest collection and sample size.

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Lack of evolutionary potential of developmental instability of front tibia length in the Indian meal moth (*Plodia interpunctella*)

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ABSTRACT. The evolutionary potential of developmental instability (DI, defined as an individual's inability to buffer its development against random perturbations) as estimated by individual asymmetry (so-called fluctuating asymmetry, small random deviations from perfect symmetry), remains a controversial subject of research. Only if DI is heritable and if it is related with fitness, can evolution be expected to occur. In this study we find no evidence for evolutionary potential of DI of the front tibia in the Indian meal moth. The heritabilities of fluctuating asymmetry and DI were negative. Individual asymmetry was not related to two fitness components: survival probability and body length. In contrast, both survival probability and body length were heritable. Body length increased with food quality but was not affected by density, whereas survival probability increased with food quality and decreased with density. Thus, effects of environmental stress and genetic background differed for fluctuating asymmetry on one hand and survival probability and body length on the other hand.

KEY WORDS: developmental instability, fluctuating asymmetry, fitness, heritability, Indian meal moth, *Plodia interpunctella.*

INTRODUCTION

The development of an individual is disturbed by random perturbations. As a consequence, its phenotype deviates from the expected one given the individual's genotype and the environmental conditions under which it developed. It is generally assumed that control mechanisms exist that buffer development against these mistakes (e.g. VAN DONGEN et al., 1999c). Developmental instability is the inability of an individual to buffer its development against these random perturbations (PALMER & STROBECK, 1992) [further called DI]. A commonly used estimator of DI is fluctuating asymmetry [i.e. small random deviations from perfect symmetry (Van Valen, 1962) further called FA]. The idea is that when trait development is perfectly buffered against perturbations, both sides of a bilaterally symmetric trait will undergo identical developmental pathways, since they are under the control of the same genes and environmental conditions, resulting in a perfectly symmetrical trait. Yet, development is never free of mistakes and perfect symmetry does not exist in nature (SWADDLE & CUTHILL, 1997). The poorer the development is buffered (i.e. high DI), the stronger deviations from symmetry are expected (i.e. high FA). DI and FA are assumed to originate from environmental and genetic effects as well as from genotype×environment interactions (MARKOW, 1995). The relative importance of these three factors, however, remains unclear, although it is critical for the interpretation of patterns in fluctuating asymmetry. The recent boost in studies of FA as estimator of quality, health or the effects of heterozygosity of individuals and populations (MARKOW, 1995; Leung & Forbes, 1996) has provoked much interest in the genetic architecture and evolutionary potential of DI (VAN DONGEN & LENS, 2000a; VAN DONGEN, 2000).

If fitness relates to particular phenotypic values, individuals that approximate these values with highest precision during ontogeny, and thus have low expected levels of DI, might have a fitness advantage (FOWLER & WHITLOCK, 1994). Furthermore, individual asymmetry might signal individual genetic quality and be used as a cue in mate selection under the 'good genes' model of sexual selection. Both the extents to which DI reflects

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individual quality and expected fitness, and the genetic variance of DI have important implications for evolutionary and sexual selection theory (DUFOUR & WEATHERHEAD, 1996). Developmental instability will be important for the genetic structure and evolutionary potential of populations only if it is related to the genotype and if it is associated with fitness (MARKOW, 1995). However, both the heritability of FA (h^2_{FA}) and DI (h^2_{DI}), as well as the relationship between DI and fitness remain controversial topics.

Recently, MØLLER & THORNHILL (1997) performed a meta-analysis to combine all available estimates of h^2_{FA}. They concluded that there is on average a small but highly significant genetic component (h^2_{FA}=0.19). However, their study has been criticized by several authors, many of them arguing that h^2_{FA} is much lower (<0.1) or even zero (LEAMY, 1997; MARKOW & CLARKE, 1997; WHITLOCK & FOWLER, 1997). Recent studies not included in the Møller and Thornhill paper seem to confirm this (VAN DONGEN et al., 1999a; VAN DONGEN, 2000 and references therein). WHITLOCK (1996) and HOULE (1997) noted that h^2_{FA} is expected to be much lower than h^2_{DI} because of the low correlation between individual asymmetry and DI. WHITLOCK (1996) [see also VAN DONGEN (1998a) and WHITLOCK (1998) for a correction of the formula] derived a way based on the hypothetical repeatability (*R*) of individual asymmetry, to transform h^2_{FA} into h^2_{DI}. This value of R estimates the proportion of variation in asymmetry that is the result of between-individual differences in the underlying DI. R, therefore, estimates how accurately individual asymmetry estimates individual DI. Similarly, GANGESTAD & THORNHILL (1999) argued that h^2_{FA} underestimates h^2_{DI} whereby h^2_{DI} may be in the order of magnitude found for many other fitness traits (i.e. h^2_{DI}=0.35-0.55). In agreement, LEAMY (1999) found a low heritability of FA (mean h^2_{FA}=0.03) but a much higher estimate for DI (mean h^2_{DI}=0.45), although neither estimates was statistically significant. Two other recent studies show that h^2_{DI} may be low as well [mean h^2_{DI}=0.09 in *Operophtera brumata* (VAN DONGEN et al., 1999a) and mean h^2_{DI}=0.04 in *Drosophila melanogaster* (WOODS et al., 1998)].

The relationship between DI and fitness is also not ubiquitous. LEUNG & FORBES (1996) performed a meta-analysis and concluded that FA-fitness relationships are fairly weak and highly heterogeneous. As for h^2_{FA}, the low correlation between individual asymmetry and DI may have obscured patterns in some studies (GANGESTAD & THORNHILL, 1999). Nevertheless, in the winter moth, VAN DONGEN et al. (1999a, 1999d) showed that fitness is not correlated with individual FA despite high values of *R*. Furthermore, individual asymmetry does not necessarily correlate with all fitness components (e.g. UENO, 1994; VAN DONGEN et al., 1999d).

As yet there is no theoretical framework that predicts under which conditions FA is expected to provide a reliable estimate of individual or population fitness, '(genetic) quality' or 'health'. This seriously hampers the general use of FA (BJORKSTEN et al., 2000) and calls for more detailed experiments (VAN DONGEN & LENS, 2000b). Investigating the evolutionary potential requires information on both the heritabilities of FA and DI as well as their relationships with fitness. Therefore, we estimate h^2_{FA}, h^2_{DI}, and the relationship between DI and fitness simultaneously for the Indian meal moth (*Plodia interpunctella*). In addition, we study two fitness traits. We performed full-sib breeding experiment estimating the effect of genotypic and environmental (density and food quality) factors on fitness and asymmetry. In this way we determined the relative contributions of environment and genotype to variation in FA, which allows us to evaluate the applicability of FA as an estimate of individual and/or population level 'quality'. Statistical power is an important, yet mostly ignored, issue in studies of FA. We propose a simulation approach to determine maximal values of h^2_{FA} and h^2_{DI}, and maximal effects of environmental conditions on population level FA.

MATERIAL AND METHODS

Breeding experiment

Thirty virgin females were randomly paired with 30 virgin males for copulation. Females were allowed to lay eggs on a feeding medium that consisted of wheatflour (77%), yeast (15%) and glycerol (8%) (further called OPTIMAL). Small, recently hatched caterpillars were transferred to experimental jars containing 2g of food. This food was freshly prepared and not replaced during the development of the caterpillars. The food did not show any obvious signs of decay during the growth period of the caterpillars. Three types of food were used: OPTIMAL, WHEAT (wheatflour only), and POTATO (potato meal only). Caterpillars were placed at three different densities: 5, 10 and 20. Thus, progeny of each family were partitioned over nine jars corresponding to the different food quality - density combinations. These treatments could not be replicated within families because number of offspring per female was too low. Both WHEAT and POTATO diets are expected to cause nutritional stress (GAGE & COOK, 1994). Yeast forms an important protein source in the artificial diet. In addition, potato meal contains nearly zero protein and fat resources. The relatively high densities are expected to increase nutritional stress even further.

Emerging moths were stored at -80°C and were sexed and measured afterwards. For the offspring of 20 families we measured body length to the nearest 0.1 mm, and determined length of the tibia of the front legs twice to the nearest 0.033 mm as outlined in detail in VAN DONGEN et al. (1999a; 1999c). We carried out an initial screening of different traits (the tibias of all legs and the length of a wing vein), hoping to be able to analyse different traits as suggested by SWADDLE (1997). However, this was not

possible because the development of the different legs was interdependent (VAN DONGEN et al., 1999c) and wing vein length could not be measured accurately. Without a thorough understanding of the mechanisms of this correlated development, a multivariate analysis of the genetic background of FA and DI of different traits could be misleading. Survival was expressed as the proportion of caterpillars surviving to the adult stage in the different family-by-treatment combinations.

Body length (i.e. length from the top of the head to the tip of the abdomen) is used as an indirect measure of fecundity and mating success (PODOLER, 1974; GREENFIELD, 1982; GAGE & COOK, 1994). If individual asymmetry is related to fitness in the Indian meal moth, we predict that population level FA should be highest in the treatments that exhibit the lowest survival during development, while individual asymmetry and body length should be inversely correlated. If FA is size-dependent, a positive association is expected.

Statistical analysis

Fluctuating asymmetry

We separated real FA from measurement error (ME) by mixed regression (VAN DONGEN et al., 1999b). The distribution of the signed asymmetry (left minus right trait value averaged over the two within-subject repeats) was tested for normality (using the Shapiro-Wilks' statistic, NETER et al., 1990) and for kurtosis in particular (following SOKAL & ROHLF, 1995). A positive kurtosis is expected when individuals differ in their underlying DI since the signed asymmetry is a mixture of different normal distributions with zero means and different variances (VAN DONGEN, 1998A; 1998B; GANGESTAD & THORNHILL, 1999). The hypothetical repeatability of individual asymmetry was obtained following VAN DONGEN (1998a). Data from all treatments and from both sexes were pooled, because this reveals the maximal degree of variation in DI. Individual asymmetry was estimated as the unsigned asymmetry. These values were used for subsequent analyses.

Genetic and environmental effects

Survival probability, body length, tibia length, and individual tibia asymmetry were analyzed by mixed model ANOVA. Each individual observation was entered in the analysis. Food quality, density and sex were treated as fixed effects, whereas family and its interaction with food quality and density were treated as random effects. The fixed-effects model the overall differences averaged across all families. Variance components of the random effects estimate genetic variance and genotype×environment interactions (where different genotypes respond to the environment in different ways, LYNCH & WALSH, 1997) respectively. The three-way random-effects interaction (i.e. family×food-quality×density) was added to the model as well. This variance component is confounded with common environment effects, because the individual food-by-density combinations were not replicated within families. However, by adding it to the model, the other variance components become unconfounded because possible common environment effects are explicitly modeled (unpublished simulations by S. Van Dongen). Significance of variance components was tested by likelihood ratio test and fixed effects were tested by traditional F-tests, adjusting the denominator degrees of freedom by Satterthwaite's procedure (details in VERBEKE & MOLENBERGHS, 1997). Heritabilities were calculated as twice the variance between families divided by the total variance (FALCONER & MACKAY, 1996). All analyses were performed in SAS (version 6.12). Body length, tibia length and individual unsigned asymmetry (the latter after logarithmic transformation) were nearly normally distributed (Shapiro Wilks' $W>0.95$) and were analyzed with PROC MIXED in SAS, assuming normality of the error terms. Survival probability was analyzed with the macro GLIMMIX, using logit link function and binomial error structure (e.g. CRAWLEY, 1993). This macro uses a quasi-likelihood approach (WEDDERBURN, 1974), can be obtained from the SAS server (http://www.sas.com/) and is described in LITTELL et al. (1996).

Power considerations

Because individual asymmetry is only weakly correlated with the underlying DI, expected heritabilities are small (WHITLOCK, 1996; HOULE, 1997; GANGESTAD & THORNHILL, 1999). Therefore, statistical power will be low and needs to be considered when negative results are found. In particular, we determined the maximal value of h^2_{DI} that could have led to the observed heritability. This can be done by generating data under a variety of alternative hypotheses (i.e. $h^2_{DI}>0$) and testing if this positive heritability is significantly larger than the observed value. We simulated data with a hypothetical repeatability and degree of FA equal to the observed values under a range of values for h^2_{DI} and determined the probability of obtaining the observed result just by chance (see below for details). If this probability was smaller than 5%, h^2_{DI} was considered significantly larger than the observed heritability. The level of h^2_{DI} for which this probability equals 5% is the maximal value. All simulations were performed in SAS (version 6.12) and the probabilities were determined from samples of 10,000 simulations.

Population level FA is expressed as a variance (or the average of the individual unsigned asymmetry values, which is equivalent, Whitlock, 1996). The estimation of a variance is subjected to large sampling variation, and the statistical comparison of different variances has low statistical power (PALMER, 1996; VAN DONGEN, 1999a). Therefore, we performed simulations in SAS to determine the maximal difference in population level FA between the different treatments (see results section for details).

RESULTS

Fluctuating asymmetry

Mixed regression analysis showed that 96% of the variation in tibia length reflected between-individual variation (σ^2=0.145). Real asymmetry explained 3% of the total variation (σ^2=0.004) and was significantly different from zero (χ^2_1=243, p<0.0001). The remaining 1% of variation (σ^2=0.002) reflected measurement error. There was no indication of directional asymmetry ($F_{1,431}$=0.34, p=0.6). The signed FA was nearly normally distributed (W=0.98), but had a small, yet statistically significant positive kurtosis (kurtosis=1.6, SE=0.23, t_{430}=6.8, p<0.001), indicating significant between-individual heterogeneity in the presumed underlying DI. The hypothetical repeatability of individual single trait asymmetry equaled 0.2.

Genetic and environmental effects

Survival probability decreased significantly with increasing densities and decreasing food quality, whereas body length decreased significantly with food quality only (Table 1, Fig. 1). In both cases, there was no interaction between the effects of food quality and density. Females were significantly larger than males. Individual unsigned asymmetry (after log transformation) did not differ between the different treatments or between males and females (Table 1, Fig.1).

TABLE 1

Overview of tests of fixed and random effects of the mixed ANOVA models for survival, body length, tibia length and log-transformed tibia unsigned asymmetry. (*: p<0.05; **: p<0.01; ***: p<0.001; significant effects are indicated in bold)

	Survival	Body length	Tibia length	Tibia unsigned FA
Source				
FIXED FACTORS				
Density	$\mathbf{F_{2,171}=8.3}^{**}$	$F_{2,30}$=1.8	$F_{2,189}$=0.09	$F_{2,424}$=0.5
Food	$\mathbf{F_{2,38}=20.8}^{***}$	$\mathbf{F_{2,19}=9.5}^{**}$	$\mathbf{F_{2,191}=43.4}^{***}$	$F_{2,424}$=0.1
Density×food	$F_{4,1103}$=1.2	$F_{4,350}$=0.8	$F_{4,191}$=2.87	$F_{4,318}$=0.3
Sex	-	$\mathbf{F_{1,558}=39.5}^{***}$	$\mathbf{F_{1,190}=150.9}^{***}$	$F_{1,323}$=0.7
Sex×density	-	$F_{2,546}$=0.5	$F_{2,184}$=1.42	$F_{2,340}$=0.1
Sex×food	-	$F_{2.525}$=0.4	$F_{2,184}$=0.04	$F_{1,169}$=0.3
RANDOM EFFECTS				
Family	$\mathbf{\sigma^2=0.49}^{**}$	$\mathbf{\sigma^2=0.04}^{**}$	$\mathbf{\sigma^2=0.009}^{**}$	σ^2=-0.005
Family×density	σ^2=0.04	σ^2=0.03	σ^2=0.002	σ^2=-0.003
Family×food	σ^2=0.03	σ^2=0	σ^2=0.000	σ^2=-0.008
Family×density×food	$\mathbf{\sigma^2=0.68}^{**}$	$\mathbf{\sigma^2=0.05}^{**}$	σ^2=0.000	σ^2=-0.0001
Residual	σ^2=0.87	σ^2=0.22	σ^2=0.047	σ^2=0.27

Survival probability, body length and tibia length varied significantly among the different families but there were no significant genotype×environment interactions. Broad sense heritabilities equaled 0.48, 0.24 and 0.32 respectively. There appeared to be family×food-quality×density three-way interactions (Table 1), which are, however, likely to reflect common environment effects.

Individual unsigned asymmetry appeared to have no genetic component and there was no indication of genotype×environment interactions. All variance components were slightly smaller than zero, which may be the result of sampling variation (LYNCH & WALSH, 1997) but could also stem from negative within-family correlations (NELDER, 1954; THOMPSON, 1962; COCKERHAM, 1973; FALCONER & MACKAY, 1996). The broad sense heritability of FA equaled -0.036. Since this heritability is negative, a transformation applying the hypothetical repeatability is meaningless.

Individual unsigned asymmetry was weakly, but significantly positively correlated with individual body length (r_s=0.17, N=430, p=0.0002). Therefore, patterns in asymmetry to some extent might reflect patterns in body length. After removing the effect body length (a measure of individual quality, see above) by dividing individual asymmetry by body length, tibia length was no longer correlated with individual asymmetry (r_s=-0.01, N=430, p=0.8). Following Leung (1998) (i.e. rule 9) we divided individual asymmetry by tibia length to obtain a size-corrected asymmetry estimate. Re-analyzing this new asymmetry-parameter did not change the overall results.

Power considerations

Heritability of FA and DI

In order to generate data under a range of positive values of h^2_{DI}, we sampled data for 20 families with sample

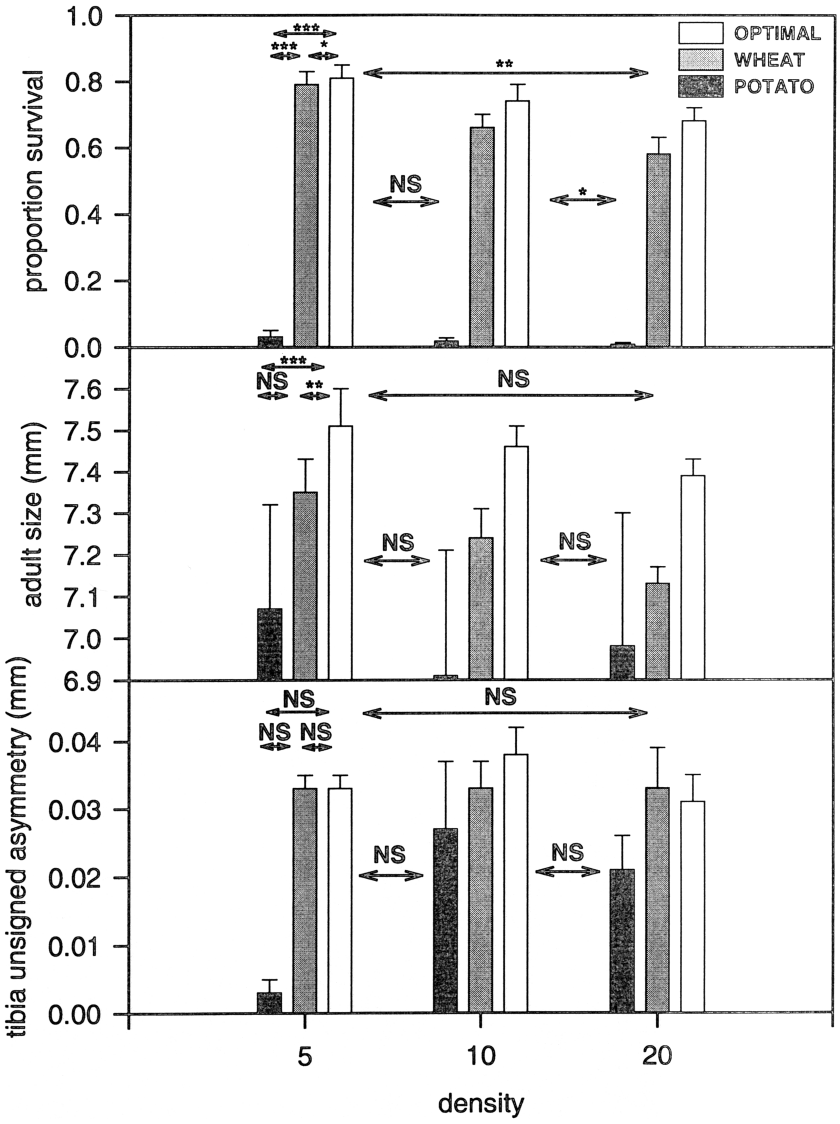

Fig. 1. – Survival probability, mean body length and mean individual unsigned asymmetry for different food qualities and densities (error bars indicate the standard error of the mean). Statistical significance of the different effects is indicated above the arrows (NS: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$). Arrows between the different food qualities indicate effects averaged over the three densities since there was no food×density interaction (Table 1).

sizes, levels of DI, and heterogeneity in DI (i.e. hypothetical repeatability) equal to the observed values. Between-individual heterogeneity in DI was partitioned within and between families at different proportions, reflecting different heritabilities. For each value of h^2_{DI}, 10000 samples were generated, and the proportion of samples where the h^2_{FA}£-0.036 (i.e. observed h^2_{FA}) was used as the significance level, testing if the simulated h^2_{DI} was larger than the observed heritability just by chance alone. *P*-values were smaller than 0.05 for all $h^2_{DI}\geq 0$ (Fig. 2), indicating that h^2_{DI} is significantly negative. Obviously, a negative heritability does not indicate negative additive genetic variance. By definition, variances cannot be negative, however, their estimates can. It is usually assumed that negative variance components are the result of sampling variance (Lynch & Walsh, 1997), but negative within-family correlations may result in negative estimates as well (Nelder, 1954; Thompson, 1962; Cockerham, 1973; Falconer & Mackay, 1996). The significantly negative between-family variance in individual asymmetry found here suggests that asymmetry values are negatively correlated within families and not the result of statistical noise (i.e. random sampling variation).

Environmental effects on DI

Food quality had a strong effect on survival probability (Table 1, Fig. 1). If any, the strongest effect of environmental stress on FA would be expected at this level. However, only 14 moths survived in the POTATO treat-

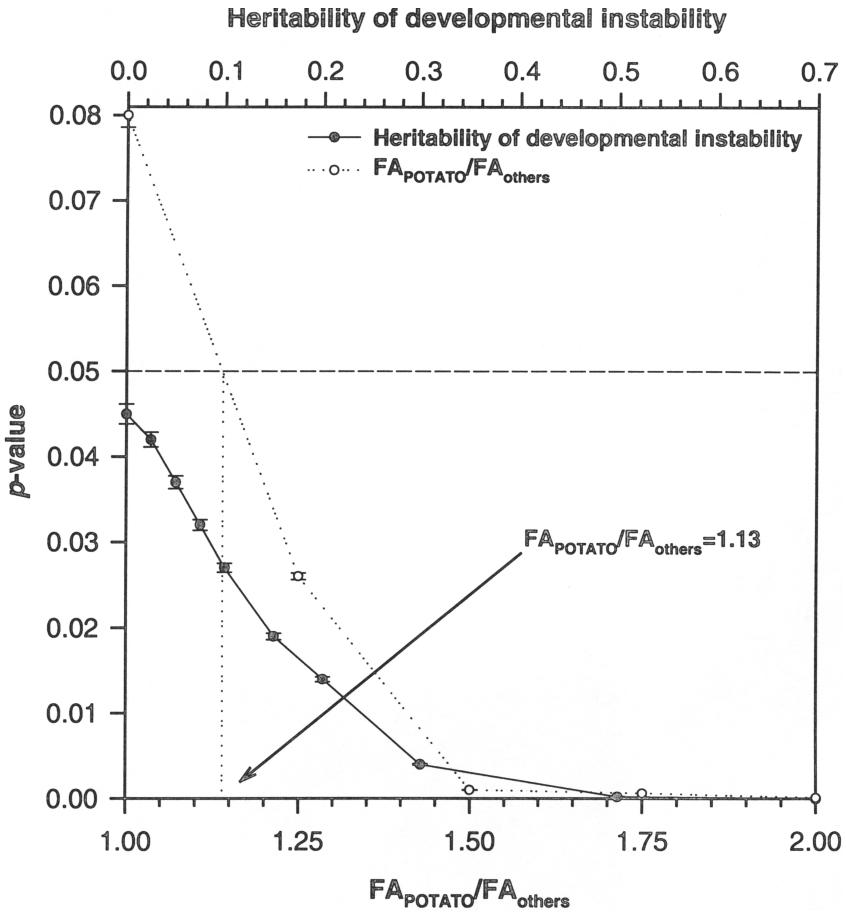

Fig. 2. – *P*-values of different alternative hypotheses testing for different values of h^2_{DI} (solid line and filled symbols) and for different ratios of population level unsigned asymmetry differences (i.e. moths reared on POTATO vs. all others) (dotted line and open symbols). Simulations of size 10,000 were performed to generate the empirical distributions (i.e. $h^2_{DI}>0$ and increased developmental instability on the food source with the lowest survival probability: $FA_{POTATO}/FA_{others}>1$). 95% CIs of the *p*-values were obtained following CRAWLEY (1993). The ratio corresponding to a *p*-value of 0.05 is indicated.

ment and statistical power to detect an effect on FA is small. Average individual unsigned asymmetry equaled 0.020 (N=14) for moths reared on POTATO compared to 0.033 (N=416) for all others. Thus, the difference in FA, although not statistically significant, is opposite to the expectations. We generated data for different degrees of heterogeneity in FA where FA for the POTATO reared moths was higher compared to the others ($FA_{POTATO}/FA_{others}\geq1$, i.e. heterogeneity in FA in the expected direction). For each level of heterogeneity we generated 10,000 samples of two populations with sizes 14 and 416 and used the proportion of samples where FA_{POTATO}/FA_{others} was smaller than 0.61 (i.e. 0.02/0.033) as p-value. In each simulation we incorporated a hypothetical repeatability of 0.2 (see above) because it has been shown to influence statistical power (VAN DONGEN, 1999b). This analysis indicates that FA_{POTATO} is maximally 13% larger than FA_{others} (Fig. 2) since larger differences would make the observed outcome very unlikely to have occurred by chance. Heterogeneity is expected to be even lower for the other comparisons since their effects on survival and body length were much weaker and statistical power much higher.

DISCUSSION

In this study we show that individual asymmetry of the front tibia does not provide a reliable estimate of individual quality in the Indian meal moth. In spite of significant between individual heterogeneity in DI (i.e. positive hypothetical repeatability), the heritability of DI was negative. This cannot be attributed to the fact that we worked with a culture reared in the laboratory since all other traits, including tibia length showed a significant broad sense heritable component. In addition, tibia asymmetry did not co-vary with fitness as manipulated by different nutritional qualities. Power analyses showed that possible undetected effects were likely to be very small. Because DI is not heritable in this population, we can rule out the hypothesis that under the presumed adverse food and den-

sity conditions only 'genetically superior' individuals with low DI would survive obscuring effects of stress. We therefore conclude that, in contrast to body length and survival probability, there is no evolutionary potential of tibia DI in this population of the Indian meal moth.

Heritabilities from full-sib breeding experiments are confounded with non-additive effects. They should be considered as upper bounds of narrow sense heritability only, and therefore cannot explain the negative heritabilities of FA and DI. Dominance effects may play an important role in both DI (LEAMY et al., 1997; 1998) and fitness traits (e.g. GARCÍA *et al.*, 1994) and may inflate broad sense heritabilities by over 30% (CRNOKRAK & ROFF, 1995). The main advantage of performing a full sib breeding design, especially considering the small heritabilities typically found for FA, is its increased power. Yet, maternal effects may obscure patterns of interest. Thus some caution is needed in interpreting the obtained results.

Our analyses show that tibia FA is not influenced by genetic or nutritional factors. Nevertheless, the hypothetical repeatability was significantly larger than zero. Hence, DI varied between individuals independent of genotype and food quality. In crowded situations, larvae of the Indian meal moth interact socially, emit secretions from their mandibular glands, and may show aggressive interactions and even become cannibalistic (GAGE, 1995 ; ANDERSON & LÖFQVIST, 1996). If in any of these interactions some individuals become dominant over others and if these interactions influence DI, it may be the source of the between-individual variation in DI observed. Possibly, small random initial differences in developmental rate may induce dominance of some individuals over others and in that way may lead to variation in DI at the individual level. This could also explain why h^2_{FA} was negative. If in each or many experimental treatments dominance of some individuals over others occurs and induces variation in DI, within-treatment (and also within-family) variation in individual asymmetry is increased, whereas the between family component remains unchanged or may even decrease. This would result in a negative correlation between asymmetries of individuals within jars and families and thus a negative value of h^2_{FA} (NELDER, 1954; THOMPSON, 1962; FALCONER & MACKAY, 1996) as observed in this study. We therefore could conclude that levels in DI in the front tibia of Indian meal moths may appear to be determined by social interactions irrespective of genotype and nutritional conditions.

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SHORT NOTES

The karyotype of the Formentera island garden dormouse, *Eliomys quercinus ophiusae*

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KEY WORDS: Eliomys quercinus, Karyotype, Biogeography, Balearic Islands.

The present terrestrial mammal fauna of the Balearic archipelago is the result of human introductions having occurred merely during the Holocene (1,2). Along with the wood mouse (*Apodemus sylvaticus*), the garden dormouse, *E. quercinus,* is the most ancient immigrant mammal on the archipelago. Its presence has been reported on all the four main islands but it is now extinct in Ibiza (2). Subfossil remains date back at least to 6000 y. BP in Mallorca and Menorca (2,3). Commercial exchanges are known to have occurred since that time between the human populations of the archipelago and those living around the Mediterranean Sea, including African coasts (4). Therefore, the geographic origin of the populations inhabiting the various islands may be different as well as their genetic characteristics. This assumption is reinforced by the fact that the endoparasitic spectrum of the garden dormouse differs from one island to another (5).

Morphology also differs; two distinct subspecies have been described from the area:

E. q. gymnesicus Thomas, 1903 from Menorca is included in the "*quercinus* group", the ventral part of its tail being entirely white. Though considered similar to the central European form, its subspecific status was retained in view of the geographic isolation of the island population (6,7). The form of Mallorca has been included in that subspecies (8,9). However, more recently, it has been considered as a transitional form between the "*lusitanicus*" and the "*quercinus*" groups, most of the specimens having a typical black ring at the end of the tail.

E. q. ophiusae Thomas, 1925 is the biggest form of the garden dormouse known in the world. Undoubtedly, it belongs to the "*lusitanicus*" group. Its geographic distribution is restricted to the island of Formentera .

However, there is no correspondence between these morphotypes and the five karyotypes described in the garden dormouse (genus *Eliomys*) either in Europe (*E. quercinus*: 2n = 48, 50, 52 or 54,), northern Africa (*E. melanurus*: 2n = 46) or Israel (*E. melanurus*: 2n = 48) (10, 11, 12, 13,14).

As far as the Balearic islands are concerned, a diploid number of 2n = 48 has been reported (15 and not 16, as stated in 12). Unfortunately, no details are given about the precise location (island) of the capture site and the source of information is not mentioned.

Therefore, we here report our analyses of the karyotype of the garden dormouse from Formentera, endeavouring to throw some light on the origin of the settlement of the species in this area.

Two specimens from Formentera were cytologically analysed. Microscope slides for observation of the chromosomes in somatic metaphases were prepared by direct treatment of the bone marrow (17). G-banding was obtained by the technique of Seabright (18). The diploid number (2n) and chromosome morphological characteristics were analysed using a Leica Q500 image analyser and Leica Chantal and Qwin software.

The karyotype of both specimens has a diploid number of 2n = 48 (fig. 1). It is composed of four pairs of large sized subtelocentric chromosomes, eight pairs of meta and ten pairs of submetacentric chromosomes, and one pair of acrocentric chromosomes. The X chromosome is large metacentric and Y is punctiform. The G-band pattern of the largest autosome pair shows complete band correspondence between karyotypes from Spain (15,16), mainland Italy (10,12) and Dalmatia (19) and is well differentiated from the pattern observed in the Israeli dormice (11).

Since the karyotype of North African garden dormice is 2n = 46 (12, 20, 21) while the 2n = 48 karyotype has only been reported in southern Europe (either in the Iberian

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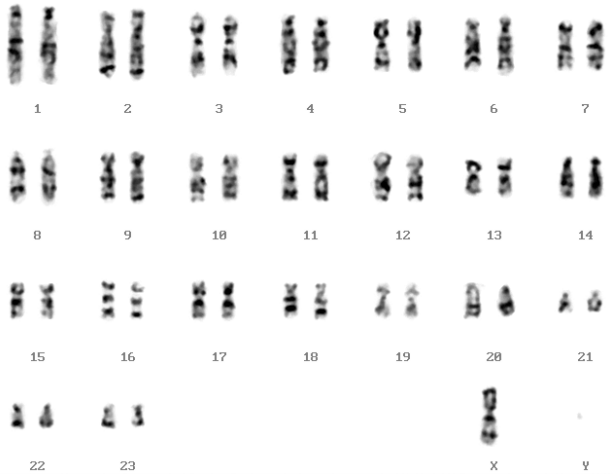

Fig. 1. – Karyogramme of a male garden dormouse (*Eliomys quercinus*) from the Formentera island (Balearic Islands, Spain). The chromosomes are numbered according to their homology with those presented in ref. 15. Sex chromosomes are in the bottom right hand corner.

Peninsula (16, 22), Italy (10,11), Dalmatia (23), Romania (24) or in some islands (Sicily, 10; Lipari, 25; Corsica, 26)) it can be assumed that the origin of the Formentera dormouse is southern Europe. Furthermore, the presence of only one acrocentric pair indicates a correspondence with the Iberian karyotypes (15, 16, 22) since the central Mediterranean *Eliomys* karyotypes are characterised by three pairs of acrocentric autosomes (12, 19). Therefore, it seems that the Garden dormouse of Formentera has been introduced there from mainland Spain, as was also the wood mouse to Ibiza, Menorca and Mallorca (27).

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Phylogenetic nomenclature: the end of binominal nomenclature?

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In phylogenetic nomenclature the name of a clade is defined ostensively by specifying an ancestor and all of its descendants in a hypothesised phylogeny. This new system of nomenclature was proposed and elaborated in a seminal series of papers by DE QUEIROZ & GAUTHIER (1, 2, 3), and has received a positive reception from many systematists (references in 4), though a few authors have rejected it (5, 6). The rules for this new system are now worked out in a new code, the PhyloCode, still in preparation. The draft version can be found on http ://www.ohio.edu/phylocode. However, no consensus has yet been reached on the format for species names. The draft version of the code only governs the rules for naming supraspecific taxa (i.e. clade names), and not species names as yet.

CANTINO et al. (4) proposed and discussed 13 different ways for naming species, all fundamentally different from the Linnean binomen. The drawbacks of the Linnean binomina have been thoroughly discussed (references above, 7, 8). Even though CANTINO and his co-authors (4) did not reach agreement on the form of a species name, all seven authors of this paper agreed that the old Linnean binomen should not be used in phylogenetic nomenclature. A "converted name" should replace the "old" binominal Linnean species name.

CANTINO et al. (4) divided the 13 methods into two major groups: (1) the converted name to be based only on the species epithet of the Linnean binomen. These epithet-based methods seem to be favoured by the majority of the people directly involved in the making of the PhyloCode (see 4, p.804); (2) the converted name to be based on the entire Linnean binomen (distinguishable or not from the original). A thorough discussion on the advantages and disadvantages of the various methods has been made (4), including some of the issues considered further on. The authors of that paper thus deserve full credit for the original ideas. All I want to do here is take a firm position in favour of the binomial-based (or binomen-based) conversion and make a choice from the methods proposed.

One of the most important properties of a name is its uniqueness (for the sake of simplicity I use the terms unambiguous and unique interchangeably, as uniqueness is the easiest way to make a name unambiguous). Non-unique names can lead to confusion and ambiguity, and are therefore less useful in scientific communication. If a code of biological nomenclature is to provide a universal language to speak unambiguously about organisms, it should avoid non-unique names. Universal uniqueness should be inherent to the name of a species so that just by mentioning the name and only the name, one can speak about one and only one species of all the living organisms. Such universal uniqueness would be inherent to binomial-based converted names, since the pre-existing codes have ensured the uniqueness of the correct Linnean binomina, e.g. by the rules of priority in cases of homonymy. There may be some overlap between the different existing codes as to genus names (e.g. animals and plants can have the same genus names), but organisms with exactly the same Linnean binomen are rare. In contrast, epithet-based converted names without a numeric portion (see further) would lead to numerous cases of ambiguity. A quick look through lists of the Belgian flora and fauna reveals that *arvensis* is a specific epithet for at least 24 plants and two animals (a bird and a beetle). This would mean that already in the Belgian flora and fauna alone, at least 26 species would be called *Arvensis*. To keep these epithet-based names unique, it was proposed that a unique number be added to the name (e.g. *Arvensis1, Arvensis2*, etc.). Although in theory a good solution, in practice the use of such names would be very difficult, if only because numbers are much harder to remember than are names. Only within a small field of specialists could numbers be practical. For someone working on Lamiaceae phylogeny, it would not be too difficult to remember that e.g. *Arvensis26* is the old *Stachys arvensis, Arvensis33* is the old *Acinos arvensis* and *Arvensis69* is the old *Mentha arvensis*, and thus know to which species the name refers. However, for an ecologist or a biology student, or an amateur biologist for that matter, lists of names would become an entangling collection of numbers. To diminish this problem, the addition of a "taxonomic address" was proposed, and in cases such as the *Arvensis* example, *Stachys* (for example) could be added to the numbered name as a "taxonomic address". However, is it not much simpler to choose a method that precludes ambiguity, such as a binomial-based conversion method?

One of the advantages of epithet-based names is that they would automatically be stable after conversion. The

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name would never change, which would be a boon to people working with or interested in nature, but not specifically in taxonomy or phylogeny. However, this stability can also easily be achieved by using binomial-based names. In fact, most of the binomial-based methods proposed in (4) result in stable converted names (as illustrated in 4, Table 3, column three). Even though a binominal name would be based on the entire Linnean binomen, this does not mean that it would have the same properties as a Linnean binomen (7). In Linnean binomina, the first name is the genus name, expressing relationships with other species having the same genus name (and thus variable with our progressing views on relationships). In binomial-based converted names, the first name should not imply any relationship. It would not be a genus name, it would just be a forename (or praenomen) (8), and therefore it would not need to change with changing hypotheses of relationships. Opponents of binomial-based methods fear that people would not be able to distinguish between the "old" Linnean binomen and the "new" binomial-based name, and would still think of the praenomen as referring to some kind of relationship (9). Solutions can be found to that problem. First: the binomial-based name should be clearly different in form from the Linnean binomen. The Linnean way of writing a name is so well established after 250 years, that any change would immediately be noticed. Dropping the capital and replacing the space between genus name and species epithet by a hyphen or dot (e.g. *stachys.arvensis* or *stachys-arvensis*) would immediately make it clear to everyone that the new name is not a Linnean binomen. It would also be possible to drop the capital **and** the space (*stachysarvensis*) but this could lead to almost unpronounceably long names. Second: the use of praenomina as clade names could be prohibited (as in method C in 4). In this case, if there is a species called *stachys-arvensis*, there should be no clade with the name *Stachys*. This, however, would lead to the replacement of all the old genus names, and thus the abandonment of many names we are familiar with. For practising taxonomists this might be an argument to reject phylogenetic nomenclature completely, and from this viewpoint it seems better not to adopt this convention. But even then, by introducing a few conventions, binomial-based names and epithet-based names could have the same properties after conversion: stability and ease of recognition from the Linnean binomen from which they were derived. Binomial-based names would then be equivalent to uninomina in practice. The converted name would not be a binomen in the Linnean sense, but just a hyphenated uninomen (7).

An obvious advantage of the binomial-based conversion system is that it would change the "old" names only in the way of writing them, hence ensuring continuity with the presently existing names. This would certainly also ease the acceptance of the new code by the scientific community not involved in systematics, and by amateur biologists. On the other hand, as long as the present codes of nomenclature remain valid (and the different codes will co-exist for a good while), species names governed by the present codes can be altered because of generic changes (example in 4, p. 804). The stable binomial-based name then would become different from the Linnean binomen, and this is seen as a disadvantage by those in favour of epithet-based names. This divergence is of minor importance however, since the binomial-based name would retain the old epithet. Moreover, if the generic realignment also included a change of the epithet (because of secondary homonymy), the binomial-based name would differ completely from the Linnean binomen, but also the epithet-based converted uninomen would differ from the new epithet of the Linnean binomen. However, because of the common practice of indicating the older synonyms when a new species name is proposed, the once converted name could easily be located.

From all the above, it should be clear that converted names would be best as binomial-based. Such a conversion method would ensure uniqueness of the name, cause minimal disruption with the old nomenclature, and enhance the acceptance of the new method by the scientific community. To reduce the risk that one might think of the praenomen as implying relationships, the converted species name should be clearly distinguishable from the Linnean binomen, but remain easy to read and pronounce. For the sake of simplicity, all names in a nomenclature system should have the same form, converted names as well as new names. Of the methods proposed by CANTINO et al. (4), only two meet the requirements discussed above, namely methods B & C (and F to a lesser extent). The methods differ in that praenomina can (method B) or cannot (method C) be used as clade names. Method C is preferable from a theoretical point of view since it reduces the risk of misunderstanding the praenomen as implying relationships. However, method B is preferable from a practical point of view, as many familiar names can be retained. A very common concern of practising taxonomists about phylogenetic taxonomy is that it would replace many old names by new ones, at the moment based on a misconception (10). However, adoption of method C would legitimise this concern.

To conclude: for these reasons, the best conversion method to adopt in the future is method B of CANTINO et al. (4), although with a slight adaptation. I would suggest conversion of the name by dropping the capital (even when starting a sentence) **and** adding a dot or hyphen (*stachys-arvensis*), instead of choosing between one of both as was suggested (*stachys arvensis* or *Stachys-arvensis*). This would serve to make the distinction between the converted name and the Linnean binomen more obvious.

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