

Enameloid microstructure in the oldest known chondrichthyan teeth

H. Botella,¹ P. C. J. Donoghue² and C. Martínez-Pérez¹

¹Department of Geology, University of Valencia, Dr Moliner 50, 46100 Burjassot, Valencia, Spain; ²Department of Earth Sciences, University of Bristol, Wills Memorial Building, Queen's Road, Bristol, BS8 1RJ, UK

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Abstract

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Previous studies on tooth enameloid microstructure in several chondrichthyan taxa spanning the phylogeny of the group provided support for the homology of chondrichthyan tooth enameloid. This hypothesis requires that a single crystallite enameloid (SCE) monolayer must be present in the teeth of the most primitive chondrichthyan. However, the dental microstructure of the earliest sharks has yet to be investigated. We have studied the tooth enameloid microstructure of the two oldest tooth-bearing shark species currently known, *Leonodus carlsi* Mader (1986) and *Celtiberina maderi* Wang (1993), from the lowermost Lochkovian (Lower Devonian) of Spain. Our study demonstrates the presence of a SCE monolayer in the teeth of both species. These results show that a superficial cap of SCE is present in the oldest shark teeth known, which suggest its presence in the most basal chondrichthyans.

Dr C. Martínez-Pérez, Department of Geology, University of Valencia, Dr Moliner 50, 46100 Burjassot, Valencia, Spain.
E-mail: Carlos.Martinez-Perez@uv.es.

Introduction

A recent examination of tooth enameloid microstructure in several chondrichthyan taxa spanning much of the phylogeny of the group has provided evidence for the homology of chondrichthyan enameloid (Gillis and Donoghue 2007). All of the teeth of stem-neoselachians studied, with the exception of some xenacanthiforms (but see also Hampe and Long 1999), possess an enameloid capping layer consisting of a monolayer of individual crystallites or single crystallite enameloid (SCE), lacking any degree of microstructural differentiation within the layer. Some Triassic species of the Hybodontiformes *Hybodus* (Reif 1977), *Acrodus* and *Polyacrodus* (Cuny *et al.* 2001) have a distinct two-layered enameloid microstructure. The teeth of modern sharks (non-batoid neoselachians) show a fully differentiated triple-layered enameloid, comprised of an outermost shiny layer enameloid (SLE) in which component crystallites are randomly orientated (not organized into bundles), and two internal layers where the crystallites are organized in bundles arranged in preferred orientations (see Reif 1973, 1977).

The analysis of the distribution of these different enameloid microstructure types within alternative hypotheses of

chondrichthyan phylogenies suggest that a SCE monolayer must be already present in the most primitive chondrichthyan teeth, whereas the acquisition of the complex triple-layered tooth enameloid of neoselachians emerged progressively along the neoselachian stem (Gillis and Donoghue 2007). However, the oldest and/or phylogenetically most basal chondrichthyan genera were not included in their study (e.g. *Leonodus*, *Celtiberina*, *McMurdodus*, *Doliodus*, *Antarctilamna*, *Pucampella* and others). Therefore, a study of the enameloid microstructure of these taxa could provide more definitive evidence on the plesiomorphy of the chondrichthyan tooth SCE. Under these assumptions we have studied the tooth enameloid microstructure of the two oldest tooth-bearing shark species known, *Leonodus carlsi* Mader (1986) and *Celtiberina maderi* Wang (1993), from the Lower Devonian of Spain.

Material and Methods

All of the material studied was recovered as isolated elements from the lowermost Lochkovian (Lower Devonian) of two areas in the Iberian Chains (Spain); the Axial Depression of the Camaras River (Carls 1988) and the Axial Depression of

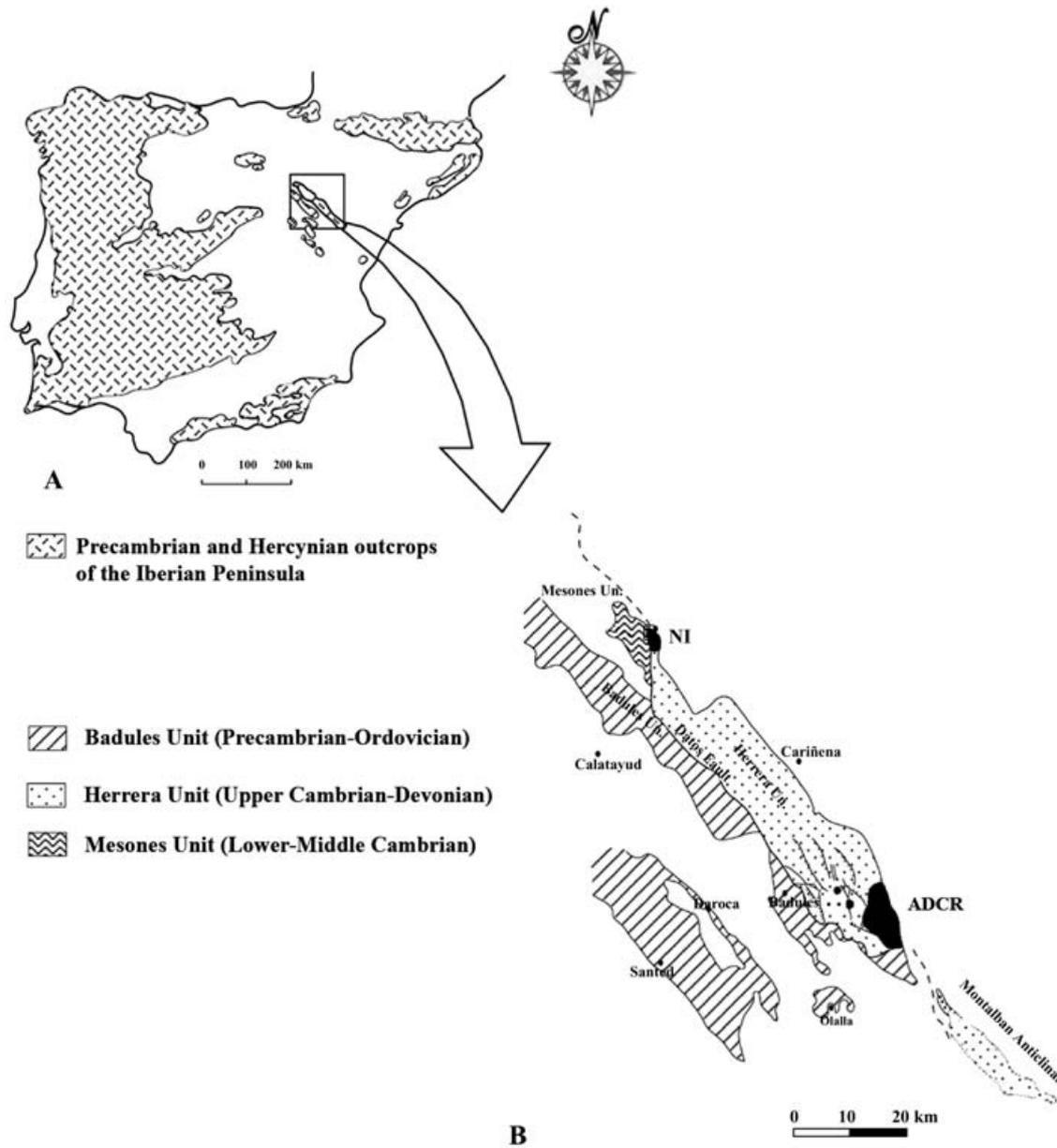


Fig. 1—**A.** Geographical setting of the studied area with indication of the distribution of Precambrian and Palaeozoic rocks in the Iberian Peninsula. —**B.** Geological map of Palaeozoic rocks in the Iberian chains with the two studied areas (Devonian outcrops in black) indicated. NI, axial depression of Nigüella (Valenzuela-Ríos 1989); ADCR, the axial depression of the Camaras River (Carls 1988).

Nigüella (Valenzuela-Ríos 1989) (Fig. 1). For a detailed geological and faunal succession see Carls (1988, 1999) and Carls *et al.* (2002).

For the histological study the teeth were embedded in a transparent polyester resin and subsequently sectioned along transverse or longitudinal planes. Afterwards, the cut surfaces were ground and then etched using 0.1 M HCl for 5–10 s. Each sample was repolished and etched as many times as necessary to elucidate the inner microstructure of the teeth. We also studied the microstructure by etching artificial fractures created using an entomological needle.

The whole teeth and etched surfaces were imaged using a Philips XL-30 scanning electron microscope. The studied specimens are housed in the Museum of Geology at the University of Valencia.

Results

Leonodus carlsi

Teeth of *L. carlsi* have a general diplodont morphology (Fig. 2A–C), possessing linguo-labially elongated (up to

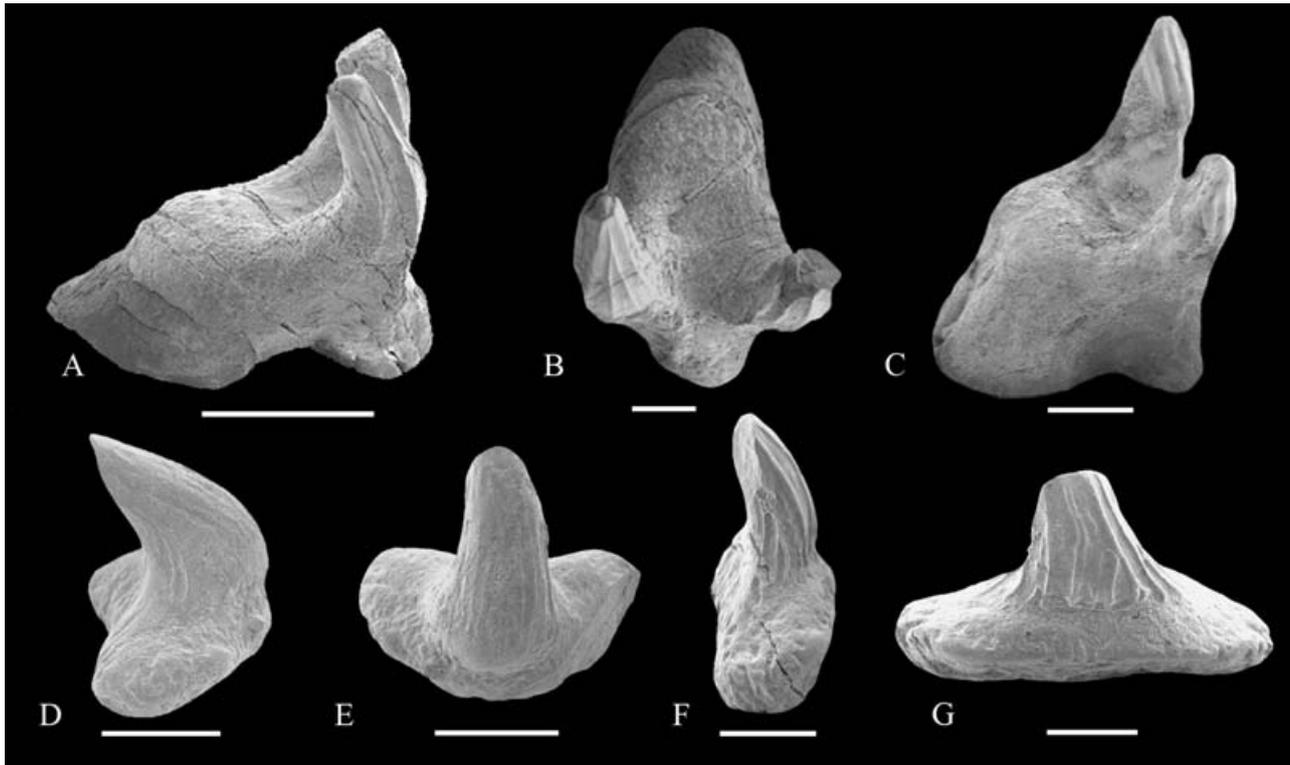


Fig. 2—Teeth of *Leonodus carlsi* Mader (1986) (A–C) and *Celtiberina maderi* Wang (1993) (D–G) from the Lochkovian (Lower Devonian) of Spain. —A, B. Museum of Geology at the University of Valencia (MGUV) 14861 (A lateral view, B upper view); —C. MGUV 14863 lateral view; —D, E. MGUV 19338 (D lateral view, E upper view); —F, G. MGUV 19339 (F lateral view, G frontal view). Scale bars for A, C, F, G, 500 μ m; B, 200 μ m; D, E, 1 mm.

5 mm) bases with a characteristic peanut-shape. The cusps are pointed, lingually arched and placed in the first labial third of the base. The height of the cusps varies between 0.5 and 3 mm and they are rounded or oval in cross-section. For more detailed morphological and histological descriptions see Mader (1986; pl. 5, fig. 1–11; text-fig. 13).

The tops of the cusps exhibit a homogeneous monolayer of SCE that is distinct from the underlying dentine core (Fig. 3A,B). No microstructural differentiation is observed across the enameloid layer. A sharp enameloid–dentine junction is obvious and numerous tubules (derived from odontoblast cell processes) cross the junction and extend into the enameloid (Fig. 3C). At higher magnification, individual crystallites are clearly discernable within the enameloid layer. Crystallites are elongate, approximating 0.5 μ m in length, and appear randomly orientated (Fig. 3D). The enameloid capping layer covers only the upper half of the cusps and it is notably thicker on the labial than on the lingual side of the teeth (Fig. 3A).

Celtiberina maderi

Celtiberina maderi teeth exhibit a relatively simple morphology with a mesio-distally extended base that is basally flattened,

with a single conical cusp that is curved lingually (Fig. 2D–G); some specimens possess small incipient cusplets lateral to the median cusp (Wang 1993; pl. V, figs 1a,c, 2a and 3a). For a detailed morphological and histological description, see Wang (1993; pl. IV, figs 1–7; text-figs 13 and 14).

The teeth of *C. maderi* possess a differentiated cover of hypermineralized tissue that is readily distinguishable from the underlying core of osteodentine (Fig. 3E–H), although it appears to be present only on the labial surface (Fig. 3E,F). The enameloid layer does not exhibit microstructural differentiation. Rounded crystallites are poorly discernable within the enameloid matrix (see Fig. 3G,I) but the enameloid cap is nonetheless distinct from the underlying osteodentine because it lacks the tubule-rich fabric characteristic of dentine. The enameloid–dentine junction is distinct even though it is permeated by the processes of odontoblasts. The dentine core is composed of osteodentine with numerous denteons surrounded by pallial orthodentine (Fig. 3H).

Discussion

Our analysis shows that a superficial capping monolayer of hypermineralized tissue is present in the cusps of the oldest

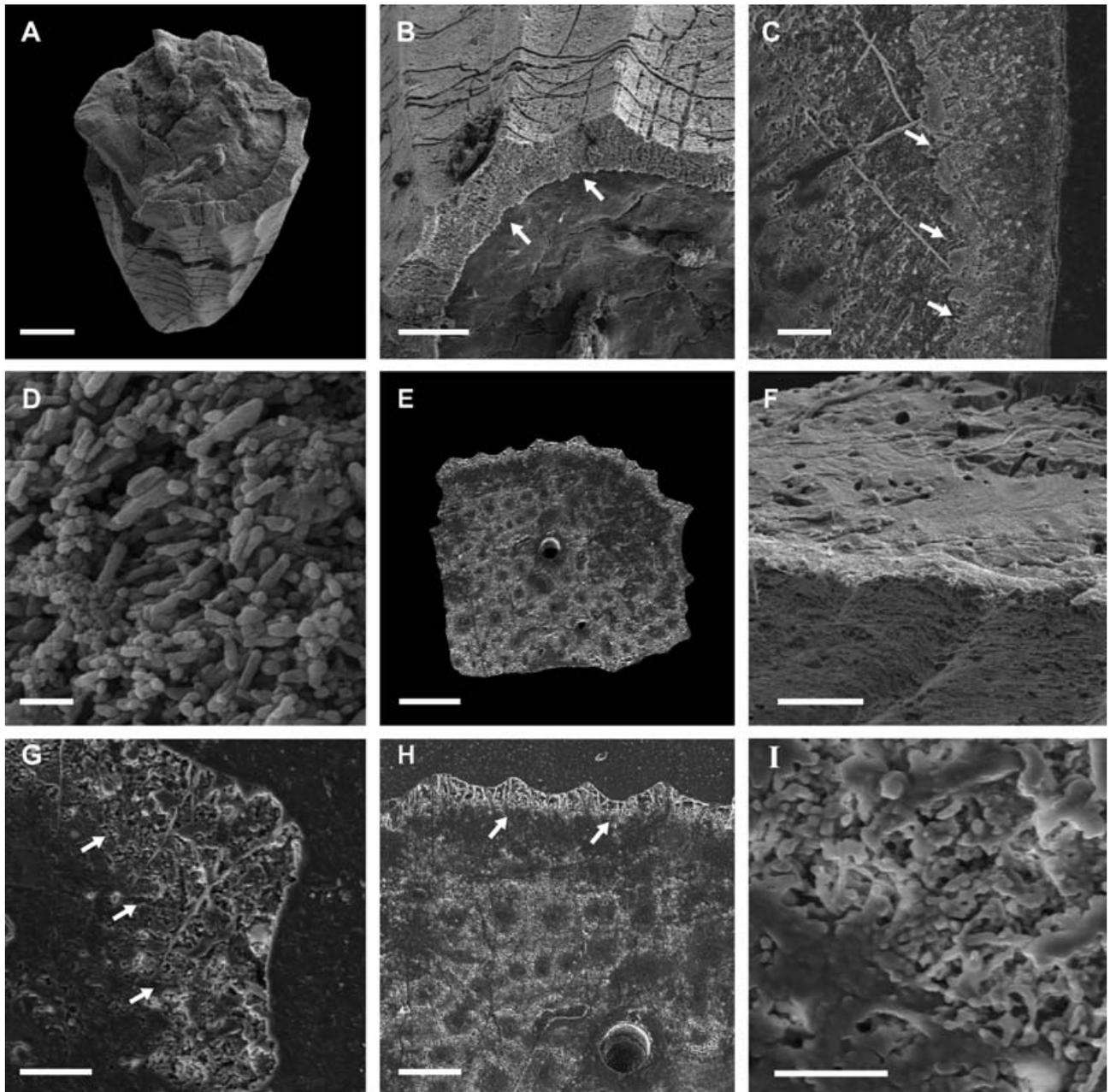


Fig. 3—Tooth enameloid microstructure of *Leonodus carlsi* Mader (1986) (A–D) and *Celtiberina maderi* Wang (1993) (E–I). —A, B. General view of a cross-fracture of a cusp of *L. carlsi*, Museum of Geology at the University of Valencia (MGUV) 19340, a distinct enameloid outer layer is clearly discernible from the underlying dentine core, showing a sharp border between them. —C. Detail of the single crystallite enameloid monolayer of MGUV 19344 in cross-section. Note the numerous remnant spaces of odontoblast cell processes extending through the enameloid–dentine junction. —D. Elongated hydroxyapatite crystallites, with random orientation, making the enameloid monolayer of MGUV 19340. —E. Overview of MGUV 19341 in cross-section. —F. Fresh fracture of the main cusp of MGUV 19342. —G. Detail of the enameloid monolayer of MGUV 19341. Individual crystallites are vaguely discernible, but no microstructural differentiation is apparent within the layer. —H. The dentine core of MGUV 19341 is composed of a centre of osteodentine with numerous denteons. —I. Detail of the single crystallite enameloid monolayer of MGUV 19341 in cross-section where individual rounded crystallites are poorly discernible and randomly orientated. All elements were etched using 10% hydrochloric acid always for less than 10 s. Scale bars for E, 250 µm; A, F, H, 100 µm; B, D, 50 µm; C, G, 25 µm; I, 10 µm. Arrows denote the enameloid–dentine junction.

known chondrichthyan teeth, from *L. carlsi* and *C. maderi* from the lowermost Lochkovian (Lower Devonian) of Spain. There is no evidence of microstructural differentiation across the enameloid layer in either species. In *L. carlsi* the enameloid monolayer consists of individual mineral crystallites randomly orientated, i.e. a SCE microstructure, and presents numerous tubules that nearly reached the surface of the cusps. This layer was previously identified as ‘durodentine’ by Mader (1986; fig. 13) but his figure shows few and short dentine tubules. In *C. maderi* individual crystallites are not clearly discernable, however, the position of the hypermineralized capping layer, its homogeneous structure (without any evidence of a double- or triple-layered structure) and the presence of a clear enameloid–dentine junction indicate the presence of SCE. Our results therefore corroborate the hypothesis that SCE is a primitive feature of the teeth of chondrichthyans (Gillis and Donoghue 2007).

Leonodus was first classified as a xenacanthiform (Mader 1986) but has subsequently been referred to the *Antarctilamna–Wellerous* clade (Soler-Gijón and Hampe 2003; Ginter 2004), which has been considered the sister-group of all remaining chondrichthyans (Ginter 2004, 2005). *Celtiberina* was originally assigned to the hybodontids (Wang 1993) but, curiously, it has not been included in any posterior tooth-based phylogeny of basal chondrichthyans (e.g. Ginter and Ivanov 1996; Hampe and Long 1999; Ginter 2004, 2005). This may be the case because some authors have not been entirely convinced that *Celtiberina* is a tooth and it has been suggested that it could instead be a modified dermal denticle (e.g. Turner 2004), perhaps because its base is weakly developed (Ginter 2004, and personal communication). However, in addition to its generalized tooth-like morphology, most specimens of *C. maderi* show clear evidence of wear resulting from tooth function. The extent of such wear varies from cusps without wear (Fig. 2D,E) to highly worn cusps (Fig. 2F,G). Similar degenerative functional wear has been documented in isolated and articulated teeth of Upper Devonian cladodont sharks from the Cleveland Shale (Newberry 1889; Williams 1992, 2001) and it is also observed in the teeth of *Leonodus* (e.g. Fig. 2C).

Zangerl (1981; see also Ginter 2004) proposed the idea that the Middle Devonian multicuspidate cladodont teeth could have evolved from a simple monocuspid tooth morphology with a laterally expanded base (although at the time of his writing, these primitive cladodont teeth were unknown). In fact, this morphology coincides exactly with the teeth of *C. maderi*. Moreover, the teeth of *C. maderi* are closely comparable, in terms of the internal microstructure of the dentine core, with some ‘cladodont’ sharks described by Gillis and Donoghue (2007), having a prominent centre of osteodentine with numerous denteons, bordered by orthodentine (Fig. 3H). This fact, together with the morphological characters discussed above and the structure of the tooth base (not as porous as typical hybodonts and firmly attached to the crown), suggests that *C. maderi* could belong

to a basal group of chondrichthyans, rather than be allied with Hybodontiformes, as proposed by Wang (1993). The study of chondrichthyan-like scales and spines that frequently occur in the same stratigraphic levels from which the teeth of *C. maderi* were recovered (Botella *et al.* in preparation), may provide decisive information on *Celtiberina* affinities.

Although *L. carlsi* and *C. maderi* are currently the oldest known tooth-bearing sharks, in terms of geological age, they are far from being the oldest reported chondrichthyans. Shark-like body scales have been reported from rocks of Ordovician (Sansom *et al.* 1996) and Silurian (Karatajuté-Talimaa *et al.* 1990; Karatajuté-Talimaa and Novitskaya 1992; Karatajuté-Talimaa 1995, 1997; Sansom *et al.* 2000; Märss and Gagnier 2001; Märss *et al.* 2002, 2006; Turner 2004) age. However, evidence of their chondrichthyan affinity is limited to one or just a few scale-based synapomorphies that may well represent crown–gnathostome symplesiomorphies (Donoghue *et al.* 2003). Nevertheless, even setting aside these reports, the existence of Ordovician stem-osteichthyans (Karatajuté-Talimaa and Predtechenskyj 1995) indicates the establishment of chondrichthyans by this time, indicating an extensive prehistory to the chondrichthyan dentition, the fossil remains of which, upon discovery, will further test the homology of chondrichthyan and osteichthyan tooth enameloid and its microstructure.

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