

Morphology and structure of the scales in the Gadiformes (Actinopterygii: Teleostei: Paracanthopterygii) and a comparison to the elasmoid scales of other Teleostei

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Abstract: Despite the commercial importance of the Gadiformes, many aspects of their classification are weakly established and disagreements exist on the phylogenetic relationships of various families. An attempt to determine the systematic significance of the scales is made in studying the morphology and the structure of the scales of 17 species belonging to nine families which were examined using light and electron microscopy. The scales are elasmoid and of the cycloid type in all families except in the Macrouridae in which they are of ctenoid or spinoid type. All these scales show the characteristics of typical Teleostei: they are made of acellular mineralized tissues, and the plywood-like structure of the basal plate is orthogonal or at least intermediate in *Melanonus zugmayeri* and *Raniceps raninus*. The reduction of the basal plate to only one ply in the scales of *Muraenolepis orangiensis* is concomitant with the reduced size of the scale. Collagen "TC" fibers crossing the plywood-like structure were only found in the basal plate of the scales of *Lota lota*, the only species completely confined to freshwater. A preliminary phylogenetic analysis suggests that *Lota lota* should be excluded from the Gadidae. The superficial ornamentations of the scales are thought to be related to the swimming. The morphological and structural characteristics of the gadiform scales could be considered at least as family-dependent.

Résumé: Morphologie et structure des écailles chez les Gadiformes (Actinopterygii : Teleostei : Paracanthopterygii); comparaison avec les écailles élasmoïdes d'autres Téléostéens. Malgré son importance, notamment du point de vue commercial, l'ordre des Gadiformes fait partie des Téléostéens dont la classification et les relations phylogénétiques sont toujours controversées. L'apport éventuel d'une étude morphologique et structurale des écailles à leur classification et aux relations phylétiques est envisagé. L'étude effectuée en microscopie photonique et électronique concerne les écailles de 17 espèces réparties dans neuf familles. Les écailles sont élasmoïdes et de type cycloïde sauf chez les Macrouridae où elles sont de type cténoïde ou spinoïde. Néanmoins, toutes les écailles examinées sont celles de Téléostéens typiques, elles sont constituées de tissus minéralisés acellulaires, le contreplaqué de la plaque basale est de type orthogonal sauf chez *Melanonus zugmayeri* et *Raniceps raninus* où il est de type intermédiaire. Chez *Muraenolepis orangiensis*, la réduction de la plaque basale à une seule strate est concomitante de la réduction de la taille des écailles. Des fibres de collagènes "TC" traversant les strates du contreplaqué n'ont été mises en évidence que dans la plaque basale de *Lota lota*, seule espèce de cet ordre inféodée à l'eau douce. Une analyse phylogénétique préliminaire suggère que l'espèce *Lota lota* devrait être retirée du taxon Gadidae. Les ornements superficiels des écailles qui présentent les aspects morphologiques les plus variés sont les parties les plus soumises aux contraintes mécaniques lors de la nage. Les particularités morphologiques et structurales des écailles des Gadiformes pourraient être des critères à prendre en compte pour l'établissement de relations phylétiques, au moins au niveau de la famille.

Keywords: Morphology, Structure, Elasmoid scales, Gadiformes, Ultrastructure, Mineralization

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Introduction

The Gadiformes are an important commercial group representing over one quarter of the world's marine fish catches. They are a large group which belongs to the superorder of Paracantopterygii. Surprisingly, the classification and the phylogeny of this important order are still debated. Indeed, systematists disagree about the number of families that should be recognized in this taxon; 12 families are distinguished by some (Cohen et al., 1990; Nelson, 1994; Kottelat, 1997), whereas others recognize only 10 families (Markle, 1989; Eschmeyer 1998). It is noteworthy that molecular studies have not led to a consensus on the phylogeny within this order, and they are entirely lacking for many families. Markle (1989) used morphological features to propose a phylogeny of the Gadiformes.

The scales of the Gadiformes were not often considered for establishing systematic relationships, despite their diversity. The scale morphology was used in only one family: the Macrouridae (Okamura, 1970; Iwamoto, 1989). The scales of species such as *Macrourus berglax*, which bear spines, were called spinoid scales and distinguished from regular ctenoid scales (Roberts, 1993). Besides these studies, few reports have been devoted to the morphology and the structure of scales of the Gadiformes (Iwamoto, 1989). Therefore, little data have been gathered from the literature (review in Meunier & Castanet, 1982; Zylberberg et al., 1992).

However, the significance of the scales in vertebrate classification has been recognized for a long time, first, at the level of higher taxa (Agassiz, 1834-1844; Goodrich, 1907; Kerr, 1952, 1955; Bertin, 1958; Schultze, 1977), and subsequently, at the family level, for some teleosts. Studies carried out on Engraulidae and Salmonidae (Kobayashi, 1952-1955), Platycephalidae (Hugues, 1981), Mormyridae (Meunier & Castanet, 1982), Osteoglossidae (Meunier, 1984a; Meunier & Castanet, 1982) and Cichlidae (Lippisch, 1995; Roberts, 1993) indicated that morphological features such as the surface ornamentations (Kobayashi, 1952-1955; Delamater et al., 1972; Delamater & Courtney, 1974; Lanzing & Higginbotham, 1974; Hugues, 1981; Bonwick et al., 1989; Roberts, 1993; Meunier, 1997), structural features of the basal plate (Waterman, 1970; Fouda, 1979; Onozato & Watabe, 1979; Olson & Watabe, 1980; Meunier & Castanet, 1982, Meunier, 1987-88) and different mineralization processes (Brown & Wellings, 1969; Lanzing & Wright, 1976; Schönborner et al., 1979, 1981; Yamada & Watabe, 1979; Zylberberg & Nicolas, 1982; Meunier, 1984b) might be useful for systematic analyses.

Thus, the aim of this study was to determine the morphology and the structure of the scales within the Gadiformes, to compare the data obtained with those of

other elasmoid scales of Teleostei, and to determine whether or not there is phylogenetic information in the characters derived from our observations. Morphological and structural studies have been carried out using light microscopy, transmission and scanning electron microscopy and microanalysis techniques on the scales of 17 species distributed in nine families of Gadiformes and in three non-gadiform species.

Material and methods

Material

The present study was carried out on scales removed from specimens kept in formalin in the collections of the Museum National d'Histoire Naturelle de Paris: MNHN (*). For some species, scales were removed from specimens commercially obtained and fixed in formalin (**), and, for few species, fresh scales were extirpated from living individuals (***)).

Salmoniformes: *Onchorhynchus mykiss**** (Walbaum, 1792). TL (Total Length): 250 mm

Beryciformes: *Hoplosthetus atlanticus**** (Colett, 1889); (IFREMER "Talus 99" April-May 1999). TL: 536 mm.

Ophidiiformes: *Ophidion barbatum* * Linnaeus, 1758; MNHN 1967-885. TL: 158 mm.

Gadiformes:

- Ranicipitidae: *Raniceps raninus* * (Linnaeus, 1758); MNHN 1974-25. TL: 245 mm

- Macrouridae: *Coryphaenoides rupestris** Gunner, 1765; (Thalassa Mission. P. Geistdoerfer, May 1975). TL: 570 mm

*Macrourus berglax** Lacepède, 1801; (Thalassa Mission P. Geistdoerfer, May 1975). TL: 470 mm

*Trachyrhynchus trachyrhynchus** (Risso, 1810); (Thalassa Mission P. Geistdoerfer, May 1975). Preanal length: 195 mm.

*Trachyrhynchus murayi**** Günther, 1887; (IFREMER "Talus 99" April-May 1999). Preanal length: 250 mm.

- Moridae: *Mora moro** (Risso, 1826); MNHN 1988-606. TL: 303 mm.

- Melanonidae: *Melanonus zugmayeri** Norman, 1930; MNHN 1987-1589. SL (Standard Length): 240 mm

- Bregmacerotidae: *Bregmaceros maclellandii* * Thompson, 1840; MNHN 3059. TL: 85 mm

- Muraenolepididae: *Muraenolepis marmoratus* * Günther, 1880; MNHN 1987-663. TL: 335 mm

Muraenolepis orangeiensis * Vaillant, 1888; MNHN 1985-449. TL: 150 mm

- Phycidae: *Gaidropsarus vulgaris**** (Cloquet, 1824); (Station Biologique, Roscoff). SL: 220 mm.

- Merlucciidae: *Merluccius merluccius*** (Linnaeus, 1758). TL: 500 mm.
- Gadidae: *Molva dypterygia** (Pennant, 1784). TL: 378 mm.

*Lota lota** (Linnaeus, 1758); MNHN 1982-103. TL: 250 mm.

*Merlangius merlangus**** (Linnaeus, 1758); (Station Biologique, Roscoff). TL: 370 mm.

*Pollachius pollachius**** (Linnaeus, 1758); (Station Biologique, Roscoff). TL: 400 mm.

*Trisopterus luscus**** (Linnaeus, 1758); (Station Biologique, Roscoff). TL: 240 mm.

The classification follows Nelson (1994) and Kottelat (1997), who included *Lota lota* within the Gadidae. Recently, *Lota lota* was no longer considered to belong to the sub-family Lotinae, but to constitute a separate family: the Lotidae (Eschmeyer, 1998).

The Macrouridae and the Gadidae are the two most specious families. Thus, more specimens were examined in these two families than in other families, which contain only few species or are monospecific, such as the Ranicipitidae.

Whenever possible, the scales were extirpated from the right flank of the specimen below the first dorsal fin, in order to avoid differences due to the location of the scales.

Methods

Some scales were stained in toto with 1% alizarin red solution, dehydrated, cleared in toluene and mounted on slides in a synthetic resin.

Other scales were dehydrated and embedded in a polyester resin. Transverse and longitudinal ground sections were obtained with a sawing machine (Isomet), giving sections 120-150 μm thick. These sections were ground to obtain 80 μm thick slices which were observed with transmitted natural and polarized light. The ground sections were also used in order to examine the degree of mineralization on microradiographs which were obtained using a high resolution X-ray film (Kodak SO 343) and an X-ray apparatus (CGR-Sigma 2060) operating at 30 KV with an intensity of 10 mA. The distance of the sample from the focus was 20 cm and the exposure time was 18 minutes.

For morphological studies of the surface ornamentations, some scales were cleaned with a 1% sodium hypochlorite solution, dried, and gold-coated. The scales were viewed with the Jeol-JSM-840 scanning electron microscope (SEM) at an operating voltage of 17 KV. To study the mineralizing front, scales were prepared with concentrated hypochlorite solution to destroy the unmineralized collagen fibers. Then, they were washed, dried and processed for SEM.

The spatial organisation of the collagenous network of the basal plate was processed on frozen scales which were broken, then dried and gold-coated for SEM.

For transmission electron microscopy (TEM), the fresh and the formalin-fixed scales were either fixed in a 0.1 M sodium cacodylate buffer containing 2% paraformaldehyde, 2.5% glutaraldehyde (pH 7.2) or fixed and demineralized with 0.1M EDTA added to the fixative which was changed every day during a week. Most samples were post-fixed in 1% osmium tetroxide in the cacodylate buffer. Few samples that were not post-fixed in osmium tetroxide were used for microanalysis of the mineral components. All specimens were dehydrated and embedded in Epon. Semi thin sections (1 μm thick) were stained with toluidine blue and observed with a light microscope in order to select areas for subsequent TEM examination. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined in a Philips TEM 201 transmission electron microscope at an operating voltage of 80 KV.

The distribution of several mineral components such as calcium, phosphorus, magnesium, fluorine, strontium and barium was studied using analytical ion microscopy. Sections of about 1 μm thick were obtained from specimens fixed with the aldehyde fixative only and embedded in Epon. They were collected on a gold support and analyzed with a Cameca SMI 300 with a O_2^+ primary bombardment and an intensity of the primaries of 7.5 μA . We have coded a matrix of 20 taxa and 10 characters

We have performed a search for the shortest trees of our data matrix (Table 1) using the heuristic algorithm of PAUP* 4 (Swofford, 1998) with the following settings : 20 random addition sequences, 7 trees held at each step, TBR swapping, maxtrees set at 10 000. The bootstrap analysis was done using the following settings: 100 replicates, heuristic search, simple addition sequence, maxtrees at 4000, TBR swapping.

Results

Morphology

The scales of all species examined in this study belong to the elasmoid type as defined by Bertin (1944). Indeed, they are thin, transparent, flexible and lamellar plates which are almost always imbricated. Thus, the posterior field, the exposed area of the scale lying below the epidermis differs from the anterior and lateral ones, covered by the adjacent scales. The anterior part is deeply inserted within the dermis. In *Muraenolepis orangeiensis*, the small scales are not imbricated, but lie side by side, forming a mosaic.

Each scale is located within a papilla, the scale pocket, which is surrounded by the scale pocket cells (Whitaker, 1986). The scales do not contain cells; the scale-forming cells or scleroblasts (Klaatsch, 1890) line the scale surface.

The gadiform scales are circular, ovoid, oblong or quadrangular, depending on the species. Their sizes also

Table 1. Matrix of the morphological and structural characters used in the phylogenetic analysis.

Tableau 1. Matrice des caractères morphologiques et structuraux utilisés dans l'analyse phylogénétique.

Taxa	Characters									
	1	2	3	4	5	6	7	8	9	10
<i>Oncorhynchus mykiss</i>	0	0	0	0	2	0	0	0	0	0
<i>Hoplostethus atlanticus</i>	0	0	0	0	0	0	0	0	0	0
<i>Ophidion barbatum</i>	0	2	1	0	2	1	0	0	0	1
<i>Trisopterus luscus</i>	0	2	1	0	2	0	0	0	0	0
<i>Raniceps raninus</i>	0	0	1	2	2	0	1	1	0	1
<i>Mora moro</i>	0	0	1	2	2	0	0	0	0	0
<i>Bregmaceros mccllellandii</i>	0	0	1	1	2	0	0	0	0	1
<i>Muraenolepis orangiensis</i>	1	2	1	0	2	0	n/a	1	1	1
<i>Merluccius merluccius</i>	0	0	1	0	2	0	0	0	0	1
<i>Melanonus zugmayeri</i>	0	0	1	0	2	0	1	1	0	1
<i>Trachyrhynchus trachyrhynchus</i>	0	0	0	0	1	0	0	0	0	0
<i>Trachyrhynchus murayi</i>	0	0	0	0	1	0	0	0	0	0
<i>Lota lota</i>	0	0	1	0	2	1	0	0	0	1
<i>Coryphaenoides rupestris</i>	0	0	0	0	0	0	0	0	0	1
<i>Macrourus berglax</i>	0	0	0	0	0	0	0	0	0	0
<i>Muraenolepis marmoratus</i>	1	2	1	0	2	0	0	0	0	1
<i>Gaidropsarus vulgaris</i>	0	1	1	2	2	0	0	0	0	1
<i>Molva dypterygia</i>	0	2	1	0	2	0	0	0	0	0
<i>Merlangius merlangus</i>	0	2	1	0	2	0	0	0	0	0
<i>Pollachus pollachius</i>	0	2	1	0	2	0	0	0	0	0

Oncorhynchus mykiss, *Hoplostethus atlanticus*, and *Ophidion barbatum* are outgroup taxa.

n/a: not applicable.

List of the morphological and structural characters used in the matrix

- Scale arrangement: imbricated (0), side by side (1)
- Localization of the radii: absent (0), two radii parallel to the antero-posterior axis (1), in the whole surface of the scale (2)
- Localization of the circuli: restricted to the anterior field (0), in the whole surface of the scale (1)
- Denticles on the crests of the circuli: denticles absent (0), restricted to the circuli of the anterior field (1), on the whole circuli of the scale (2)
- Number of the cteni: more than three cteni (0), reduced to three cteni (1), none (2)
- TC fibers in the basal plate: absent (0), present (1)
- Organization of the basal plate: orthogonal plywood (0), intermediated plywood (1)
- Mineralization of the basal plate: more than one ply (0), one ply (1)
- Development of the basal plate: more than one ply (0), single ply (1)
- Localization of the focus: subcentral (anterior or posterior) (0), central (1)

Liste des caractères morphologiques et structuraux pris en compte pour établir la matrice

- Disposition des écailles: imbriquées (0), non imbriquées (1)
- Disposition des radii: absents (0), deux radii parallèles à l'axe antéro-postérieur (1), présents sur toute la surface (2)
- Disposition des circuli: présents uniquement dans le champ antérieur (0), présents sur toute la surface de l'écaille (1)
- Denticules au sommet des circuli: pas de denticules (0), présents uniquement sur les circuli du champ antérieur (1), présents sur tous les circuli de l'écaille
- Nombre des cténi: plus de trois cténi (0), au nombre de trois (1), pas de cténi (2)
- Fibres TC dans la plaque basale: absentes (0), présentes (1)
- Organisation de la plaque basale: contreplaqué orthogonal (0), contreplaqué intermédiaire (1)
- Etendue de la minéralisation dans la plaque basale: plus d'une couche de contreplaqué (0), une seule couche (1)
- Développement de la plaque basale: plus d'une couche (0), une seule couche (1)
- Place du foyer: subcentral (antérieur ou postérieur) (0), central (1)

Figure 1. *Melanonus zugmayeri*. SEM. **a.** General view. **b.** Detail of the posterior field (insert of Fig. 1a) showing circuli with smooth crests.

Figure 2. *Raniceps raninus*. SEM. **a.** General view. **b.** Detail of the lateral field (insert of Fig. 2a) showing circuli with crests ornamented with denticles (arrows).

Figure 3. *Merlangius merlangus*. **a.** General view of a whole mounted scale stained with alizarin red. **b.** SEM. Detail of the central area of the scale showing the radii arising from the focus towards the margin. **c.** SEM. Detail of a radius with mineralized globules (arrows).

Figure 4. *Bregmaceros mccllellandii*. **a.** General view of a whole mounted scale stained with alizarin red. **b.** SEM. Detail of the central area showing the change in morphology of the crests of the circuli which bear denticles in the anterior field and become gradually smooth in the posterior one. **c.** SEM. Detail of the lateral area of the Fig. 4b showing unmineralized spaces between the adjacent circuli.

Figure 5. *Merluccius merluccius*. **a.** General view of a whole mounted scale stained with alizarin red. **b.** SEM. Detail of the posterior field with the ridges forming a complicated network. **c.** SEM. Detail of the network formed by the ridges which coexist with mineralized globules (arrow).

Figure 6. *Gaidropsarus vulgaris*. **a.** General view of a whole mounted scale stained with alizarin red showing two radii. **b.** SEM. Detail of the posterior field showing a radius (R) and the numerous denticles on the circuli.

Figure 7. *Lota lota*. General view of a whole mounted scale stained with alizarin red. Note the regular rings formed by the circuli.

Figure 1. *Melanonus zugmayeri*. MEB. **a.** Vue générale. **b.** Détail de la Fig. 1 montrant les circuli à crêtes lisses du champ postérieur.

Figure 2. *Raniceps raninus*. MEB. **a.** Vue générale. **b.** Détail de la Fig. 2a montrant des circuli avec des denticules dans les champs latéraux (flèches).

Figure 3. *Merlangius merlangus*. **a.** Vue générale d'une écaille colorée par le rouge d'alizarine. **b.** MEB. Détail de la partie centrale de l'écaille montrant des radii issus du foyer et orientés vers la périphérie. **c.** Détail d'un radius où se trouvent des globules minéralisés (flèches).

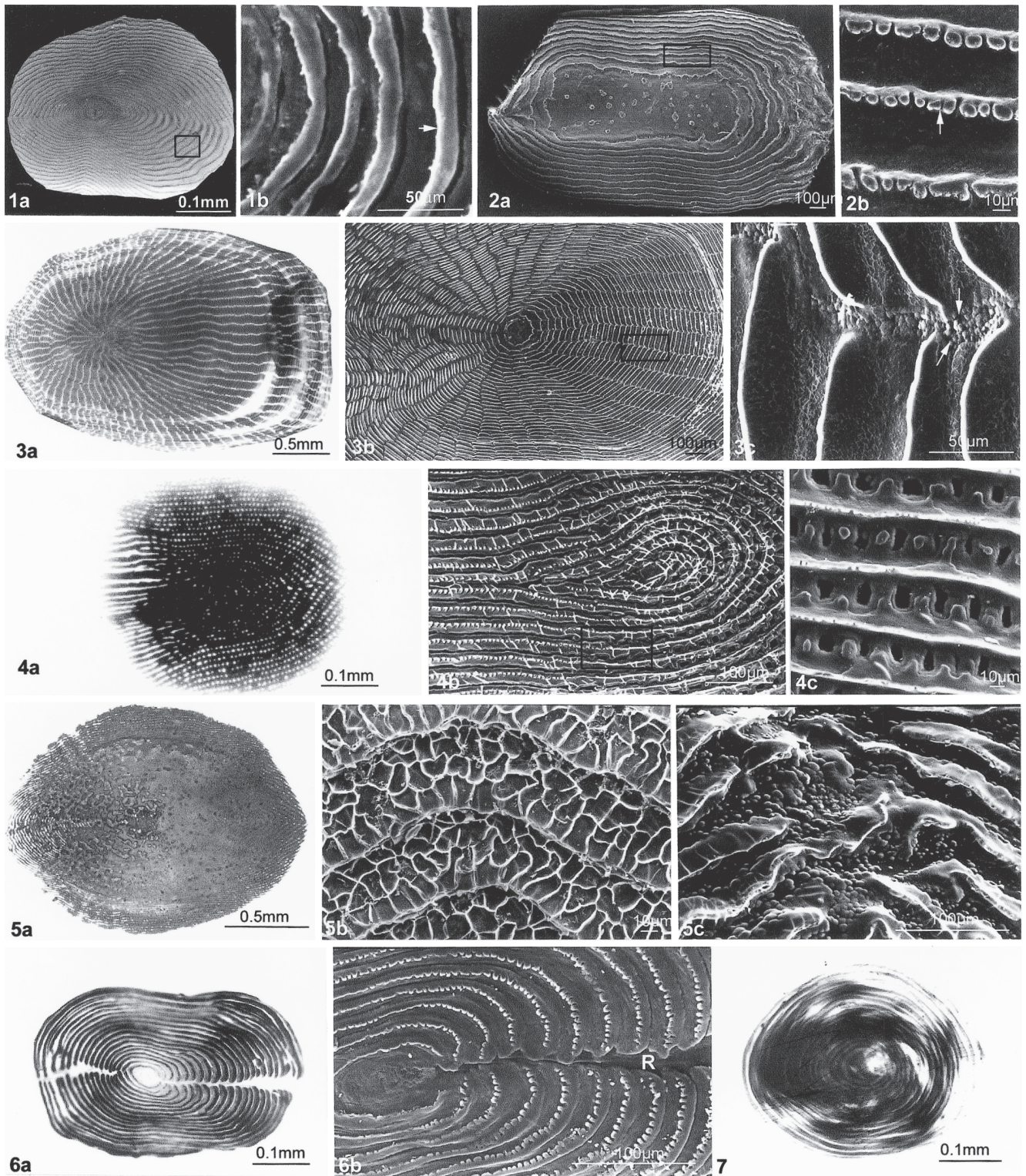
Figure 4. *Bregmaceros mccllellandii*. **a.** Vue générale d'une écaille colorée par le rouge d'alizarine. **b.** MEB. Détail de la partie centrale de l'écaille montrant les modifications d'aspect des crêtes pourvues de denticules dans le champ antérieur et devenant progressivement lisses dans le champ postérieur. **c.** MEB. Détail du champ latéral montrant des espaces non minéralisés entre les circuli adjacents.

Figure 5. *Merluccius merluccius*. **a.** Vue d'une écaille entière colorée par le rouge d'alizarine. **b.** MEB. Détail du champ postérieur où les replis forment un réseau complexe. **c.** MEB. Détail du réseau où se trouvent également des globules minéralisés (flèche).

Figure 6. *Gaidropsarus vulgaris*. **a.** Vue d'une écaille colorée par le rouge d'alizarine. **b.** MEB. Détail du champ postérieur montrant un radius (R) et les denticules présents sur les circuli.

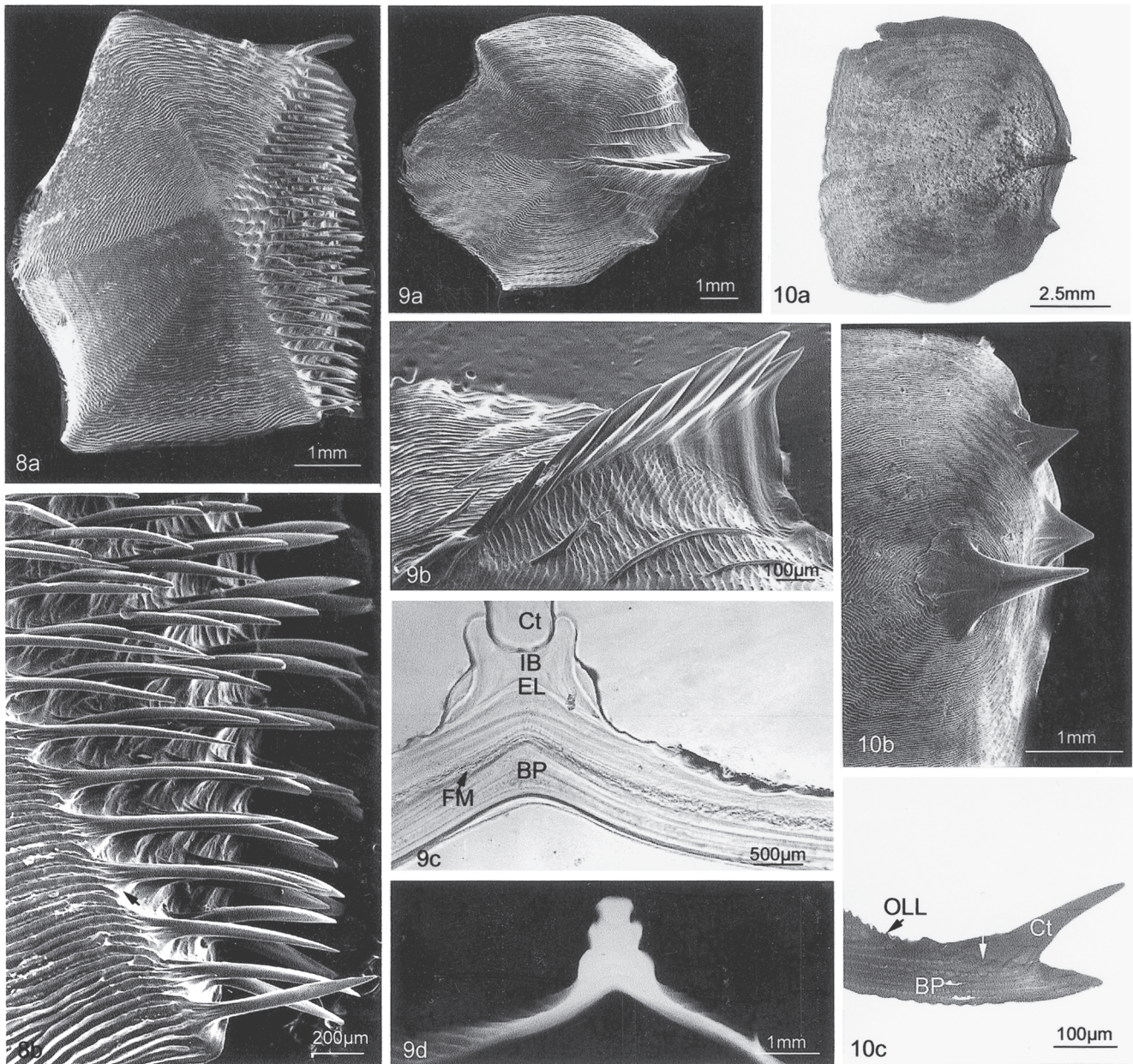
Figure 7. *Lota lota*. Vue d'une écaille colorée par le rouge d'alizarine. Noter la forme régulière des anneaux constitués par les circuli.

appeared to vary not only according to the families, but also according to the species within a family. The Macrouridae



have the largest scales; the antero-posterior axis of their scales reaches 1 cm. They are the only ones which develop ctenoid and spinoid scales. Cycloid scales are found in all other Gadiformes examined (Figs 1-10).

The ornamentation of the surface is mainly represented by the circuli, which are variably developed but always present. Circuli are concentric crests surrounding the focus of the scales, which was not always located in the central



area of the scale. It is found in the anterior part in the scales of *Merlangius merlangus* but in the posterior part in the scales of *Bregmaceros maclellandii*. The crests are regular, smooth and more widely spaced in the posterior field than in the anterior one in *Molva dypterygia*, *Melanonus zugmayeri*, *Merluccius merluccius*, *Trisopterus luscus*, *Pollachius pollachius*, *Muraenolepis marmoratus*, and *Muraenolepis orangiensis* (Figs 1a-b, 3b-c, 5b, 6a-b). The scales of *Lota lota* are the only ones which show regularly spaced circuli around the focus (Fig. 7). In *Mora moro*, *Raniceps raninus* and *Gaidropsarus vulgaris*, the apices of the crests that ornament the whole surface of the scale bear

denticles of about 2 to 3 μm long which are oriented towards the focus (Figs 2a-b, 4b, 6b).

The scales of *Bregmaceros maclellandii* show circular crests parallel to the scale margin in the lateral and posterior fields. The crests become approximately oriented along the antero-posterior axis in the anterior field, where they are perpendicular to the scale margin (Figs 4b-c). These circuli are separated by spaces perforated by unmineralized square areas (Figs 4a-c). Denticles are restricted to the crests of the circuli of the anterior field. Transitional stages are located in the lateral areas (Figs 4b-c).

Figure 8. *Coryphaenoides rupestris*. SEM. **a.** General view showing the regular distribution of the cteni on the posterior field. **b.** Detail of the posterior field showing the continuity between the lateral cteni and the circuli ornamenting the outer surface of the scale.

Figure 9. *Macrourus berglax*. **a.** SEM. General view showing the well-developed cteni in the centre of the posterior field compared to the small lateral ones. **b.** SEM. Note the development of the central cteni and the thinness of the lateral ones. **c.** Standard transmitted light. Transverse ground section showing the "intercalary bone" (*IB*) between the ctenus (*Ct*) and the external layer (*EL*). (*BP*) basal plate. **d.** X-ray microradiograph of a transverse ground section of a scale showing no difference between a homogenous mineralization in the external layer and the spine.

Figure 10. *Trachyrhynchus trachyrhynchus*. **a.** General view of a whole mounted scale stained with alizarin red. **b.** SEM. Detail of the posterior field showing the conical cteni located on the circuli of the outer surface of the scale. **c.** Semi-thin cross section of the scale showing that the spine (*Ct*) is set on the scale external layer (arrow). (*BP*) basal plate; (*OLL*) outer limiting layer.

Figure 8. *Coryphaenoides rupestris*. MEB. **a.** Vue générale montrant la répartition régulière des cténi sur le champ postérieur. **b.** Détail du champ postérieur montrant la continuité entre les cténi latéraux et les circuli.

Figure 9. *Macrourus berglax*. **a.** MEB. Vue générale du champ postérieur montrant des cténi beaucoup plus développés dans le centre que dans les parties latérales. **b.** MEB. Notez la différence de taille des cténi beaucoup plus grands au centre que dans les parties latérales. **c.** Eclairage standard. Lame mince montrant l'os intercalaire (*IB*) entre le cténus (*Ct*) et la couche externe (*EL*). (*BP*) plaque basale. **d.** Microradiographie d'une lame mince transversale d'une écaille. Aucune différence du degré de minéralisation n'est perceptible entre la couche externe et le cténus.

Figure 10. *Trachyrhynchus trachyrhynchus*. **a.** Vue générale d'une écaille colorée au rouge d'alizarine. **b.** MEB. Détail du champ postérieur montrant des cténi de forme conique, situés sur les circuli. **c.** Coupe semi-fine d'une coupe transversale montrant que le cténus (*Ct*) repose sur la couche externe (flèche). (*BP*) plaque basale ; (*OLL*) couche limitante externe.



Merluccius merluccius has scales ornamented with a network of branched ridges (Fig. 5b). Mineral globules of 2.5 to 5 μm in diameter are present in the vicinity of the ridges (Fig. 5c). The circuli are interrupted by grooves, the radii which arise from the focus and reach the scale margin. The radii are distributed on the whole scale surface in *Trisopterus luscus*, *Merlangius merlangus*, *Muraenolepis marmoratus* and *Pollachius pollachius* (Fig. 3). The radii are crossed by circular grooves in the scales of *M. merlangus* (Fig. 3). In *Gaidropsarus vulgaris*, the radii consist of only two grooves parallel to the antero-posterior axis of the scale (Fig. 6).

The ctenoid and spinoid scales of the Macrouridae have numerous concentric circuli with slightly crenated crests (Figs 8a, 9a, 10a), but radii are lacking. The scales of the three species of Macrouridae differ in respect with their cteni and spines. Numerous cteni cover the whole posterior field of the scales of *Coryphaenoides rupestris*, where they are regularly distributed in parallel rows (Fig. 8a). Each ctenus is of about 1.5 mm in length and fusiform with a sharp end. The lateral cteni which are in the vicinity of the circuli appear to be connected with them (Fig. 8b). The spines of the scales of *Macrourus berglax* are restricted to the central area of the posterior field. The central row consists of three or four massive spines which are built on to one another. This row is flanked on each side by one or two rows of smaller isolated spines. These thin spines are sharply pointed, and grooves are found along them (Figs 9a-b).

In *Trachyrhynchus trachyrhynchus*, the posterior field of the scales is ornamented with three massive conical spines. The central one (about 1,5 mm in length) is in the vicinity

of the focus and two spines (about 0,7 mm in length) are lateral. All are oriented towards the posterior margin of the scale (Fig. 10b). A longitudinal groove is observed on the anterior side of the central spine only (Fig. 10a-b). The spines are connected to the scale surface through a circular basis which appears to be set on an underlying scale surface where circuli are present (Fig. 10b).

Structure

The elasmoid scales of the Gadiformes are composed of two main layers: the superficial layer and the basal plate. The superficial layer is composed of two superimposed strata: the outer limiting layer and the external layer. The basal plate is made up of isopedin, a type of lamellar bone (Goodrich, 1907; Meunier, 1987). The various layers of the scales are made of acellular tissues which show various mineralizing processes (Figs 11-15). In scales freshly fixed for TEM, the superficial scleroblasts appeared as isolated cells distributed on the surface according to the superficial ornamentation. These cells have a central ovoid nucleus. The basal scleroblasts are very flat cells with a flat nucleus. They are connected to each other to form an uninterrupted layer lining the basal surface of the scale.

The superficial layers

The outer limiting layer constitutes the outer surface of the scale; it is restricted to the posterior field. In demineralized sections, the organic matrix of this layer appears as a delicate meshwork composed of thin microfibrils. Abundant electron-dense material makes up electron-dense lines which are distinct within this layer. The microfibrils are also organized in a radiating pattern. The crystal deposit reflects

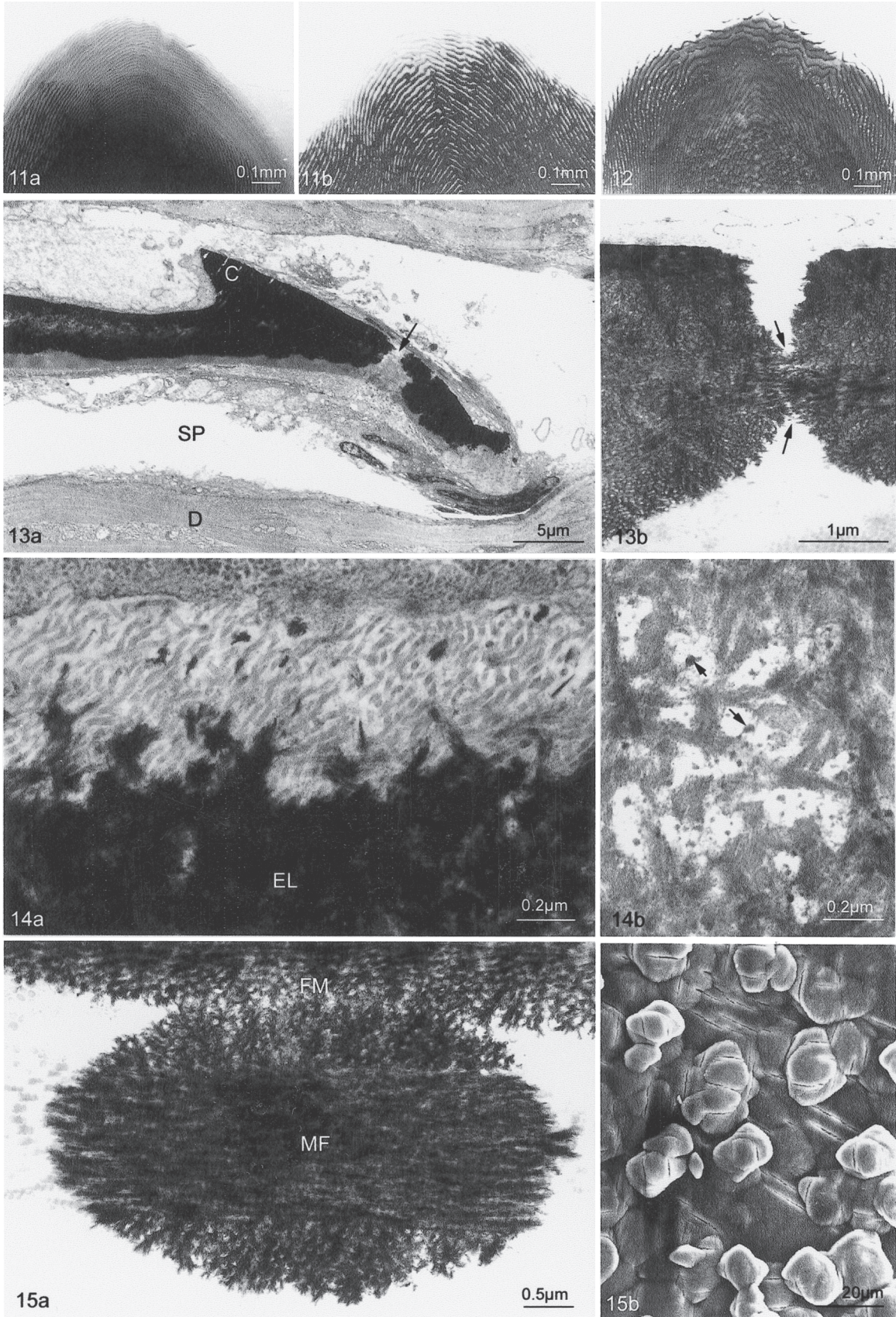


Figure 11 *Merluccius merluccius*. **a.** Posterior field of a whole mounted scale stained with alizarin red. **b.** Detail of the scale margin showing the mineral deposit at the level of the circuli.

Figure 12 *Mora moro*. Posterior field of a whole mounted scale stained with alizarin red showing the first mineral deposit in the circuli at the scale margin.

Figure 13 *Trisopterus luscus*. TEM. **a.** Anterior margin of the scale inserted within the dermis. (*C*) circulus; (*D*) dermis; (*SP*) scale pocket. Note that the mineral of the scale margin is separated from the central mineralized external layer by a narrow unmineralized space (arrow). **b.** Detail of the mineral deposit ensuring the junction between two adjacent circuli (arrows).

Figure 14 *Trisopterus luscus*. TEM. **a.** Mineral deposit in the external layer. Crystals are located close to cells, then fuse to form a mineralized external layer (*EL*). **b.** Demineralized section. Electron-opaque granules (arrows) are distributed among the collagen fibrils in the external layer.

Figure 15 *Trisopterus luscus*. **a.** TEM. Detail of a Mandl's corpuscle (*MF*) ahead of the mineralizing front (*FM*) showing that the mineral deposit is oriented by the collagen fibrils within the basal plate as well as in the Mandl's corpuscle. **b.** SEM. Mandl's corpuscles of different sizes.

Figure 11 *Merluccius merluccius*. **a.** Champ postérieur d'une écaille colorée par le rouge d'alizarine. **b.** Détail de la partie périphérique de l'écaille montrant le dépôt minéral au niveau des circuli.

Figure 12 *Mora moro*. Champ postérieur d'une écaille colorée par le rouge d'alizarine montrant les premiers dépôts de minéral dans les circuli à la périphérie de l'écaille.

Figure 13 *Trisopterus luscus*. MET. **a.** Partie périphérique du champ antérieur inséré dans le derme. (*C*) circulus ; (*D*) derme ; (*SP*) poche de l'écaille. Le dépôt minéral de la périphérie est séparé de celui de la partie centrale par d'étroites zones non minéralisées (flèche). **b.** Détail des zones de jonction minéralisées reliant deux circuli déjà minéralisés (flèches).

Figure 14 *Trisopterus luscus*. MET. **a.** Minéralisation de la couche externe. Les cristaux se forment à proximité des cellules et fusionnent pour assurer la minéralisation de toute la couche externe (*EL*). **b.** Coupe déminéralisée. Des granules opaques aux électrons (flèches) sont répartis entre les fibrilles de collagène de la couche externe.

Figure 15 *Trisopterus luscus*. **a.** MET. Détail d'un corpuscule de Mandl (*MF*) en avant du front de minéralisation (*FM*) montrant que les cristaux sont orientés par les fibrilles de collagène dans la plaque basale et dans les corpuscules de Mandl. **b.** MEB. Corpuscules de Mandl de tailles variées.

the organization of the fibrillary matrix. At the scale surface, the radial arrangement of the crystals according to that of the microfibrils forms the globular concretions found at the surface of scales in various species (Fig. 5c).

The external layer is located below the outer limiting layer in the posterior field and at the surface of the anterior field. Elevations of the external layer are responsible for the formation of the circuli which mineralize before the intervening spaces (Figs 11-13). The external layer is made of thin collagen fibrils about 20-30 nm in diameter arranged in a loose network within which electron-dense granules are present (Fig. 14b). In developing scales, the mineralization of the external layer starts at the periphery (Figs 11-12). The crystals appear first at the level of the marginal scleroblasts which line the newly formed circulus (Fig. 14a). They are associated with the dense granules and are not oriented along the collagen fibrils which are passively mineralized. At the level of the forming circulus, the crystals aggregate to form clusters. The clusters increase in size by agglomeration of newly formed crystals. Then, the clusters fuse, the circulus become entirely mineralized (Fig. 13a). In some species such as *Gaidropsarus vulgaris*, which has small scales, the mineralization occurs only at the level of the circuli, the spaces between two adjacent circuli remains unmineralized, as observed using secondary ionic microanalysis (Fig. 16). In the other species which have larger scales, the mineralization develops in the space between the circuli when the latter are mineralized and the

external layer become entirely mineralized (Figs 13b, 16). Microanalysis of these two kinds of scales shows that the mineral deposit contains the same elements which are similarly distributed whatever its extent is.

The mineral deposit occurs first in the external layer, subsequently in the basal plate, and finally in the outer limiting layer. No mineralization is found at the level of the radii, where no outer limiting layer is present, and where the external layer is obviously reduced or is even lacking.

Cteni and spines

Even though the cteni and the spines differ in shape, number, and development in the four species of Macrouridae examined (*Coryphaenoides rupestris*, *Macrourus berglax*, *Trachyrhynchus trachyrhynchus*, and *T. murrayi*), they show a similar organization at the ultrastructural level. They are composed of collagen fibrils of about 30 nm diameter, similar to the collagen fibrils forming the external layer (Figs 18, 19). Within the cteni and the spines, the collagen fibrils are oriented in the same direction as the axis of the spine (Fig. 19), except at the distal end, where the fibrils curve towards the center of the apex. However, the mineral deposit appears to be oriented by the collagen fibrils in the cteni and in the spines, unlike the external layer, where the mineral deposit is not related to the collagen fibrils. We did not observe an outer limiting layer at the surface of the cteni and spines. The scleroblasts are set around the spines, which do not contain any cell (Fig. 18).

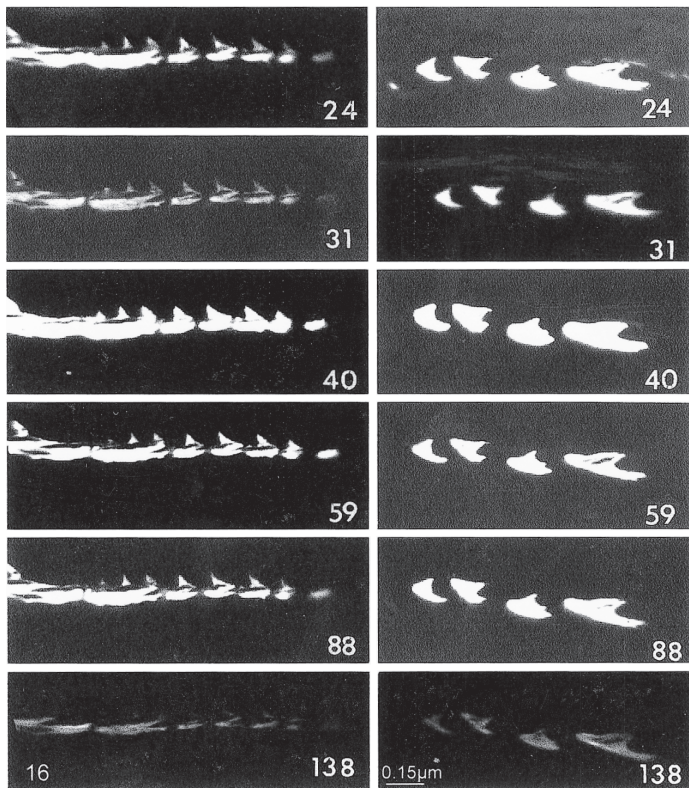


Figure 16. Analytical ion microscopy showing the distribution of Mg (M = 24), P (M = 31), Ca (M = 40), F (FCa M = 59), Sr (M = 88), and Ba (M = 138) in sections of the well-developed and mineralized scales of *Trisopterus luscus* (left) and in the minute scales of *Gaidropsarus vulgaris*, where the mineralization is confined to the circuli (right). Note the similar distribution of all the mineral components identified.

Figure 16. Microanalyse ionique secondaire montrant la répartition de Mg (M = 24), P (M = 31), Ca (M = 40), F (FCa M = 59), Sr (M = 88), et Ba (M = 138) dans des coupes d'écaillés de grande taille et bien minéralisées de *Trisopterus luscus* (à gauche) et d'écaillés de petite taille de *Gaidropsarus vulgaris* (à droite) où la minéralisation est limitée aux circuli. Dans les écaillés des deux espèces étudiées, tous les éléments identifiés ont une localisation identique.

The attachment of the cteni and spines on the scale surface is characteristic for each of the four species. In *Coryphaenoides rupestris*, the cteni are extensions of the external layer. In *Macrourus berglax*, an "intercalary bone" (Delamater & Courtney, 1973) forms a hinged junction between the scale and the spine (Fig. 9c). In *Trachyrhynchus trachyrhynchus*, the three spines are laid down on the surface of the scale. In the peripheral areas of the spine basis, the underlying scale surface shows circuli exhibiting both the outer limiting layer and the external layer when observed at light and electron microscopic levels (Figs 10c, 17). In *M. berglax*, *T. trachyrhynchus* and *T. murrayi*, TEM observations reveal that the outer limiting layer becomes

Figure 17. *Trachyrhynchus trachyrhynchus*. TEM. Peripheral area of the basal part of a spine (Ct) set on the outer limiting layer (OLL) and the external layer (EL). (BP) basal plate.

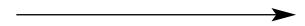
Figure 18. *Macrourus berglax*. TEM. In the central area of the ctenus, the outer limiting layer (OLL) becomes thinner and finally disappears, and the collagen matrix of the spine (Ct) contacts the external layer (EL). (BP) basal plate.

Figure 19. *Trachyrhynchus murrayi*. TEM. **a** Transverse, and **b** longitudinal sections through a spine showing the spatial organization of the collagen fibrills.

Figure 17. *Trachyrhynchus trachyrhynchus*. MET. Zone périphérique de la partie basale d'un cténus (Ct) reposant sur la couche limitante externe (OLL) et sur la couche externe (EL). (BP) plaque basale.

Figure 18. *Macrourus berglax*. MET. La couche limitante externe (OLL) devient de plus en plus mince et finalement disparaît sous la partie centrale du cténus (Ct) qui repose alors sur la couche externe (EL). (BP) plaque basale.

Figure 19. *Trachyrhynchus murrayi*. MET. Coupes **a** transversale, et **b** longitudinale d'un cténus montrant l'organisation des fibrilles de collagène



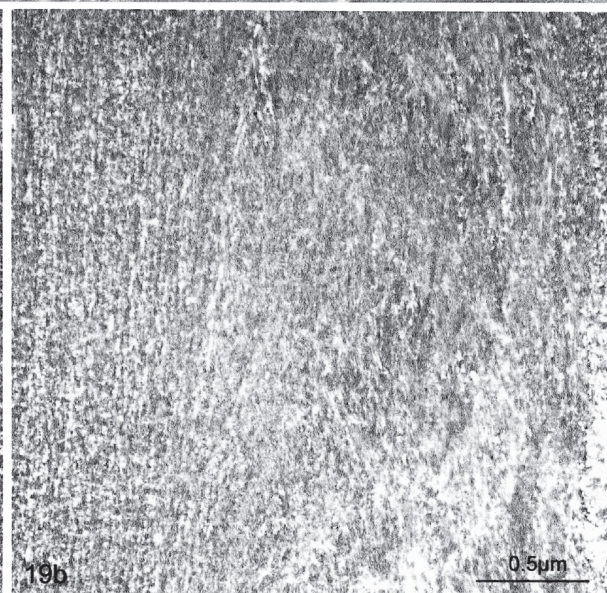
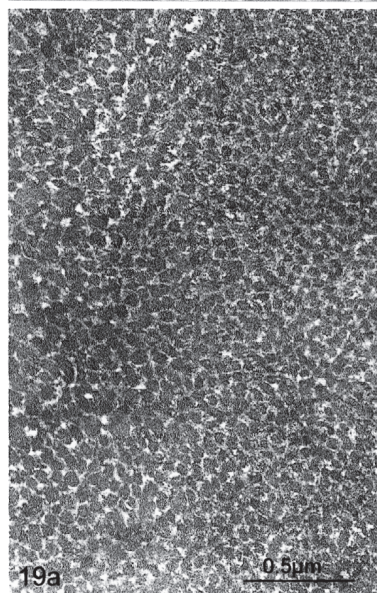
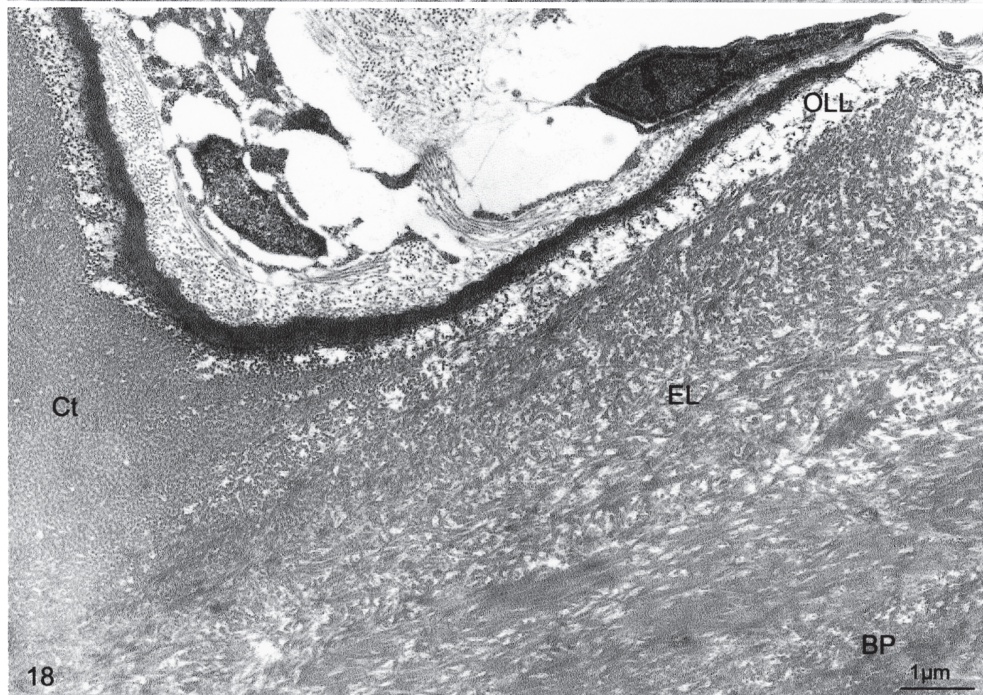
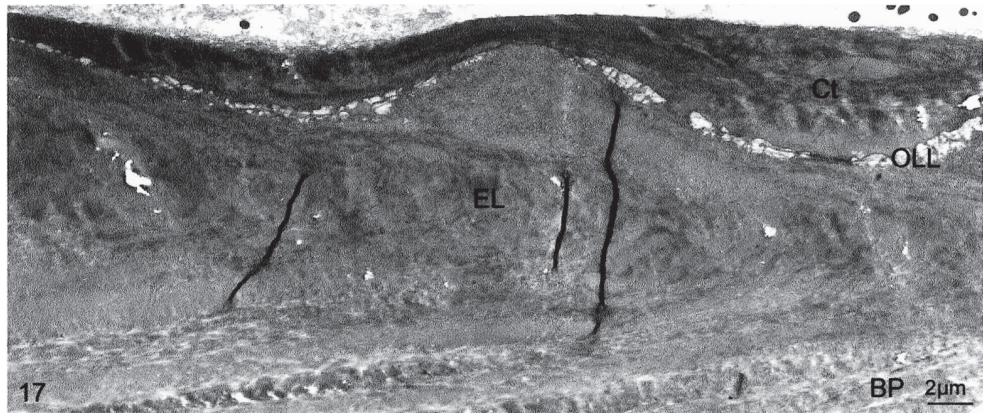
progressively thinner from the periphery to the center of the spine basis, and finally disappears in the central area where the collagen fibrills of the external layer and of the spine are in contact (Fig. 17). X-ray micrographs do not show any difference in the extent of the mineral deposit between the cteni, the spines and the external layer (Fig. 9d).

The basal plate

The basal plate composed of isopedin is the main part of the scale. It shows a lamellar structure (Fig. 20). The thick collagen fibrills (about 100 nm in diameter) are arranged in superimposed plies. In each ply, the closely packed collagen fibrills are parallel to each other (Figs 21, 22). Their direction varies from one ply to the next forming a plywood-like structure (Fig. 21). In most species examined, two adjacent plies are arranged at a right angle forming an orthogonal plywood (Figs 21, 22). In the basal plate of *Melanonus zugmayeri* and *Raniceps raninus*, the rotation angle is slightly different from a right angle. Therefore, the plywood is of an intermediate type (Meunier & Castanet, 1982).

The small scales of *Muraenolepis orangiensis* do not overlap, and are arranged side by side (Fig. 23a). They show a basal plate composed of collagen fibrills oriented in only one direction (Fig. 23b).

The basal plate of *Lota lota* is composed of two kinds of collagen fibrills. Thick collagen fibrills (100 nm in diameter) are organized in an orthogonal plywood-like structure (the isopedin). Thin collagen fibrills (about 30 nm in diameter) form sheets which are perpendicular to the plywood-like structure. These fibrills, called TC fibres (Onozato &



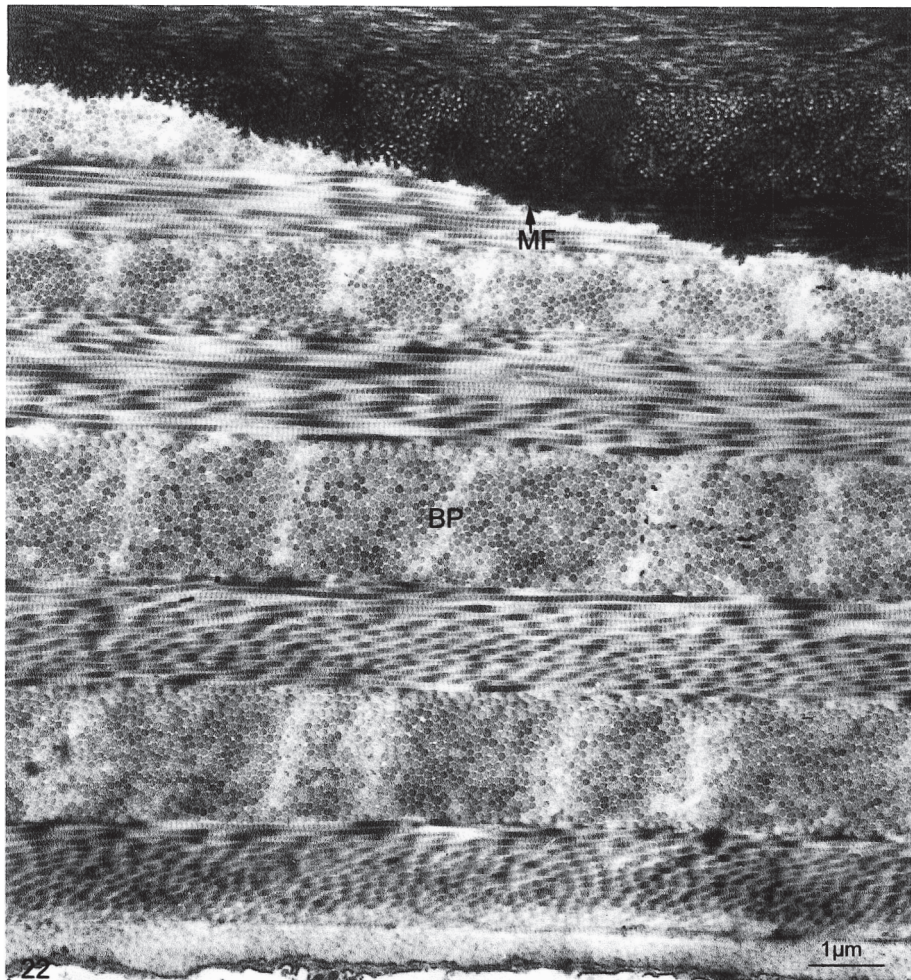
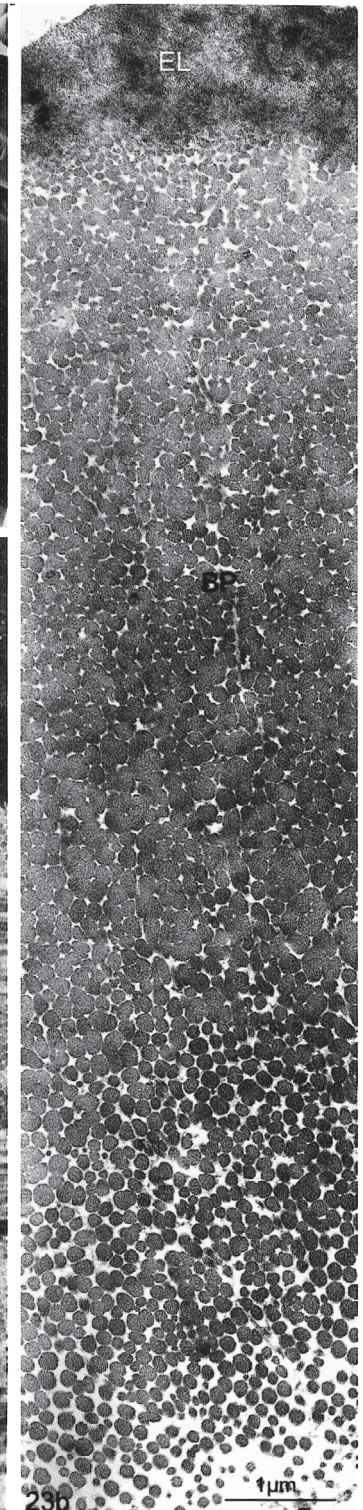
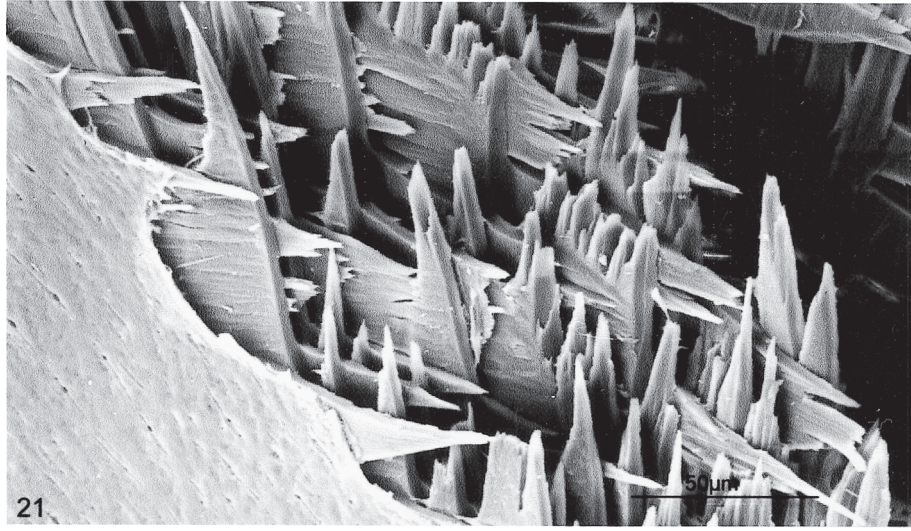
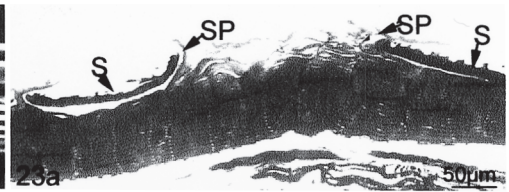
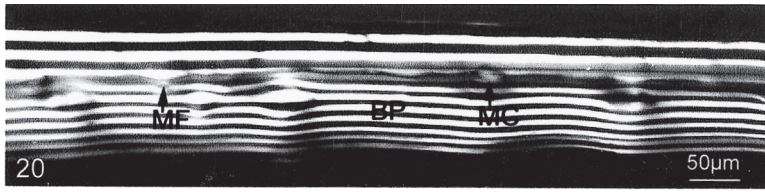


Figure 20. *Trachyrhynchus trachyrhynchus*. Ground section of the basal plate (BP) examined with polarized light. The alternative dark and clear layers indicate that the plywood is orthogonal. (MC) Mandl's corpuscle; (MF) mineralizing front.

Figure 21. *Macrourus berglax*. SEM. Broken freeze-dried scale showing the lamellar structure of the basal plate. The direction of the fibrils of adjacent plies are at right angles to one another.

Figure 22. *Macrourus berglax*. TEM. Spatial organization of the collagen fibrils in the basal plate (BP), where they form an orthogonal plywood-like structure. (MF) mineralizing front.

Figure 23. *Muraenolepis orangiensis*. **a.** Semi-thin cross section of the integument, stained, showing two scales (S), located in their pocket (SP). Note that these small scales do not overlap. **b.** TEM. The basal plate (BP) is made of one ply of collagen fibrils oriented parallel to the external layer (EL).

Figure 20. *Trachyrhynchus trachyrhynchus*. Lame mince de la plaque basale (BP) observée en lumière polarisée. La disposition des couches alternativement claires et sombres est due à la présence d'un contre-plaqué orthogonal. (MC) Corpuscules de Mandl ; (MF) Front de minéralisation.

Figure 21. *Macrourus berglax*. MEB. Fracture d'une écaille congelée montrant la structure lamellaire de la plaque basale. Les directions des fibrilles de collagène de deux couches adjacentes sont orthogonales.

Figure 22. *Macrourus berglax*. MET. Les fibrilles de collagène sont organisées en un contre-plaqué orthogonal dans la plaque basale (BP). (MF) front de minéralisation.

Figure 23. *Muraenolepis orangiensis*. **a.** Coupe semi-fine de la peau, colorée au bleu de toluidine, montrant deux écailles, chacune enfermée dans sa poche. Noter que ces écailles de petite taille ne se recouvrent pas. **b.** MET. La plaque basale (BP) ne comporte qu'une seule couche de fibrilles de collagène orientées parallèlement à la couche externe (EL).



Watabe, 1979), are involved in the first stages of mineralization, and their presence is responsible for the peculiar aspect of the mineralizing front which resembles inverted trees.

In the basal plate, the crystals are always oriented by the collagen fibrils and the mineralization is isotropic as in a bony tissue. The mineralization of the basal plate starts in the collagen fibrils forming the older plies of the basal plate, that are adjacent to the external layer. Then, the mineralization progresses towards the inner part of the scale. Moreover, in all species, the basal plate shows mineralized corpuscles (Mandl's corpuscles: Baudelot, 1873) ahead of the mineralizing front (Fig. 15). These corpuscles fuse, forming larger corpuscles, and then fuse with the mineralizing front as the mineralization progresses. The mineralization is also isotropic within the Mandl's corpuscles (Fig. 15a). Thus, their shape is dependent on the type of plywood-like structure, and on the number of plies which are mineralized (Fig. 15b).

Discussion

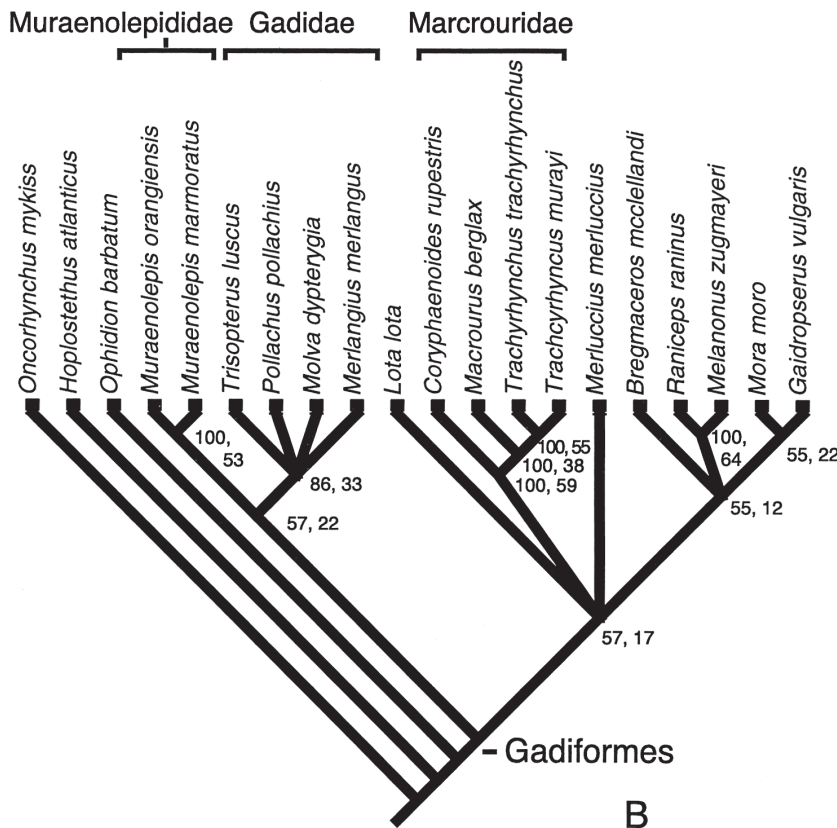
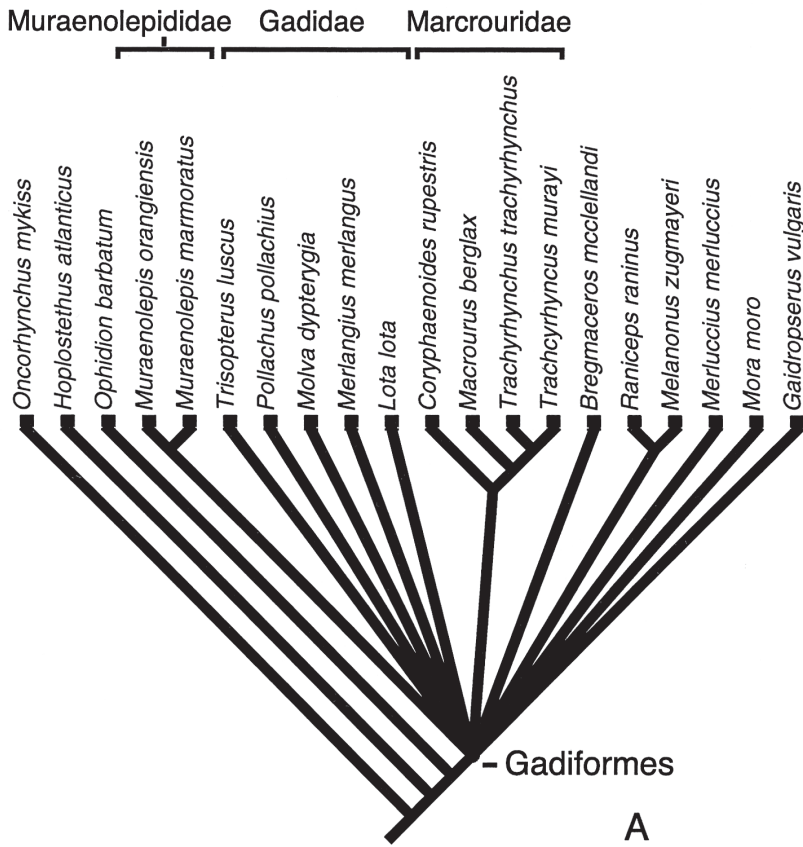
Comparative structure of the scales of the Gadiformes

The morphological diversity of the elasmoid scales of the Gadiformes might have been related to varied arrangements or extents of the layers forming the scales, but the fine structure of each of these layers shows a great uniformity among the families belonging to this order. As previously described in the elasmoid scales of teleosts, the outer limiting layer does not contain collagen fibrils, except when it is crossed by anchoring bundles made of parallel collagen fibrils which arise from the external layer and extend

through the dermis to the dermo-epidermal junction (Zylberberg & Meunier, 1981; Sire, 1985). It should be noted that the outer limiting layer could not be considered as a bony tissue (because of the absence of collagen fibrils) or as an enamel (because no epidermal involvement was demonstrated). It represents a mineralized tissue which is also found in the elasmoid scales of the Gymnophiona (Amphibians) (Zylberberg & Wake, 1991) and in the osteoderms of squamates (Levrat-Calviac & Zylberberg, 1986). Thus, this tissue could be characteristic of the mineralized plates developed in the dermis of vertebrates, whatever their origin (review in Zylberberg et al., 1992).

The external layer covers the whole outer scale surface. It is the first layer formed and mineralized during scale ontogenesis. The peculiar mineralization of this layer consists of a deposit of crystals forming clusters associated with dense granules. Its collagen fibrils are not involved in the first stages of the mineral deposit. This mineralization process might be compared to a spheritic mineralization described by Ørvig (1968), who considered this process as a precursor of the isotropic mineralization characteristic of bony tissues. The isotropic mineralization represents the "ultimate stages" in the phyletic processes occurring in the mineralization of vertebrate connective tissues, according to Ørvig (1968).

Among the Gadiformes which were examined, the Macrouridae is the only family which exhibits ctenoid or spinoid scales (Roberts, 1993). The cteni or the spines of the four species of Macrouridae differ in number, shape, and arrangement. Moreover, within the genus *Coryphaenoides*, the shape of the cteni was shown to be species-dependent (Iwamoto & Shcherbachev, 1991). Nevertheless, at the ultrastructural level, the cteni and the spines appear to be



constituted of thin collagen fibrils arranged in a similar spatial pattern, whatever the shape and the development of the cteni and spines are. The collagen fibrils of the cteni and spines resemble those of the external layer with which they were contiguous in the scale of *Coryphaenoides rupestris*. In the other three species, *Macrourus berglax*, *Trachyrhynchus trachyrhynchus* and *T. murrayi*, the basal part of the spines is set on the posterior surface of the scale, where the outer limiting layer is still present, at the margin of the spine basis, and it separates the matrix of the spine from that of the external layer. The outer limiting layer disappears progressively towards the central area of the spines,

Figure 24. Phylogenetic signal in the scales of Gadiformes. **A.** Strict consensus of the most parsimonious trees. **B.** 50% majority-rule consensus tree of the 77 most parsimonious trees obtained by a heuristic search. The numbers next to the nodes indicate the percentage of the shortest trees that support these nodes and the bootstrap percentage, respectively. For the bootstrap, the following settings were used: 100 replicates, heuristic search, simple addition sequence, maxtrees set at 4000, TBR branch swapping. *Oncorhynchus mykiss*, *Hoplostethus atlanticus*, and *Ophidion barbatum* are outgroup taxa. A topological constraint specifying the relationships between the three outgroup taxa and the ingroup was enforced.

Figure 24. Signal phylogénétique des écailles des Gadiformes. **A.** Consensus strict des arbres les plus parcimonieux. **B.** Consensus de majorité (à 50 %) des 77 arbres les plus parcimonieux obtenus par la recherche heuristique. Les nombres près des nœuds indiquent le pourcentage des arbres les plus parcimonieux qui renferment ces clades et le pourcentage de bootstrap. Pour le bootstrap, les options suivantes ont été sélectionnées : 100 répliquats, recherche heuristique, séquence d'addition simple, maxtrees égal à 4 000, remaniement des branches TBR. Les extra-groupes sont *Oncorhynchus mykiss*, *Hoplostethus atlanticus*, et *Ophidion barbatum*. Une contrainte topologique spécifiant les relations entre les trois extra-groupes et l'intra-groupe a été utilisée.

where the collagenous matrix of the spine contacts that of the external layer. However, it is noteworthy that in the cteni and in the spines, the mineralization appears to be inotropic.

The largest part of the scale is the basal plate, which exhibits a spatial arrangement of the collagen fibrils in a plywood-like structure often developed in the dermal skeleton, and which characterizes isopedin (Meunier, 1987). The mineral deposit is associated with the collagen fibrils, and the mineralization is inotropic.

In the basal plate of *Lota lota*, TC fibers are involved in the first stages of mineral deposit at the mineralizing front, which shows the same peculiar aspects as in other taxa where TC fibers were described, such as the Cypriniformes (Onozato & Watabe, 1979; Zylberberg & Nicolas, 1982) or the Characiformes (Zylberberg & Meunier, 1996). The peculiar pattern of the superficial ornamentation and the formation of sheets of TC fibers in the basal plate of *Lota lota*, which could be considered as an apomorphic condition, are characteristics which could be added to those which lead to set this species apart from most other Gadiformes, as suggested by Nolf & Steurbaut (1989). A recent classification proposed by Eschmeyer (1998) indicated that *Lota lota* belongs to the Lotidae, which is excluded from the Gadidae. However, Cohen et al. (1990), Nelson (1994) and Kottelat (1997) consider *Lota lota* as a Lotinae, a sub-family included within the Gadidae. Further studies are necessary to clarify the position of this species (see below).

Comparisons to other Teleostean scales

The scales of the Gadiformes show many features which are found in other Teleostei. They are made of acellular mineralized tissues, the scale-forming cells line the surface of the scale and of the cteni and spines, and the plywood-like structure of the basal plate is orthogonal in most species (but it is of an intermediate type in a few species). The orthogonal plywood was demonstrated to be a "secondary condition" of the spatial arrangement of the collagen fibrils within the basal plate of many Teleostei (Meunier & Castanet, 1982; Meunier, 1987-88). The primitive condition, a twisted plywood (Giraud et al., 1978), is restricted to basal taxa like Osteoglossomorpha, Elopomorpha, and Ostariophysi (Meunier & Castanet, 1982).

The scales of *Muraenolepis orangiensis*, which have a basal plate restricted to only one ply, offer an example of the reduction of the dermal skeleton. The small scales of *M. orangiensis* could be compared to those of the eel (Zylberberg et al., 1984). The formation of such minute scales has been related to a slender and elongate body. It has been demonstrated that the flexibility of the body is inversely proportional to the scale size (Grinberg, 1950). Moreover, these scales are not imbricated; they are located

side by side within the dermis, this latter distribution favouring the undulatory movement (Burdak, 1979). The small scales might represent an intermediate stage illustrating a trend of reduction of the dermal skeleton which disappears in some teleostean lineages (Bertin & Arambourg, 1958). In the species with an elongate body and with small scales, or with a scaleless integument, the protection is ensured by an increased thickness of the integument and a more abundant production of mucus. This is observed in Gadiformes with a slender body (*Muraenolepis orangiensis* and *Gaidropsarus vulgaris*), in the eel *Anguilla anguilla*, and in some Ophidiinae (Cohen & Nielsen, 1978) as well as in other species with a naked integument such as some Anguilliformes (*Conger conger* and *Muraena helena*), Siluriformes such as the Siluridae *Silurus glanis*, and the Ictaluridae *Ictalurus melas* (unpublished results).

Morphological features which might be of a specific value concern mainly the pattern of the outer surface.

The cycloid scales which have no grooves (radii) were considered to be closest to the ancestral stage (Kobayashi, 1953). Radii are characterized by the absence of the superficial mineralized layers, which enhances the pliability of the basal plate and gives flexibility to the scales. They probably favour swimming.

Two different kinds of ornamentations are borne by the scale surface: macroscopic ones (circuli, ridges, tubercles, spines, cteni...) and microscopic ones supported by the former, such as denticles on the crests of the circuli (Delamater et al., 1972; Lanzing & Higginbotham, 1974; Sire 1986; Bonwick et al., 1989). The denticles are mostly located in the covered anterior field of the scales, whereas the cteni or the spines are confined to the free posterior field. Thus, denticles and cteni differ in their location, their structure and their function. The denticles deeply insert within the dermis, strengthen the attachment of the scales to the dermis, and concomitantly decrease the friction between adjacent scales, whereas the cteni are involved in the formation of a boundary layer at the surface of the body, which reduces drag (Burdak, 1979).

Among the Gadiformes observed in this study, only the Macrouridae exhibit ctenoid or spinoid scales. The present data indicate that the cteni and spines were deposited on the posterior surface of the scale, on the outer limiting layer that develops last during scale ontogenesis. Thus, they substantiate the hypothesis that during ontogenesis, the cycloid type precedes the formation of the cteni (Kobayashi, 1953; Okamura, 1970; Burdak, 1979).

Phylogenetic signal in the scale characters

Denticular characteristics have been proposed as useful criteria for systematic study by Delamater & Courtney (1974), and the cteni have been considered for establishing

phylogenetic relationships in the Macrouridae (Okamura, 1970; Iwamoto, 1989). To test the idea that scale structure includes phylogenetic information, we have coded a matrix that incorporates the characters that we have described above (Table 1). The matrix comprises twenty taxa, including three outgroups, whose relationships to the ingroup (Gadiformes) were taken from the literature (Roberts, 1993). We have performed a search for the shortest trees using a heuristic algorithm of PAUP* 4 (Swofford, 1998). All characters were unordered. A topological constraint specifying the relationships between the three outgroup taxa (as shown on Fig. 24) and the ingroup was enforced. This procedure yielded 77 most parsimonious trees, with the following statistics: tree length of 22 steps; consistency index (CI) of 0.591; retention index (RI) of 0.700. Thus, there is much homoplasy in the data, but there is clearly a phylogenetic signal. This is confirmed by the g_1 statistic (-0.505), that indicates a significant left-skewed distribution of tree length (Huelsenbeck, 1991). This statistic is based on the examination of the distribution pattern of 50 000 randomly-generated trees. It is noteworthy that the shortest of these random trees required 26 steps (four more steps than the shortest trees). A strict consensus of the most parsimonious trees (Fig. 24A) includes only five clades in the ingroup (the relationships between the outgroups and the ingroup were specified using a topological constraint). A decay analysis indicates that a single step is required to collapse all the clades. One of these clades consists of *Raniceps raninus* and *Melanonus zugmayeri*, that represent two taxa (Ranicipitidae and Melanonidae) that have been suggested to be closely related (Markle, 1989). Another clade corresponds with the taxon Muraenolepididae, whose monophyly is thus supported. The last three clades correspond with the Macrouridae and subclades of the latter. These results may seem disappointing, but they are not unexpected given that our matrix includes only ten characters (not nearly enough to fully resolve the phylogeny for twenty taxa), two of which (characters 6 and 9) are phylogenetically uninformative in this context (they are reported because this anatomical data could be re-used by other authors to build their matrices). Thus, only eight characters in our matrix could potentially resolve the phylogeny of Gadiformes. However, a 50% majority-rule consensus tree offers a better resolution (Fig. 24B). While we must emphasize that the nodes present only in the majority-rule consensus tree are not robust (as shown by the bootstrap frequencies), they confirm previous suggestions that *Lota* should be removed from the Gadidae (Markle, 1989). The phylogeny of Gadiformes presented here is of modest interest in itself (because of the lack of resolution and of robustness), but we suggest that scale characters could be incorporated to a morphological matrix to attempt to resolve the phylogeny of this group.

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