Tooth replacement rates in early chondrichthyans: a qualitative approach

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LETHAIA

The continuous replacement of teeth throughout their lifetime is a common characteristic of most chondrichthyans. This process was already present in the earliest representatives of the group. It has been well established that different species of extant sharks show rapid tooth replacement rates; however, some authors have suggested that in early chondrichthyans this rate might have been much slower. Here we present a qualitative approach to analyse tooth replacement rates in the Early Devonian shark *Leonodus carlsi*, the earliest tooth-bearing shark known to date. For this, we have examined 1,103 isolated teeth from Celtiberia, Spain. Our study provides strong evidences of an extremely slow dental replacement in this primitive chondrichthyan based on three independents analyses: (1) statistical analysis of the wear degree, demonstrating that teeth remain functional for a long period of time; (2) analysis of both the histological and the morphological features of the teeth cusps suggests that this chondrichthyan used a maturation process that optimizes its function, thus worn teeth show an efficient working shape that implies their teeth remained functional for a long time after being modelled by use; and (3) estimations of size increments between teeth (Δs) of the same dental family for some recent sharks whose rates of replacement were known prove that Δs is inversely proportional to the rate of replacement ($R^2 = 0.8327$). The estimated values of tooth replacement rates obtained from Δs for *L. carlsi* and for some Late Devonian cladoselachian sharks are significatively slower than those observed in current sharks. □ *Early chondrichthyans*, *tooth replacement*, *histology*, *tooth wear*, Leonodus carlsi, *Early Devonian*.

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Owen (1866) was the first author to propose that sharks' teeth undergo a continuous process of formation, forward progression and replacement. Subsequent papers by Cawston (e.g. 1938, 1940, 1944), disagreeing with Owen's suggestion, have solely historical interest, and today zoologists and palaeontologists agree that constant and coordinated tooth replacement throughout lifetime is a feature common to all chondrichthyans (e.g., Breder 1942; James 1953; Moss 1972; Reif 1982; Castro 1983; Luer *et al.* 1990; Overstrom 1991; Shimana 1997; Becker *et al.* 2000; Williams 2001; Botella 2006). However, an interesting exception to this widely accepted idea is the odd Early Devonian chondrichthyan *Pucapampella*, which shows a single row of teeth attached directly to the jaw, and lacking the characteristic shark-like tooth families. In addition, chimaeroids do not demonstrate tooth replacement, but growing tooth plates is considered as a derived condition that evolved by fusion of the tooth families of massive, but separate, teeth present in basal holocephalans (see Janvier 1996, p. 144).

Shark teeth are organized in files (or tooth families) and rows. Each tooth family includes functional and replacement teeth, aligned perpendicular to the jaw axis. Embryonic teeth develop internally (from tooth buds produced at the dental lamina) in the lingual part of each tooth family, erupt into the mouth, and migrate to the labial part (jaw margin) until occupying a functional position, in a 'conveyor belt'-like mechanism (Williams 1992). Teeth remain functional during some time, and finally, the used tooth is shed (sometimes it can be retained), and this position is occupied by the next tooth of the same family. Rows are aligned along the jaw and consist of teeth of different families placed in equivalent positions.

Tooth replacement rates (defined as the speed at which shark teeth progress forward from one row to the next, towards the distal margin of the jaw) have been measured in some species of current sharks using different individually teeth marker techniques and visualizing periodically how those marked teeth move forward to the distal margin of the jaw (e.g. Reif *et al.*

1978; Luer *et al.* 1990). Available data on mean values of tooth replacement rates range from a minimum of 8–10 days/row in *Negaprion brevirostris* (Moss 1967) to a maximum of 5 weeks/row in *Scyliorhinus canicula* (Märkel & Laubier 1969). Another technique for estimating tooth replacement rates is that used in Breder (1942), Overstrom (1991) and Correia (1999) by collecting all the teeth shed by sand tiger sharks (*Carcharias taurus*) confined in an aquarium. In these studies, replacement rate is given in teeth/shark day, and concluded that *C. taurus* loses one tooth every two days (according Overstrom 1991) or one tooth every day (according Correia 1999). These studies reveal a fast tooth replacement in chondrichthyans. Thus, in some species, a single individual can produce thousands of teeth throughout its life.

This rapid and continuous production, together with the fact that they are composed of highly resistant biogenic apatite, are the reasons for teeth being the most abundant elements among the fossil remains of sharks. Nevertheless, the oldest fossil remains attributed to chondrichthyans are not teeth, but body scales from the Late Ordovician of Colorado (Samsom *et al.* 1996), and from the Early Silurian of Siberia (Karatajūté-Talimaa 1973, 1992) and Mongolia (Karatajūté-Talimaa *et al.* 1990); but no tooth appears associated with these scales. In fact, the oldest occurrence of unequivocal chondrichthyan teeth (*Leonodus carlsi* Mader, 1986, see below) dates from the early Lochkovian (Early Devonian), approximately 50 Myr after the appearance of the oldest chondrichthyan-like scales. The absence of chondrichthyan teeth, but not of scales, during the Late Ordovician and Silurian and the small number of chondrichthyan teeth records even through all the Early Devonian suggest that early chondrichthyans might have had a very slow tooth replacement rate (Maisey 1984; Paterson 1992 *in* Janvier 1996). Other authors, however, advocate the possibility that early (Ordovician and Silurian) chondrichthyans lack teeth (Turner 1992, 2004; Long 1995; Samsom *et al.* 1996; Williams 2001; Turner & Miller 2005), which would necessarily imply an independent origin of teeth in the clade, and may provide evidence of multiple origins of teeth among gnathostomes (Smith & Johanson 2003).

Since tooth replacement is a continuous dynamic process that occurs during the lifetime of a shark; it can only be directly observed and measured in living animals. However, some indirect evidences can provide the palaeontologist some valuable information about tooth replacement rates in fossil sharks. In the present paper we estimate a qualitative rate of tooth replacement for *Leonodus carlsi*, the earliest tooth-bearing shark known to date from the Lower Devonian of Spain, discussing also tooth replacement speed in other Devonian sharks.

Previous studies of *Leonodus carlsi*

Leonodus carlsi was originally erected by Mader (1986) for isolated diplodont teeth from the lowermost Lochkovian to Pragian (Lower Devonian) of the Iberian Chains and Cantabrian Mountains (Spain). Later, disarticulated *Leonodus* teeth were reported from the Lochkovian and Pragian of other Spanish localities (Wang 1993; Valenzuela-Ríos & Botella 2000; Botella & Valenzuela-Rios 2005) and from the Lochkovian of Belgium (Blieck & Goujet 1991).

Although some authors have proposed acanthodian affinities for *L. carlsi* (Goujet 1993; see also Janvier 1996), currently, most early chondrichthyans specialists consider *L. carlsi* to be the oldest unequivocal shark teeth of the fossil record (e.g. Long 1995; Long & Young 1995; Ginter & Ivanov 1996; Hampe & Long 1999; Williams 2001; Miller *et al.* 2003; Soler-Gijón & Hampe 2003; Turner 2004; Ginter 2004; Turner & Miller 2005; Botella *et al.* 2005; Botella 2006). However, recently Turner & Miller (2008) have suggested that teeth of *Dendrodus arisaigensis* from the Late Silurian of Canada might be the oldest shark teeth, although as these authors noted, the affinities of *Dendrodus* to the chondrichthyans still remain unclear.

Mader (1986) provided detailed morphological and histological descriptions of *Leonodus* teeth, summarized in Figure 1 and below. The base of the bicuspidate teeth is concave (noted that it is convex in the upper side and slightly concave in the basal side), wider than long, (considering the width as linguo-labial and the length as anteromedial–posterolateral direction, according to Johnson 1980) and ranges from less than 1 mm to 5 mm in width. Both 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. The cusps' cross-sections are rounded or oval. The ornamentation, extraordinarily well preserved, is limited exclusively to the upper part of the cusps. It is smooth on the lingual side, while on the labial side there are numerous chevron-shaped ridges that converge towards the top of the cusp. Histologically, teeth are formed by a spongy tissue composed of travecular dentine, crossed by numerous vascular channels; in the base and in the proximal part of the cusp there are vascular channel openings. At the tops of the cusp, an orthodentine layer (not present according Hamper & Long 1999) is covered by a relatively bulky, single crystallite enameloid layer (durodentine *sensu* Mader 1986), which clearly differentiated from the internal dentine (Botella *et al.* in press).
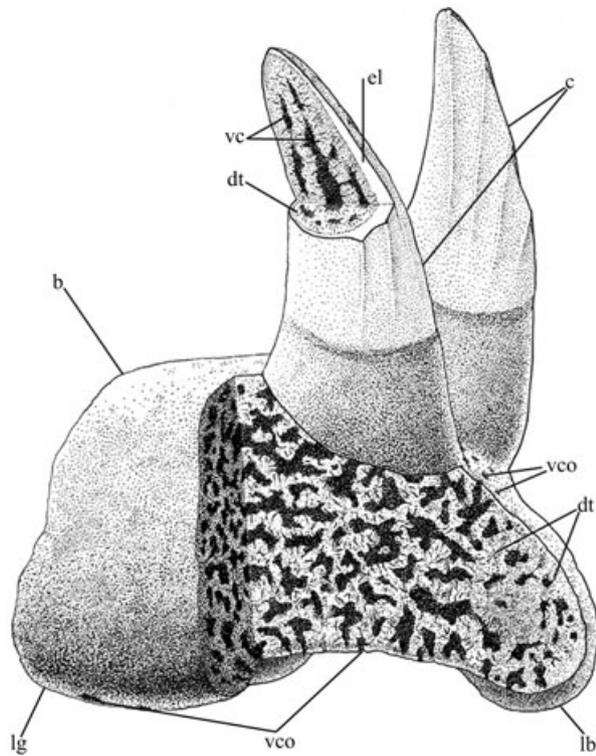
Fig. 1. Reconstruction of generalized morphology and histology of teeth of *Leonodus carlsi* Mader, 1986. Teeth are formed by a spongy tissue constituted of travecular dentine crossed by numerous vascular channels. At the top of the cusp an orthodentine layer is covered by a relatively bulky enameloid tissue layer clearly differentiated from the internal dentine, this layer being notably thicker on the labial side; b, base; c, cusps; dt, dentine tubules; el, enameloid; lb, labial process; lingual process; vc, vascular channels; vco, vascular channels openings. Not to scale.

Wang (1993) studied more than 1500 specimens from the Lower Devonian of Celtiberia (Wang 1993; table 13, pp. 100–107) and observed that some of these teeth differ from the original description of Mader (1986). In order to explain these differences Wang defined several new *Leonodus* 'species' and morphotypes such as: *Leonodus* sp. A (Wang 1993, pl. VI, figs 12–13), smaller teeth with four cusps; *Leonodus* sp. B (Wang 1993, lam. VI, figs 7–8) with a longer than wide base and two short, thin and parallel cusp; *Leonodus* sp. B → sp. A (Wang 1993, pl. VI, figs 9–11) with small, additional cusplets in the internal part of one or both main cusps; *Leonodus* sp. C (Wang 1993, pl. VIII, figs 1–3) similar to *L. carlsi* in size and shape, but with a thickening in both sides of the base associated to the cusps; *Leonodus* sp. 'Kurze' form, 'Lange' form or 'unicuspidate' form (see Wang 1993, pl. VI, fig. 14; pl. VII, figs 1–8). Besides, 14 tricuspidate teeth, with a similar base to that of *Leonodus* sp. B, were defined as *Phoebodus ancestralis* (Wang 1993, pl. VI, figs 1–6). Some authors suggest that erection of some of these species seems to be

incorrect and unnecessary (Ginter 2004; Turner 2004). We share the same opinion, and consider that most of these morphologies (or probably, all of them) must be treated as different tooth types of a unique species, *Leonodus carlsi*, showing a gradual monognathic heterodonty. Accordingly, *Phoebodus ancestralis* and *Leonodus* sp. A could belong to symphyseal teeth, *Leonodus* sp. C and typical teeth of *L. carlsi* to lateral teeth, and *Leonodus* sp. B to posterior teeth. Otherwise, some teeth assigned to *Leonodus* sp. 'unicuspidate' form can be regarded as broken or pathological teeth. Thus, based on the study of some anomalous *Leonodus* teeth, Botella (2006) provides definitive evidence of the existence of a dental lamina and continuous tooth replacement in *L. carlsi*.

Although *Leonodus* was first known only for isolated disarticulated teeth, Wang (1993; table 13) observed that, frequently, some characteristic chondrichthyan scales occur in association with *Leonodus* teeth, and assigned them to *Leonodus* sp. (Wang 1993, p. 107; pl. 8, figs 5–7). More recently, Soler-Gijón & Hampe (2003) described new semi-articulated material of *L. carlsi*, preserved in a single slab (also from the Lower Devonian of Celtiberia), which consists of numerous teeth, disarticulated fin spines, and patches of articulated scales.

Botella *et al.* (2005) describe the histological characters and the morphological variations (assignable to distinct areas of the animal's body), found in more than 200 isolated scales of *Leonodus*, and propose the association of these scales with *L. carlsi* teeth in a single natural assemblage. The new *Leonodus* material shown in Soler-Gijón & Hampe (2003) and Botella *et al.* (2005) provides definitive grounds as to their chondrichthyan taxonomic status (Turner & Miller 2005; M. Ginter, personal communication, 2006).

Material and methods

We have studied 1103 more or less complete teeth and broken cusps of *L. carlsi* from the Nogueras Formation (Lockhovian-Pragian) of Celtiberia, Spain (Carls 1988). All teeth appear as isolated elements coming from the dissolution of carbonate rocks with formic acid (5–10%). Of these, 460 come from localities in two different areas of the Iberian Chains (Fig. 2): (1) the Axial Depresion of the Cámaras River (ADCR) (Carls 1988); and (2) the Axial Depression of Nigüella (NI) (Valenzuela-Ríos 1989). The remaining teeth (643) were loaned by Professor Carls (Braunschweig University) and all come from ADCR. For a detailed geological and stratigraphical description of these two areas see Carls and Valenzuela-Ríos (2002), and for a detailed faunal succession see Carls (1999). All
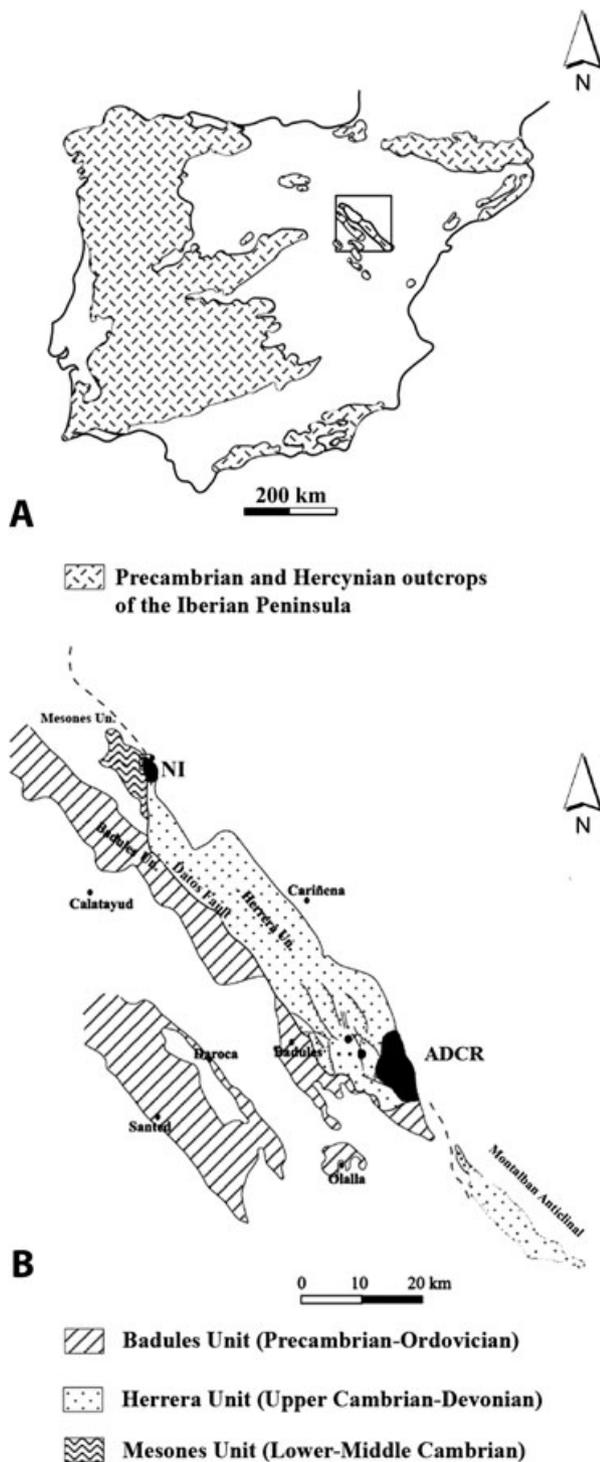
Fig. 2. A. Geographical setting of the studied area with indication of the distribution of Precambrian and Palaeozoic rocks in the Iberian Peninsula. B. Geological map of Palaeozoic outcrops in the Iberian chains pointed out the two studied areas. Devonian outcrops in black. NI, Axial Depression of Nigüella (Valenzuela-Ríos 1989); ADCR, the Axial Depression of the Camaras River (Carls 1988).

teeth referred in this paper are held in the Museum of Geology at the University of Valencia (MGUV).

For the histological study we used both a fluorescence microscope (Nikon Eclipse E800) and a scanning electron microscope (SEM). The fluorescence microscopy is based on the properties that certain substances present for absorbing the light of a specific wavelength (excitation light) and emitting light in another longer wavelength. Applications of fluorescence microscopy to investigations of dental hard tissues are based on the inherent fluorescence of teeth. If a specimen is irradiated with the desired wavelength (excitation light) and visualized using a filter that only lets through radiation of equal wavelength to that of the emitted light, then the fluorescing areas can be observed shining out against a dark background with high contrast. This method allowed us to distinct the internal structure of teeth. Studied specimens were embedded in epoxy resins, polished in oriented planes and studied in the fluorescence microscope at the Department of Cytology of the University of Valencia. For SEM investigations, a Philips XL-30, at the University of Valencia (Spain), was used.

Statistical analyses were performed with SPSS (version 15.0.1 for Windows; SPSS Inc., Chicago, IL, USA). We have used analysis of contingency tables together with the analysis of adjusted residuals. Analysis of contingency tables (see for example Quinn & Keough 2006) allows analysing the dependency or independency relationship of two kinds of attributes or variables (in our case different types of elements and the wear degree present in them). Generally, such tables have r rows, c columns and $r \times c$ cells; each cell is placed in a i-row and a j-column. Each ij-cell contains an observed frequency (n_{ij}) that must be tested against an expected frequency (E_{ij}) generated by the null hypothesis of independence. This is the characteristic structure of a chi-square test with $(r - 1)(c - 1)$ degrees of freedom. However, when the null hypothesis is rejected, this does not happen for all the cells. Hence, and in order to find those cells, we have used the analysis of adjusted residuals (for a detailed description of the technique see Everitt 1977).

Tooth replacement rate in *Leonodus carlsi*

Evidence from the tooth wear

First considerations on tooth replacement rate in early chondrichthyans was given by Newberry (1889) based on the high wear observed on isolated teeth of Late Devonian cladodont sharks from the Cleveland Shale. He noted that some teeth showed their main cusps
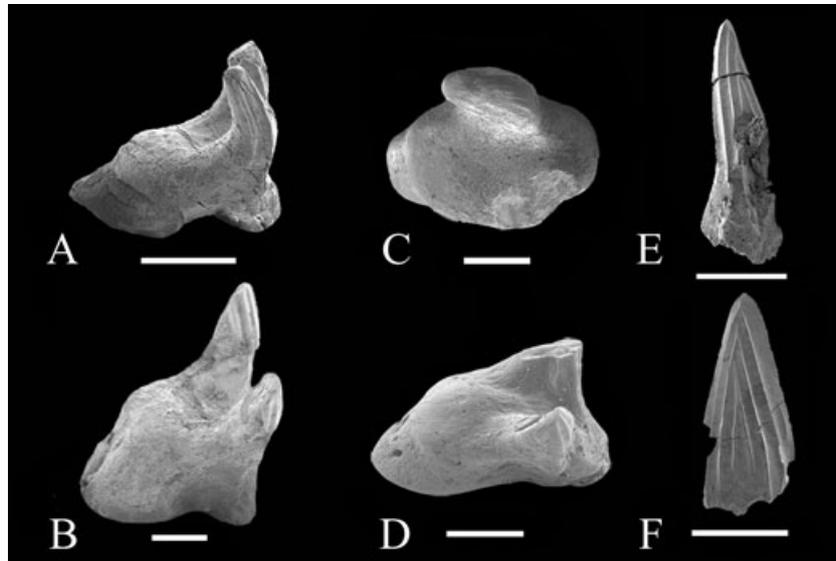
Fig. 3. *Leonodus carlsi* Mader, 1986. A. Entire tooth MGUV 14861 in lateral view. B. Entire tooth MGUV 19345 in lateral view with well-developed wear surfaces that produce an incisive top on the labial side of the cusps. C and D. Teeth with only one complete cusp and the other fractioned; C, MGUV 19346 in lateral view and D, MGUV 19347 in upper view. E and F. Isolated broken cusps of *L. carlsi* in labial view, MGUV 19846 and MGUV 19847 respectively. Scale bar, 200 µm.
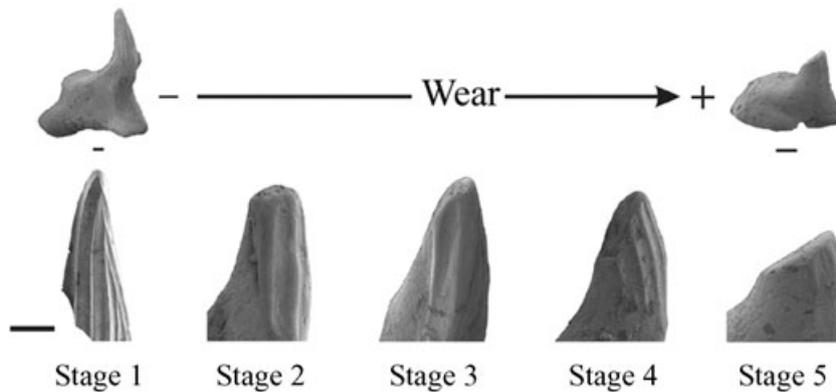

Fig. 4. Different wear present in teeth of *Leonodus carlsi* Mader, 1986. Teeth suffer a continuous wear from the moment they appear in the mouth until they are shed. The wear has been categorized in five stages for statistical analyses (Table 1). Noted that in advanced stages of wear there always remains a cutting edge inclined towards the labial part due to the unequal distribution of the enameloid tissue layer (see text). Scale bars, 200 µm.

completely worn (nearly to the base), and concluded that this implies that teeth remained functional for a long time, suggesting that tooth replacement was much slower than in modern sharks (see also Williams 1992, 2001).

Obviously, as Newberry (1889) and Williams (1992, 2001) pointed out, if tooth replacement is very slow there has to exist great differences between the wear of the functional teeth and the wear of newest formed teeth. Unfortunately, all *Leonodus* teeth found to date occur as isolated elements and do not show the true order of tooth arrangement. However, we found a great variation in the stage of wear at the cusps of *Leonodus* teeth examined, ranging from cusps without wear, to totally worn cusps (where the tops, with enamel-like tissue external layer, are completely reduced). In addition, in our collection the wear degree

of the cusps can be assessed, yielding three types of elements: (1) complete teeth (Fig. 3A, B), (2) teeth with only one complete cusp and the other fractioned (Fig. 3C, D) and (3) isolated broken cusps (Fig. 3E, F). Types 1 and 2 are constituted largely by teeth that were shed by the animal after having been functional, while the broken cusps (type 3) could originate from a tooth fracture on any of their functional stages, from the moment it appears in the mouth until the tooth is shed. It is important to note that tooth cusp breaking during the lifetime of a shark, due to feeding, is very usual (e.g. Budker 1971; Hubbell 1996; Shimana 1997). In order to facilitate the analysis of our observations, cusps are grouped into five different categories in function of their stage of wear (Fig. 4). We have quantified the wear degree in 736 specimens.

Table 1. Test of independence of the stage of wear and the type of element

	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Total
Entire teeth	$_{37.5}$ 7 $^{-6.45***}$	$_{53.6}$ 37 $^{-3.12**}$	$_{68.3}$ 93 $^{+4.36**}$	$_{17.6}$ 36 $^{+4.44***}$	$_{5.0}$ 12 $^{+3.7**}$	185
One-cusp teeth	$_{54.0}$ 17 $^{-7.05***}$	$_{77.2}$ 75 $^{-0.37}$	$_{98.3}$ 126 $^{+4.41***}$	$_{25.4}$ 37 $^{+3.03**}$	$_{7.1}$ 7 $^{-0.07}$	262
Broken cusps	$_{59.5}$ 127 $^{+12.6***}$	$_{85.2}$ 104 $^{+3.12**}$	$_{108.4}$ 56 $^{-8.18***}$	$_{28.0}$ 1 $^{-6.9***}$	$_{7.9}$ 1 $^{-3.19**}$	289
Total	151	216	275	74	20	736

$\chi^2 = 238.8$; d.f. $= 8$; $P < 0.000001$

One-cusp teeth are teeth with only one complete cusp and the other one fractioned. Each cell $_{eij} n_{ij}{}^{Rij}$, contains an observed frequency (n_{ij}), an expected frequency (e_{ij}) generated by the null hypothesis of independence, and the adjusted residual value (R_{ij}). Sign of R_{ij} indicates if the expected value is lower (positive sign) or larger (negative sign) than the observed value[25]. The significance of adjusted residuals is indicated by means of * for $0.05 > P \geq 0.01$, ** for $0.01 > P \geq 0.00001$ and *** for $0.00001 > P$.

Table 2. Increment of size in consecutive teeth (Δs) and available tooth replacement rates for species considered in the study

Species	Tooth size increment (Δs)	Mean tooth replacement rate	Reference
Mustelus canis	2.6%	10–12 days/row	Ifft & Zinn 1948
Negaprion brevirostris	3.9%	8–10 days/row	Moss 1967
Scyliorhinus canicula	10.3%	35 days/row	Märkel & Laubier 1969
Triakis semifasciata	3.1%	9–12 days/row	Reif *et al.* 1978
Ginglimostoma cirratum	7.7%	28 days/row	Reif *et al.* 1978
Carcharhinus plumbeus	8.1%	18 days/row	Wass 1973
Leonodus carlsi	61.7%	–	
Devonian cladoselachian (CMNH 9449)	29.2%	–	
Devonian cladoselachian (CMNH 5956)	23.3%	–	
Cladoselache clarki (BMNH 9272)	24.7%	–	

The rest of the material of our collection is not considered in this analysis since they it comprises isolated bases or elements showing evidence of taphonomic alteration or fresh breakage (probably during the extraction of the sediment).

This permits us to outline a null dependency hypothesis: the stage of wear is independent from the type of element that we consider. To test this hypothesis, we have carried out a statistical analysis, using contingency tables together with the analysis of adjusted residuals (Everitt 1977), indicating in which cases (cells of the table) the null hypothesis fails, in the event of being rejected.

Our results are given in Table 1. As it had been predicted, the null hypothesis is rejected with a high significance ($P < 0.000001$), therefore we can assert that both attributes are not independent. No significant differences are found between entire teeth and teeth with one cusp. In both categories, we found a greater number of teeth with a higher stage of wear (stages 2, 3 and 4) than the one that could be expected, and a smaller number with a low stage of wear. In contrast, the opposite occurs for the third category (isolated broken cusp), here the forms with little wear are over-represented and the forms with much more wear are under-represented (in all cases the statistics are highly significant, see Table 1). The more plausible explanation for these differences is a very slow rate of replacement, which causes teeth to be detached during lifetime, after having been used for a long time (rows 1 and 2), to present high wear, while isolated cusps that are fractured in earliest moments of the continuous replacement process show significantly lower wear. In addition, the results expressed in Table 2 also demonstrate that breaking of cusps occurs chiefly during the life of the animal and not during the subsequent taphonomic process. This is supported by the fact that if the cusp-break was the result of a post-mortem process, then wear in isolated cusps would not have demonstrated significant differences with those of the other two categories.

The maturational process of Leonodus teeth

Hickman (1980) described the process for which some functional structures use a maturational and degenerative route for optimizing their function (more synthetically 'of use' *sensu* De Renzi 1986). Hickman recognized that 'wear may refine rather than degrade some elements of morphological design', thus, 'the worn shape of a structure (rather than its pristine form) may be its most efficient working shape'.
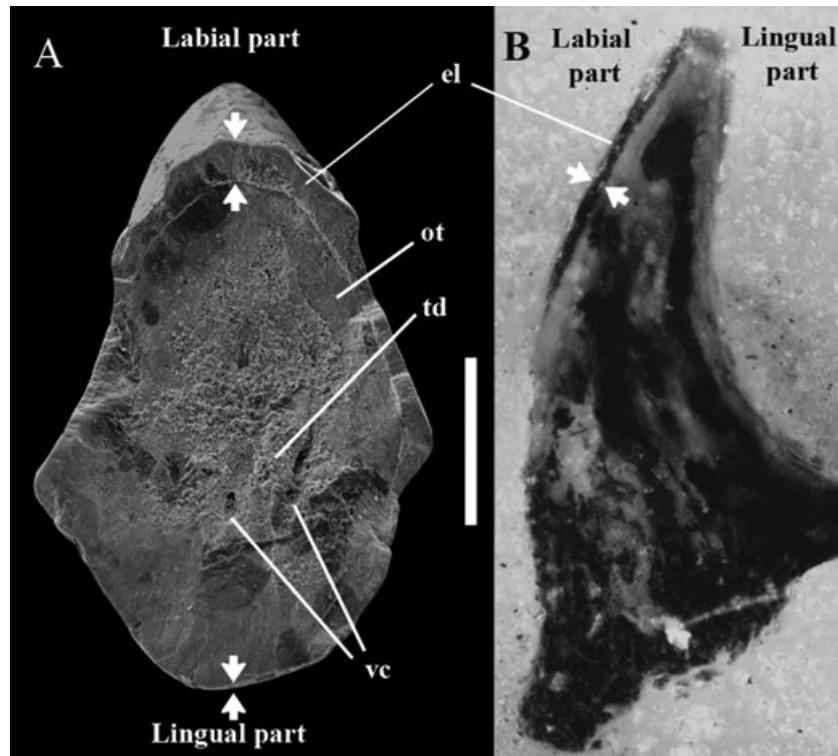
Fig. 5. Histology of teeth cusps of *Leonodus carlsi* Mader, 1986. A. Scanning electron microscope image, transverse fresh fractures of specimen MGUV 19343. B. Fluorescence microscopy (Nikon Eclipse E800) image, tangential thin section of specimen MGUV 16465; sample was excited with blue light, 420–490 nm. White arrows show the thickness of the enameloid capping layer. The thickness of the enameloid layer decreases from the labial part to the lingual part of the cusps (A). In B the lingual part of the cusp lacks completely the enameloid layer; ot, orthodentine; td, travecular dentine; el, enameloid; vc, vascular channels. Scale bar is 200 μm.

If we analyse the continuous wear process of *Leonodus* teeth reconstructed in Figure 4, it can be observed that, while the cusps become worn, an incisive top remains on the labial side and a sharp, pointing edge inclined towards the labial part is generated in middle and advanced stages of wear (stages 2 to 4); this means that efficient functional morphologies (i.e., fine penetrating and grasping cusps) are presented at any given stage of wear. This particular wear pattern could, somehow, balance the presence of a slow tooth replacement, accounting for the continual presence of an efficient dentition, regardless of how long the teeth remained in functional positions.

We propose that the generation of efficient worn shapes during wear process is a 'strategic' consequence of the exceptional, histological and morphological design of *Leonodus* teeth. As noted above, the tops of the cusp are covered by an external enameloid capping layer clearly differentiated from the internal dentine. Detailed examination of the cusp histology from both fresh fractures in SEM (Fig. 5A), and oriented fine sections in Fluorescence microscopy (Fig. 5B), demonstrates that the enameloid layer is notably thicker on the labial than in the lingual side of the teeth. In fact, the thickness of the enameloid layer decreases from the labial-most part of the cusps to the lateral sides (Fig. 5A) and in some teeth it is nearly absent in the lingual part of the cusps (Fig. 5B).

Additionally, as noted before, the ornamentation of *L. carlsi* tooth cusps consists of numerous chevron-shaped ridges, but they are only present on the labial side (see Figs 2, 3). This ornamentation with ribs or ridges has a mechanical reinforcement function for the tooth (Cappetta 1986).

As a consequence of the unequal distribution of the enamel-like tissue layer ('strategically' thicker on the labial part), asymmetrical wear is produced (greater on the lingual than on the labial side of the cusp). This generates a lingually tilted wear plane, developing an incisive top on the labial side of the cusps during wear process. Furthermore, the chevron-shaped ridges (Fig. 3F) allows for the constant presence of a sharp pointed edge in worn cusps. In other words, *Leonodus* teeth seem to 'be designed' to be sharpened by use.

This process for which functional structures use a maturational and degenerative route in order to optimize their function has never been describe in modern shark teeth, with rapid replacement rates, and provide

independent evidences of very slow tooth replacement in *L. carlsi*, since then teeth must stay in the mouth long enough to be modelled by use.

Evidence from the size increment of consecutive teeth

In addition to the renewal of worn or broken teeth, another important function of the tooth replacement process is the maintenance of teeth of appropriate size and tooth spacing during the animal growth (Luer *et al.* 1990; Williams 2001). The functional teeth size increases as the total size of the shark does (Strasburg 1963; Moss 1967, 1972; Luer *et al.* 1990). Thus, every newest formed tooth needs to be longer than the preceding one, and consequently, the teeth size of the same tooth family increases from outer to inner rows (Strasburg 1963; Moss 1972). The size difference between teeth of the same dental family is inversely proportional to the rate of replacement (Moss 1972; Williams 2001). Based on this, the size-difference between teeth from the same tooth family has been related to the rate of replacement. Accordingly, Williams (2001) proposed the presence of very slow tooth replacement in Late Devonian cladoselachian sharks, in which teeth on opposite ends of the tooth family show a great difference in size, with the youngest teeth often being twice as big as the functional one (see also Williams 1990; 1992), because this implies substantial growth in the shark before the newest formed teeth reach the functional position. In addition, Botella (2006) has suggested the presence of slow tooth replacement in *L. carlsi*, based on the discovery of a pathological tooth which consists on the fusion of two adjacent teeth within the same family where the size of the lingual tooth is 62% greater than that of the labial one (Fig. 6).

In Table 2 we estimate the size increment (Δs) in consecutive teeth for six species of recent sharks (*Mustelus canis*, *Scyliorhinus canicula*, *Triakis semifasciata*, *Ginglimostoma cirratum*, *Carcharhinus plumbeus* and *Negaprion brevirostris*), whose mean tooth replacement rates are available in the literature (see Luer *et al.* 1990 for references). Δs (in %) has been estimated, according to Moss (1972), as the average increment in the maximum widths of the exposed (enamelled) portion between consecutive teeth of the same tooth family:

$$\Delta s = [\Sigma(\text{width tooth}_{ij} - \text{width tooth}_{ij-1})/ \text{width tooth}_{ij}]/N \text{ obs.}$$

where *i* is the family number and *j* is the row number.

Teeth measurements for *Scyliorhinus canicula*, *Triakis semifasciata* and *Carcharhinus plumbeus* were
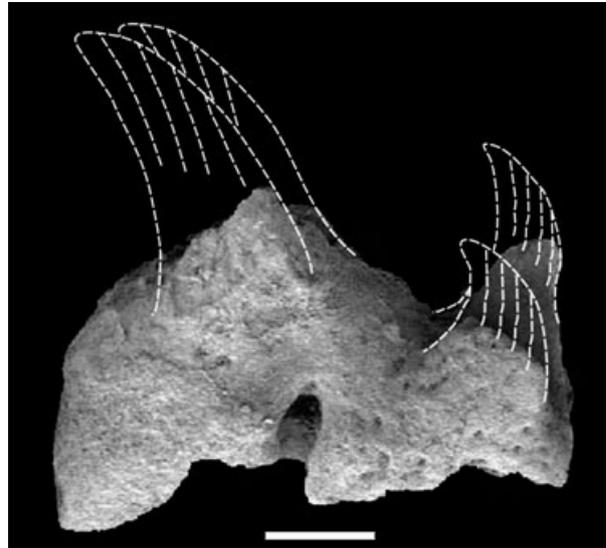

Fig. 6. Pathological fused teeth of *Leonodus carlsi* Mader, 1986, MGUV 14826, in lateral view with restoration of the cusps (in white). Scale bars equal 500 μm.

taken from carcasses coming from L'Oceanografic aquarium, Valencia (Spain) and specimens captured by commercial fisheries in the Mediterranean coast of Spain and in the Atlantic coast of Northern Uruguay. *Ginglimostoma cirratum* measurements were taken from figured material (Luer *et al.* 1990, fig. 2). The average increment in tooth size for *Mustelus canis* and *Negaprion brevirostris* was provided by Moss (1972, fig. 1) and Moss (1967, fig. 22–2) respectively.

Size increment averages are plotted against mean tooth replacement rates in Figure 7A. The relation between these variables was assumed to be linear, and the regression line was calculated by using a least-squares method. It is clear that there is a high correlation ($R^2 = 0.8327$) between size increments of consecutive teeth and tooth replacement rates for the considered species.

Additionally, in Table 2, Δs was estimated for the Early Devonian shark *L. carlsi* and for three specimens of Late Devonian cladoselachian sharks from the Cleveland Shale, USA (Williams 2001). For cladoselachian sharks the measurements were taken from specimens CMNH 5956 and CMNH 9449 figured in Williams (1992, 2001) and BMNH 9272 (*Cladoselache clarki*) figured in Claypole (1895) and Ginter (2002). For *L. carlsi* Δs was estimated from the pathological fussed teeth MGUV 14.826 (Fig. 6). Values of Δs for these fossil sharks were plotted on the Δs versus tooth replacement rate regression line obtained for the extant sharks in Figure 7B. As it can be observed available mean values of tooth replacement rates for current sharks range between
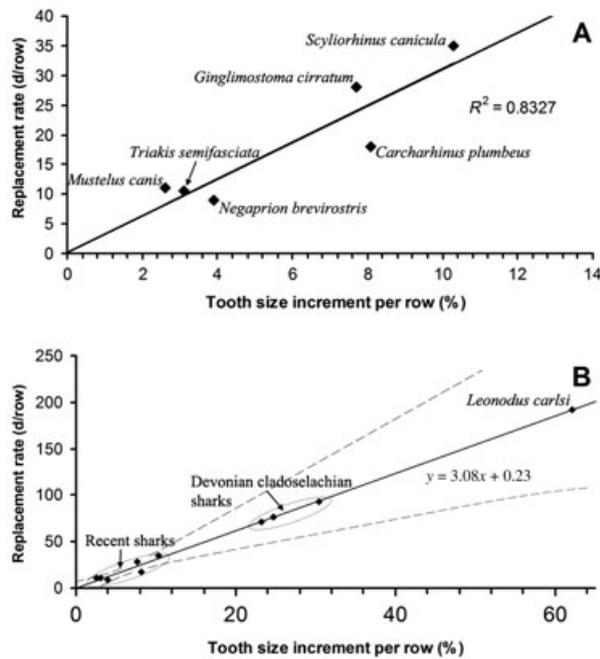
Fig. 7. A. Least squares regression of tooth replacement rates, in days/row, against increment of size in consecutive teeth (Δs), in %, for current sharks considered in our study; Δs was estimated as the average increment in width between consecutive teeth of the same family. B. Extrapolated values of tooth replacement rates, obtained from the Δs (see Table 1), for *Leonodus carlsi* and Devonian clado-selachian sharks on the regression line (black) calculated in A; dashed line indicates 95% confidence interval.

8–10 days/row in *Negaprion brevirostris* to 35 days/row in *Scyliorhinus canicula*. The extrapolated value of tooth replacement rate obtained for *L. carlsi* is around 190 days/row, which is up to 10 times slower than the mean value achieved for current sharks (18.5 days/row). Similar results are obtained for Late Devonian cladoselachian sharks, whose extrapolated values of tooth replacement rates are in the order of 75 to 85 days/row, a figure also considerably much slower than the measured rates for extant sharks. Therefore, despite of the limitations of our approach (see below), it can be stated that both *L. carlsi* and the Late Devonian cladoselachian sharks considered in this study would have had extremely slow tooth replacement rates in comparison with those of extant sharks, even considering an ample confidence interval for the obtained values (95%, see Fig. 7B). However, numerical values of tooth replacement rates obtained here for fossil chondrichthyans should be regarded as approximations at best, and it is necessary to take into account the following limitations of our approach. First, available values of tooth replacement rates in current sharks are restricted to a small number of species. Second, it has been shown that replacement rate of a given species can be affected by several factors such as diet (see Moss 1967), seasonal changes (i.e. Luer *et al*. 1990 reported punctual

tooth replacement rates up to 70 days/rows in winter for a specimen of *Ginglymostoma cirratum*) or the age of the animals (see Wass 1973). In this sense, the rates of tooth replacement seem to be faster in young individuals than in sexually mature specimens (Wass 1973; Reif *et al*. 1978) while the Δs between consecutive teeth is greater in juveniles that in mature animals (see Moss 1967, 1972; Strasburg 1963). Third, occurrences of '*in situ*' tooth families are very scarce in Devonian sharks, and the way of preservation often does not allow for the precise measurements of Δs between consecutive teeth (see Williams 2001). Thus, the only available data for *L. carlsi* come from the pathological fussed teeth MGUV 14.826. For Late Devonian cladoselachian sharks, the Δs measurements were taken from figured material (see above), although the attained measurements accurately correspond to the size increment estimations provided by Williams (2001, pp. 217, 220–21).

Notwithstanding these remarks, based on the high differences between current sharks and primitive ones showed in Table 1 and Figure 7B, it is clear that any variation in the calculated Δs can only affect slightly the numerical part of our results, keeping the qualitative aspects of our conclusions valid. Thus, this analysis, together with the other two shown above, provides strong evidences on the existence of extremely slow tooth replacement in *L. carlsi*. This very slow replacement is also evidenced for Late Devonian cladoselachian sharks, as was previously suggested by Williams (1990, 1992, 2001) although in this case the worn cusps do not show the sharp and pointed shapes as in *Leonodus* but more rounded and blunt (see Newberry 1889; Williams 1992, 2001).

Conclusions

The continuous replacement of teeth during lifetime is considered to be a feature common to most chondrichthyans (see above), and it seems to be present in the oldest tooth-bearing sharks know to date (e.g. Miller *et al*. 2003; Botella 2006). Available data of tooth replacement rates in current sharks show that they are in general rapid, ranging from 8–10 days/row in *Negaprion brevirostris* (Moss 1967) to a maximum of 5 weeks/row in *Scyliorhinus canicula* (Märkel & Laubier 1969). Nevertheless various authors have noted that tooth replacement rates of early sharks must have been much slower than those observed in current species (e.g. Newberry 1889; Maisey 1984; Paterson 1992 *in* Janvier 1996; Williams 1992, 2001). Obviously, tooth replacement rates cannot be directly

measured in fossil animals, but some indirect evidence, as we have shown in this paper, can supply some information in this respect. Thus, our study of 1,103 isolated teeth of *L. carlsi* from the Nogueras Formation (Lockhovian-Pragian, Celtiberia, Spain) provides strong evidence of an extremely slow dental replacement in this primitive chondrichthyan. This very slow rate of tooth replacement is inferred from three independent analyses:

1. Statistical analysis of the degree of wear in three different types of elements of our collection: entire teeth, teeth with only one complete cusp, and the other fractioned and isolated broken cusps. Analysis of contingency tables together with an analysis of adjusted residuals (Everitt 1977) shows that the wear degree is significantly ($P < 0.000001$) greater in the cusps of complete teeth and teeth with only one cusp than in broken isolated cusps. Broken cusps could be originated from a tooth fracture on any of the tooth-functional stages (from the moment it appears in the mouth until the tooth is shed). On the other hand, we can assume that entire teeth and teeth with only one cusp are mostly elements that were shed by the animal after having been functional. The differences we have found in wearing degree indicate that those teeth remained in functional position during long periods of time, which, in turn, indicates a very slow tooth replacement.

2. The unequal distribution of the cusp external enameloid tissue layer, which is notably thicker on the labial than in the lingual part, together with the ornamentation of the cusp-tips (chevron-shaped ridges placed only on the labial side), produces an unequal wear. Thus, while the tooth is worn down, an asymmetrical wear of the cusp is produced (less on the labial than on lingual side), and a sharp edge, inclined in a labial-lingual direction, is formed. In this regard, we can observe that teeth with a medium or advanced wear degree show a more efficient functional morphology, developing a sharp and penetrating cusp. This process, by which functional structures pass through a maturation route in order to optimize its function (Hickman 1980), also indicates a slow rate of replacement in *Leonodus*, since the tooth must remain enough time in the mouth to be 'modelled' by use.

3. Size difference between teeth of the same dental family is inversely proportional to the rate of replacement (Moss 1972; Williams 2001). We have estimated the average size increments (Δs) in consecutive teeth for some extant sharks species whose mean tooth replacement rates were available in the literature (Luer *et al.* 1990 and references therein). Our study shows a high correlation between Δs and tooth replacement rate in current sharks ($R^2 = 0.8327$). We have also estimated Δs in *L. carlsi* (from the pathological tooth MGUV 14.826, which consists of the fusion of two successive regular teeth of the same tooth family) and in three specimens of the Late Devonian cladoselachian sharks whose dental families were preserved *in situ*. The extrapolated value of tooth replacement rate obtained for *L. carlsi* is around 10 times slower than those observed in current sharks. Similar results are obtained for the Late Devonian cladoselachian sharks (up to four times the mean value of the current sharks).

Taken together, this evidence allows us to affirm that *L. carlsi* had an extremely slow tooth replacement. In addition, our analysis confirms the proposal of Williams (1992, 2001) about the presence of slow tooth replacement for Late Devonian cladoselachian sharks from the Cleveland Shale. Slow tooth replacement rate has been suggested as a common characteristic to early chondrichthyans as consequence of a still rudimentary dental lamina (Paterson 1992 *in* Janvier 1996), and could be interpreted as a primitive feature in higher (i.e., those with dental lamina) Gnathostomata. This scarce production of teeth could explain 'partially' the absence of chondrichthyan teeth in Upper Ordovician and Silurian sediments and the small number of chondrichthyan teeth records even all through the Lower Devonian.

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