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Chondrichthyans from the Spanish Triassic (westernmost Tethys) with remarks on the evolution of chondrichthyan enameloid

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Hago constar:

que la presente Tesis Doctoral titulada “**Chondrichthyans from the Spanish Triassic (westernmost Tethys) with remarks on the evolution of chondrichthyan enameloid**” que presenta **Esther Manzanares Ubeda** para la obtención del título de Doctora, ha sido realizada bajo mi dirección, con el mayor aprovechamiento.

Y para que así conste, en cumplimiento de la legislación vigente, expido el presente certificado en Valencia, a 18 de junio de 2020.

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SUMMARY

The Triassic sediments of the Iberian Peninsula has been the focus of Geological and Paleontological studies for more than a century and the stratigraphic sequence and the invertebrate assemblages are well established for a number of different basin. However, works focused on chondrichthyans faunas were non-existent until recently. As a consequence, it has been long time assumed that chondrichthyans were very scarce (or inexistent) in the coastal environments of the current Iberian Peninsula during Triassic times.

This view changed at the beginning of the last decade, with the studies of paleontologist from the department of Geology of the University of Valencia (Dra. Márquez-Aliaga, Dr. Héctor Botella, Dr. Pablo Plasencia and Dr. Martínez-Pérez). Their first studies already demonstrated that the shark faunas from Middle Triassic of the Iberian Chains (East of Spain) are comparable (in terms of diversity and abundance) with other worldwide localities of the same age, and even a number of endemic taxon were erected. Preliminary studies at Bugarra section yield abundants disarticulated remains of chondrichthyans exoesqueleton (mainly teeth and scales) including teeth of *Pseudodalatias henarejensis*, a new endemic species of pseudodalatiid. But it wasn't until 2013, when the first comprehensive taxonomic study of the chondrichthyan assemblage of several Middle Triassic sections from the Iberian Range was published. The assemblage consisted of isolated remains of seven species of five non-neoselachian shark genera (*Palaeobates*, *Hybodus*, *Pseudodalatias*, *Omanoselache*, and *Lissodus*), including a new species of hybodontiform shark, *Hybodus bugarensis*.

This assemblage represented a varied group from a paleogeographic point of view made up of common genus from Middle Triassic shark faunas of northern Europe together with species only known previously from North America and China. This disparity in the ‘paleobiogeographical’ distribution of the species was suggested to be linked with the Ladinian marine transgression, which allowed for the colonization of the shallow epicontinental seas of the Iberian Peninsula by faunas from the Germanic domain to the north and the Sephardic domain to the south of the Tethys sea. However further studies were required to test this hypothesis and to fully understand the faunal colonization patterns of the “new-developed” epicontintental seas of the Iberian seas, which can be an excellent proxy to compare differences in colonization process between differences groups in a long-time ecological context.

After this, the project of the present thesis chondrichthyan faunas found in the Betic and the Catalanonian Coastal Ranges are taxonomical similar to that described from the Ladinian of the Iberain Range , the relationship with other Triassic localities of the Tethys sea and the possible pathways for the colonization of the new shallow epicontinental seas of the western Tethys followed by coastal sharks and to compare it with other faunas, adjusting of previous palaeogeographic reconstructions of the westernmost Tethyan realm for the Middle Triassic.

Due to the cartilaginous nature of the skeleton of chondricthyans, their most common fossil remains are their teeth and scales. So, apart from the systematic classification of the chondrichthyan remains based on morphological characteristics, histological studies of the tissues that composed them has been realizing since the 19th century. Chondrichthyes teeth are covered by a thin layer of hypermineralized tissue known as enameloid with an intricate hierarchical organization. The smallest repeating structural units are individualized elongated fluoroapatite crystallites [$Ca_5(PO_4)_3F$], each formed by a number of hexagonal fluoroapatite unit cells. In a higher level of structural complexity, crystallites are arranged in parallel, forming tightly packed bundles, each with an envelope of organic matrix. Bundles with different orientations make assemblies that can be found in different layers, forming the whole enameloid layer of the teeth. The acquisition of these hierarchical structural levels has been progressive along the phylogeny of Chondrichthyans and traditionally has been related to the emergence of new feeding strategies in the group (i.e grasping and shallowing, grinding, crushing, cutting, etc). Through the last century, the study of the the micro and ultrastructure of the enameloid layer has been of great importance in order to differentiate teeth of neoselachian (batomorphs and selachimorphs), and in understanding higher-level taxonomic relationships and the evolutionary history of the group. It was assumed that the teeth of neoselachian, more precisely selachimorphs, are organized in a triple layer enameloid, whereas for batoid different works reported the presence of a single layer of TBE (Tangled Bundled Enameloid) or of SCE (Single Crystallite Enameloid). This resulted in the emergence of a debate centred around whether this ‘simplified’ enameloid is a derived or a retained ancestral character for the group. However, enameloid microstructure has never been studied in a broad phylogenetically and/or anatomical context. Accordingly, the study of the enameloid layer has demonstrated the presence of an homogeneus SCE layer in euselachian scales, the teeth of Lonchidion derenzii and most of extant batoid; whereas all Archaeobatoidae and *Rhyna ancylostoma* present a bundled enameloid

RESUMEN

1. INTRODUCCIÓN

Los sedimentos del Triásico de la península Ibérica han sido objeto de estudios geológicos y paleontológicos durante más de un siglo, por lo que la secuencia estratigráfica y las asociaciones de invertebrados fósiles están bien establecidos para una serie de cuencas diferentes. Sin embargo, los trabajos centrados en las faunas de los condrictios no existían hasta hace poco. Como consecuencia, se ha supuesto durante mucho tiempo que los condrictios eran muy escasos (o inexistentes) en los entornos costeros de la actual península Ibérica durante el Triásico.

Este punto de vista cambió a principios de la última década, con los estudios de paleontólogos del departamento de Geología de la Universidad de Valencia (Dra. Márquez-Aliaga, Dr. Héctor Botella, Dr. Pablo Plasencia y Dr. Martínez-Pérez). Sus primeros estudios ya demostraron que las faunas de tiburones del Triásico Medio de las Cadenas Ibéricas (este de España) son comparables (en términos de diversidad y abundancia) con otras localidades mundiales de la misma edad, incluso llegando a erigir varios taxones endémicos. Los estudios preliminares en la sección de Bugarra arrojan abundantes restos desarticulados del exoesqueleto de condrictios (principalmente dientes y escamas), incluidos los dientes de Pseudodalatias henarejensis, una nueva especie endémica de pseudodalatido. Pero no fue hasta 2013, cuando se publicó el primer estudio taxonómico exhaustivo del conjunto de condrictios de varias secciones del Triásico Medio de la Cordillera Ibérica. Esta asociación está compuesta por restos aislados de siete especies de cinco géneros de tiburones no neoselachianos (Paleobates, Hybodus, Pseudodalatias, Omanoselache y Lissodus), incluida una nueva especie de tiburón hibodontiforme, Hybodus bugarensis. Este conjunto representaba un grupo variado desde un punto de vista paleogeográfico compuesto por varios géneros comunes de faunas de tiburones del Triásico Medio del norte de Europa junto con especies que solo se conocían anteriormente en América del Norte y China. Se sugirió que esta disparidad en la distribución 'paleobiogeográfica' de las especies estaba relacionada con la transgresión marina del Ladiniense, que permitió la colonización de los mares epicontinentales poco profundos de la Península Ibérica por faunas desde el dominio germánico al norte y el dominio sefardí hasta el sur del mar de Tethys. Sin embargo, se requirierían más estudios para probar esta hipótesis y comprender completamente los patrones de colonización faunística de los mares epicontintentales "recién desarrollados" de los mares ibéricos, que pueden ser un excelente proxy para comparar las diferencias en el proceso de colonización entre grupos de diferencias en un largo período de tiempo dentro de un contexto ecológico.

A partir de estos antecedentes, el proyecto de la presente tesis surge de la necesidad de conocer en profundidad la diversidad de las asociaciones de condricitios de otras cuencas del Triásico de la península Ibérica, la relación con otras localidades del Triásico del mar de Tethys y las posibles vías para la colonización de los nuevos mares epicontinentales poco profundos del oeste de Tethys por tiburones costeros y para compararlo con otras faunas, ajustando las reconstrucciones paleogeográficas anteriores del reino del Tethys más occidental para el Triásico Medio.

El grupo más destacado de condricitios durante el Triásico fue el Hibodontiformes. Se les considera el grupo hermano de los neoseláceos y sus dientes presentan una amplia gama de morfologías, lo que indica la diversidad de presas de las que se alimentan. Este grupo de condrictios se distribuyó globalmente durante el Triásico, pero parecen tener más éxito en el Triásico Medio de los Palaeotethys. La Península Ibérica estaba situada en la parte más occidental del mar de Tethys, y la expansión de los Neotethys condujo al desarrollo de cinco ciclos marinos transgresivos -regresivos que cambiaron en la paleogeografía de la parte más occidental de los Tetis. Estas alternancias de los ciclos de regresión y transgresión marina implicaron frecuentes cambios ambientales en las zonas marinas de la placa ibérica. Para este doctorado, se estudiaron dos cordilleras: la cordillera Bética y la Costera Catalana. En ambos, los sedimentos de las secciones estudiadas registran los eventos del segundo y tercer ciclos marinos transgresivos-recesivos.

Debido a la naturaleza cartilaginosa del esqueleto de los condrictios, sus restos fósiles más comunes son sus dientes y escamas, debido a su alta mineralización. Por lo tanto, aparte de la clasificación sistemática de los restos de condrictios basados en características morfológicas, los estudios histológicos de los tejidos que los componen se han estado realizando desde el siglo XIX. Los dientes de los condrictios están cubiertos por una capa delgada de tejido hipermineralizado conocido como esmaltoide con una intrincada organización jerárquica. Las unidades estructurales más pequeñas que se repiten son cristalitos de fluoroapatito alargados individualizados [Ca5 (PO4) 3F], cada uno formado por una cantidad de células unitarias de fluoroapatito hexagonales. En un nivel más alto de complejidad estructural, los cristales están dispuestos en paralelo, formando haces apretados, cada uno con una envoltura de matriz orgánica. Los paquetes con diferentes orientaciones forman conjuntos que se pueden encontrar en diferentes capas, formando la capa de esmaltoide completa de los dientes. La adquisición de estos niveles estructurales jerárquicos ha sido progresiva a lo largo de la filogenia de los condrictios y tradicionalmente se ha relacionado con la aparición de nuevas estrategias de alimentación en el grupo (es decir, agarraradora, trituradora, machacadora, cortante, etc.). A lo largo del siglo pasado, el estudio de la microestructura y la ultraestructura de la capa de esmaltoide ha sido de gran importancia para diferenciar los dientes de neoseláceos (batomorfos y selacimorfos) y para comprender las relaciones taxonómicas de alto nivel y la historia evolutiva del grupo. Se asumió que los dientes de neoselaceos, más precisamente selacimorfos, están organizados en una triple capa de esmaltoide, mientras que para batoideos diferentes trabajos informaron la presencia de una sola capa de TBE (Tangled Bundled Enameloid, en sus siglas en inglés) o de SCE (Single Crystallite Enameloid). Esto dio lugar a la aparición de un debate centrado en si este esmaltoide "simplificado" es un carácter ancestral derivado o retenido para el grupo. Sin embargo, la microestructura del esmatloide nunca se ha estudiado en un contexto filogenético y / o anatómico amplio.

2. OBJETIVOS

En consecuencia, los dos objetivos principales de esta tesis doctoral son ampliar el conocimiento de la taxonomía de las comunidades de condrictios que habitaron la costa de la península Ibérica durante el Triásico Medio-Superior y la evolución de la capa de esmaltoide, más precisamente, la SCE en los dientes y escamas de hibodóntidos y batomorfos. Los objetivos específicos para desarrollar esta tesis doctoral se han dividido en dos grandes bloques bloques:

Bloque 1: Estudio sistemático y biogeográfico de los condrictios de la península Ibérica del Triásico Medio.

- Estudio sistemático de los restos de condictios recuperados de secciones de la cordillera Bética (Capítulos 3 y 4)

- Estudio sistemático de los restos de condricitios recuperados de la cordillera Costera Catalana (Capítulo 5)

- Estudio biogeográfico de los restos recuperados en las cordilleras Ibéricas, Béticas y Costero Catalanas en relación con las faunas de tiburones del mar más occidental de Tetis (Capítulo 5)

Bloque 2. Estudio de la capa de SCE en el esmaltoide de condrictios euseláceos

- Caracterización de la microestructura esmaltoide en escamas de selacios (Capítulo 6)

- Caracterización del esmaltoide en dientes de batoideos basales y actuales (Capítulos 7 y 8 respectivamente)

3. METODOLOGÍA

Debido a que la presente tesis doctoral es una recopilación de artículos científicos, todo el material y los métodos están recogidos en cada uno de los trabajos que componen el cuerpo principal, y por lo tanto, no se ha incluido un capítulo separado de metodología. Aun así, en los párrafos siguientes se incluye una explicación sintetizada de los métodos y equipos utilizados.

El material estudiado en el primer bloque de trabajos de mi tesis se obtuvo mediante la dilución ácido en ácido acético al 5-10% de diferentes rocas de las secciones estudiadas durante diferentes campañas a lo largo de varios años. Después de la dilución, los residuos se tamizaron con tamices de mallas de 2, 0,125 y 0,063 mm respectivamente, y los fósiles se recogieron con un microscopio binocular y se fotografiaron con el microscopio electrónico HITACHII S-4800 alojado en los Servicios de Microscopía de la Universidad de Valencia.

Para el estudio de la capa SCE en condrictios, se ha utilizado la técnica conocida como “lámina delgada” en los dientes y escamas. Para este bloque de trabajos, además de los fósiles recuperados, también se han estudiado dientes de batomorfos actuales cedidos por el museo de La Cau del Tauró; y dientes de batomorfos primitivos cedidos por el profesor Dominique Delsate, del Musée national d'histoire naturelle de Luxembourg. Primero, los restos se incluyen en bálsamo de Canada a 120° C durante dos horas para endurecer el bálsamo y poder orientar la muestra según el plano en el que se vaya a realizar el pulido. Posteriormente, las muestras incluidas son pulidas con carborundum (800-1200 mm) hasta alcanzar el plano de sección deseado (longitudinal o transversal). Posteriormente, los fósiles incluidos son atacados con un ácido durante un período de tiempo variable (HCl diluido al 10% con una duración de 5 a 10 s; ácido ortofosfórico diluido al 0,5% durante 1 o 2 minutos; o en algunos casos hidróxido peróxido durante un periodo de tiempo de tres horas a tres días). Para el último paso, todos los restos seccionados se recubren con una aleación de oro-paladio y se fotografían en un microscopio electrónico de barrido Hitachi S-4800 en el Servicio de Microscopios de la Universitat de València.

Una vez que los dientes y las escamas se clasifican, seccionan y fotografían adecuadamente, se alojan en el Museo de Historia Natural de la Universidad de Valencia (antiguo Museo Geológico de la Universidad de Valencia) y se les asigna un número de museo (MGVU-).

4. RESULTADOS

BLOQUE 1: ESTUDIO SISTEMÁTICO Y BIOGEOGRÁFICO DE LOS CONDRICTIOS DE LA PENÍNSULA IBÉRICA DEL TRIÁSICO MEDIO.

Capítulo 3. Condrictios del Triásico Medio-Superior restos de la Cordillera Bética (España)

En el presente estudio, describimos, por primera vez, la fauna de condrictios de varias secciones del Triásico Medio-Superior en el Dominio Bético y la comparamos con otras faunas de la misma edad descritas recientemente en la cordillera Ibérica.

Las muestras se recuperaron después de la disolución (con ácido acético al 10%) de rocas de carbonato. La asociación se compone de siete especies pertenecientes a seis géneros (Hybodus plicatilis, Omanoselache bucheri comb. nov., Omanoselache contrarius comb. nov., Lonchidion derenzii, Lissodus aff. L. lepagei, Pseudodalatias henarejensis y cf. Rhomaleodus budurovi), la mayoría de ellos. no nesoseláceos. Los restos de condrictios aparecen en niveles que datan de Ladiniense al Carniense de acuerdo con bivalvos, ammonoides y conodontos.

Los hallazgos son comparables, en términos taxonómicos, a la fauna de condricitios del Ladiniense de la Cordillera Ibérica que se describió recientemente, aunque los condrictios parecen notablemente menos abundantes en el Dominio Bético. La mayoría de las especies encontradas también se encuentran en la Cordillera Ibérica, con la excepción de Lonchidion derenzii y cf. Rhomaleodus budurovi, que se encuentran en la Sección Boyar, fechada como Carniense. El pequeño tamaño de todos los dientes recuperados, pertenecientes probablemente a especímenes jóvenes, sugiere que los ambientes epicontinentales muy poco profundos registrados en las rocas del Triásico Medio-Superior del Dominio Bético podrían usarse como áreas de cría.

Capítulo 4. Lonchidion derenzii, sp. nov., un nuevo tiburón Lonchidion (Condrictios, Hibodontiformes) del Triásico superior de España, con comentarios sobre el esmaltoide en Lonchidiidae

Lonchidiidae representa una de las familias más diversas y controvertidas de los Hibodontiformes, el grupo hermano de los neoseláceos.

En este estudio, describimos por primera vez la nueva especie asignada a la familia Lonchidiidae, Lonchidion derenzii sp. nov., basado en dientes recuperados del Triásico Superior (Carniense) de España. El material estudiado pertenece a la sección Boyar, ubicado en el sureste de la Cordillera Bética y fechado como Carniense gracias a la asociación de bivalvos, conodontos y polen. El nivel desde el que se recuperaron los restos registra un ambiente marino de plataforma poco profundo con una gran influencia continental. Lonchidion derenzii, sp. nov., representa el registro más antiguo de este género en España y puede considerarse como el registro más antiguo de este género en Europa.

Capítulo 5. Tiburones del Triásico Medio de la Cordillera de la Costa Catalana (NE España) y patrones de colonización faunística durante la transgresión del mar de Tethys hacia el oeste

Los cambios paleogeográficos que ocurrieron durante el Triásico Medio en el dominio del mar de Tethys más occidental fueron gobernados por una transgresión marina hacia el oeste del Océano de Tethys. La transgresión inundó amplias áreas de la parte oriental de Iberia, formando nuevos ambientes epicontinentales de aguas poco profundas, que posteriormente fueron colonizados por diversas faunas, incluidos los condrictios. La transgresión es registrada por dos sucesivos ciclos transgresivo-regresivos: (1) Anisiense medio-superior y (2) Anisiense superior-Carniense inferior. Aquí, describimos la fauna de condricitios recuperada de varias secciones estratigráficas del Triásico Medio (Pelsoniense-Longobardiense) ubicadas en la cuenca Costera Catalana (Tethys más occidental). El conjunto consiste en dientes aislados de las especies *Hybodus plicatilis*, *Omanoselache bucheri*, *O. contrarius* y *Pseudodalatias henarejensis*. Nuestros datos complementan una serie de estudios recientes sobre faunas de condrictios de las cuencas marinas del Triásico Medio-Superior de la península Ibérica, lo que nos permite evaluar los patrones de colonización faunística. Los tiburones que alcanzaron el este de Iberia durante el primer pulso transgresor (Anisiense) muestran fuertes afinidades con la bioprovincia sefardí (relacionada con el océano Neotethys), mientras que un aumento posterior en la diversidad de condrictios (durante el pulso transgresivo Ladiniense) se debe a la llegada de taxones con afinidades a faunas europeas (Paleotethys). Esto contrasta con los patrones de colonización de invertebrados y conodontos evaluados recientemente, donde las asociacines del Anisiense medio-superior están compuestos de taxones con afinidad a la bioprovincia alpina / germana, mientras que las faunas de la bioprovincia sefardí solo alcanzaron las aguas poco profundas de la península ibérica durante el segundo (Ladiniense) pulso transgresor. Aquí se explican las diferencias entre los patrones de colonización de los condrictios frente a los invertebrados y los conodontes sobre la base de diferentes estrategias de dispersión. Nuestro hallazgo sugiere la existencia, durante el Anisiense, de conexiones entre Paleo y NeoTethys a través del microcontinente Cimmerio occidental, que sirvió como vías para la entrada de los condrictios desde la bioprovincia sefardí.

BLOQUE 2. ESTUDIO DE LA CAPA SCE EN EL ESMALTOIDE DE CONDRICTIOS

Capítulo 6. La microestructura del esmaltoide de escamas en euseláceos (Condrictios)

La microestructura del esmaloide de los dientes de condrictios se ha estudiado durante décadas y ha demostrado ser una herramienta taxonómica útil. Los cambios en la organización esmaltoide se han relacionado con la aparición de nuevas estrategias tróficas y la radiación mesozoica del “Crown group” de los neoseláceos. Sin embargo, en contraste con la abundancia de estos datos sobre el esmaltoide dental, las descripciones del esmaltoide de las escamas de condricitios son casi inexistentes. Se describen Hibodontiformes mesozoicos y neoseláceos actuales, incluidos batoideos y tiburones. Se muestra que una capa gruesa de esmaltoide de cristalito único (SCE) cubre todas las escamas estudiadas. Aunque el esmaltoide de las escalas claramente no alcanza altos niveles de diferenciación microestructural presente en el esmaltoide dental de algunos neoseláceos, encontramos cierto grado de organización, como cristales orientados, diferenciación en subcapas y la presencia de conjuntos mal estructurados de haces paralelos con cristales densamente dispuesto. Como las escamas carecen de funciones de alimentación de los dientes, sugerimos que la aparición de la organización / diferenciación microestructural del esmaltoide en condrictios puede entenderse como consecuencia de un proceso de autoorganización más que como presión adaptativa.

Capítulo 7. Nuevas ideas sobre la microestructura esmaltoide de los peces batoideos (Condrictios)

Los dientes de condrictiso están cubiertos con un tejido hipermineralizado conocido como esmaltoide. Su microestructura muestra una organización jerárquica que ha aumentado su complejidad estructural desde un esmaltoide monocristalino homogéneo (SCE) en los primeros condrictios hasta el complejo esmaltoide multicapa que se encuentra en los tiburones modernos (que consiste en haces de cristalitos dispuestos en patrones intrigantes). Análisis recientes de la microestructura del esmaltoide en peces batoideos, centrados en Myliobatiformes y taxones fósiles, apuntan a la presencia de un esmaltoide multicapa agrupado (o fibroso), una condición propuesta como plesiomórfica para Batoidea. En este trabajo, proporcionamos más análisis del esmaltoide para una selección de taxones que cubren la filogenia de los batoideos. Nuestro análisis SEM muestra una capa superficial de SCE, donde los cristales individualizados son claramente discernibles, cubriendo la corona de los dientes en la mayoría de las especies estudiadas. Se encontró un esmaltoide empaquetado de doble capa solo en un Rhinoidei, Rhina ancylostoma Bloch & Schneider, 1801. Llegamos a la conclusión de que la condición más extendida entre los batoideos existentes es una SCE monocapa sin ningún tipo de diferenciación microestructural, probablemente plesiomórfica al menos para el “crown group” de batoidea. Sugerimos que el complejo esmaltoide presente en otros batoideos es un carácter convergente que ha aparecido repetidamente durante la evolución de los batoideos, probablemente como una adaptación mecánica hacia las dietas durófagas moderadas.

Capítulo 8. Sobre la microestructura del esmaltoide de Archaeobatidae (Neoseláceos, Condrictios)

En este estudio, presentamos nuevos datos sobre la microestructura esmaltoide de la familia batoidea más antigua, Archaeobatidae.

Primero, todos los dientes se atacaron superficialmente con HCl al 10% durante 5 s y se fotografiaron en el SEM. Posteriormente, los mismos dientes fueron incrustados en bálsamo de Canadá, pulidos y atacados nuevamente con HCl al 10% para revelar la microestructura del esmaltoide antes de ser fotografiados por segunda vez.

La capa esmaltoide de Archaeobatidae consiste en un esmaltoide cristalino superficial simple (SCE) con un esmaltoide de empaquetado paralelo (PBE) en todos los taxones estudiados, pero solo en Toarcibatis y Cristabatis existe un esmaltoide empaquetado enmarañado (TBE) debajo. La complejidad estructural y la diversidad encontrada en Archaeobatidae son comparables a las descritas recientemente en otros batoideos fósiles. Nuestros datos sugieren una tendencia general a la "simplificación" en el esmaltoide de batoideos hasta el esmaltoide monocristalino homogéneo que está presente en la mayoría de los batomorfos actuales; lo que contrasta con la creciente complejidad estructural presente en los selacimorfos.

5. CONCLUSIONES

Esta tesis se ha centrado en el estudio de los condrictios durante el Triásico Medio-Superior y la evolución de la capa de esmaltoide, más precisamente, la SCE en los dientes y escamas de hibodóntidos y batomorfos., proporcionado una serie de nuevos resultados en este sentido. Que se resuemn a continuació.

El primer bloque de trabajos ha demostrado que las faunas de condrictios que se encuentran en las cordilleras Bética y Costero Catalana son taxonómicamente comparables a las descritas en el Ladiniense de la cordillera de Ibérica, aunque los restos son considerablemente menos abundantes que en la cordillera Ibérica. Las asociaciones fósiles están compuesas por seis géneros (Hybodus, Omanoselache, Lissodus, Lonchidion, Pseudodalatias y Rhomaleodus) y siete especies (H. plicatilis, O. bucheri, O. contrarius, L. aff. L. lepagei, L. derenzii, P. henarejensis y cf. R. budurovi). De esos taxones, P. henarejensis es endémica de la Península Ibérica; y Lonchidion derenzii y cf. Rhomaleodus budurovi son endémicos de la Cordillera Bética. Además, ambos taxones están restringidos a los niveles de la sección Boyar, datada como Carniense según el registro fósil. Las comunidades de condrictios descritas en esta tesis estaban dominadas en gran medida por pequeños tiburones costeros durófagos con dentaduras aplastantes bien adaptadas para la caza de crustáceos y moluscos (capítulos 3, 4 y 5 repectivamente).

Además, la colonización de los condrictios durante el Triásico fue dirigida dos pulsos transgresivos del

mar de Tethys. Los tiburones que alcanzan el NE de Iberia durante el primer pulso transgresivo (Amiriense medio-superior) del mar de Tethys muestran claras afinidades con los taxones de la bioprovincia sefardí, relacionados con el Océano Neotethys (es decir, Omanoselache). El notable aumento en la diversidad de condrictios que ocurre en E Iberia durante el segundo pulso transgresivo (Ladiniense) se debe a la llegada de nuevos taxones con afinidades claras con las faunas europeas del Océano Palaeotethys (es decir, Paleobates angustissimus, Lissodus, Hybodus, Rhomaleodus o Pseudodalatias). Este patrón de colonización es inverso a los evaluados recientemente para otros grupos fósiles. Por lo tanto, los bivalvos, los foraminíferos, los braquiópodos y las faunas de conodontos del Anisiense del este de Iberia están compuestos casi exclusivamente de taxones de la bioprovincia alpina / germánica. En contraste, los invertebrados y los conodontos de la bioprovincia sefardí alcanzan las aguas poco profundas de la Península Ibérica por primera vez durante el segundo pulso transgresor (Ladiniense). Las diferencias existentes entre los patrones de colonización se explican sobre la base de sus diferentes estrategias de dispersión: la dispersión de bivalvos, amonites y foraminíferos, está determinada en gran medida por las corrientes oceánicas, mientras que la dispersión de los tiburones costeros depende de la vagilidad adulta (muy baja) y requiere hábitats apropiados continuos.

Es por ello, que proponemos la existencia de corredores estrechos, probablemente a corto plazo, de aguas poco profundas entre Paleo y Neo-Tethys durante el Anisinese; el microcontinente Cimmeria habría actuado como barrera para las corrientes oceánicas en el incipiente Neotethys; y durante el siguiente aumento del nivel del mar (Ladiniense), el mar de Tethys inundó vastas áreas del microcontinente Cimmeria más occidental permitiendo que las corrientes marinas del Neotethys se ex-

tiendan hacia el Palaeotethys llegando a los mares epicontinentales ibéricos (Capítulo 5).

En el bloque dos, queda demostrado mediante la aplicación de técnicas de lámina delgada para el estudio de los restos fósiles *Lonchidion derenzii*, sp. nov., demuestra la presencia de una SCE. Estudios previos sobre la microestructura es,altoide de otros taxones de la familia Lonchidiidae también han reportado la presencia de una capa de SCE homogénea en *Lissodus* angulatus, *Lissodus minimus* y *Lissodus* aff. L. *lepagei*. Por lo tanto, un esmaltoide formado por cristales sin ningún tipo de disposición en fibras (o haces) es la condición generalizada entre Lonchidiidae. Este tipo de ultrastuctura es la más extendida entre los dientes de los hibodontiformes, aunque existen algunas excepciones en las que se pueden encontrar "haces sueltos" de cristales (Capítulo 4). Por otra parte, el esmaltoide que se encuentra en algunas escamas de hibodontiformes (*Lobaticorona*, *Glabrisubcorona* y *Coniunctio*) y neoseláceos demuestra la presencia de una parte externa de SCE compacta y la parte interna de SCE, con algunos cristaless densamente dispuestos y paralelos a la superficie de la escama (Capítulo 6). Teniendo en cuenta que las escamas carecen de funciones de alimentación de los dientes, la aparición de una incipiente organización / diferenciación microestructural en el enameloide de la escala (es decir, la orientación preferida de los cristalitos o "subcapas") no puede vincularse con ninguna función adaptativa obvia y podría entenderse como una consecuencia de procesos de autoorganización.

Con respecto a la estructura del esmaltoide en batoideos, el análisis de la microsestrucutra de sus dientes ha demostrado que un SCE que carece de diferenciación microestructural es la condición más extendida entre los batoideos actuales, siendo probable que este sea el carácter pleisiomórfico al menos para el “crown group” de batoideos. El Rhinoidei Rhina ancylostoma es la única excepción conocida. El enameloide de doble capa de este taxón tiene una parte más externa en la que los cristalitos son individualizados y altamente compactados y una parte más interna donde los cristalitos se organizan en haces de orientación variable (Capítulo 7). Las unidades SCE + BCE identificadas en Archaeobatidae difieren del esmaltoide en triple capa de los tiburones modernos tanto en la compactación de los haces (siendo más compacta y bien definida en los tiburones moernos) como en la disposición de los componentes PBE, RBE y TBE de los tiburones (Capítulo 8). La presencia de un esmaltoide con haces de cristales organizados de forma compleja en Archaeobatidae, la familia de batomorfos más antigua conocida, y muchos otros batomorfos antiguos sugiere la evolución de un esmaltoide complejo en sus dientes antes de la dicotomía entre Batomorphii y Selachimorpha. Los datos disponibles sugieren una tendencia general a la "simplificación" en el enameloide batoideos desde el esmaltoide organizado de forma compleja presente en Archaeobatidae y varios otros batomorfos antiguos hasta la capa homogénea de SCE presente en muchos otros taxones fósiles, así como en la mayoría de los linajes recientes. Esto contrasta con la creciente complejidad estructural presente en los selachimorfos.

La aparición de una estructura del esmaltoide compleja sugiere que el esmaltoide agrupado presente en algunos batoideos (Archaeobatidae, *Rhina ancylostoma*, *Rhynchobatus* sp., *Ptychotrygon* sp. y *Parapalaeobates* cf. *atlanticus*) es un carácter convergente que apareció más de una vez durante la evolución de los batoideos, probablemente como una adaptación mecánica hacia dietas durófagas moderadas.

INTRODUCTION

CHONDRICHTHYAN DIVERSITY AT TRIASSIC TIMES: AN OVERVIEW

The Permo-Trias mass extinction was one of the most severe crisis experienced by life on Earth (Benton, 1995; Scheyer *et al.*, 2014). The common paradigm persists in a slow and progressive step-by-step recovery for both the marine and terrestrial biotas which ended with the replacement of the “Palaeozoic Fauna” with the “Modern Fauna” (Chen and Benton, 2012). The recovery of the Triassic ecosystems is believed to have lasted between eight to nine million years in the oceans, with low to high trophic levels rebuilt through the Early to Middle Triassic and more complex ecosystems appearing well into the early Middle Triassic (Anisian) (Chen and Benton, 2012; Scheyer *et al.*, 2014).

Among the marine vertebrates that survived the Permian mass extinction are the cyclostoms, the chondrichthyans and the osteichthyans; whereas other marine groups, such as placoderms and “Acanthodii” did not survive this event (Benton and Pfretzschner, 2007; Hurley *et al.*, 2007; Bratvold, 2016). Most of the chondrichthyans that originated in the Paleozoic, such as symmoriids, petalodontiforms, stenacanthids and brasonelliformes were extinct by the end of the Permian. Following this extinction, the surviving chondricthyans underwent a great radiation that led to the apparition of most of the modern shark families by the end of the Mesozoic (Long, 2010) (Figure 1).

The most prominent group during the Triassic was the Hybodontiformes. Although the first record (doubtful pers. obsv.) of this group came from the Late Devonian (Ginter

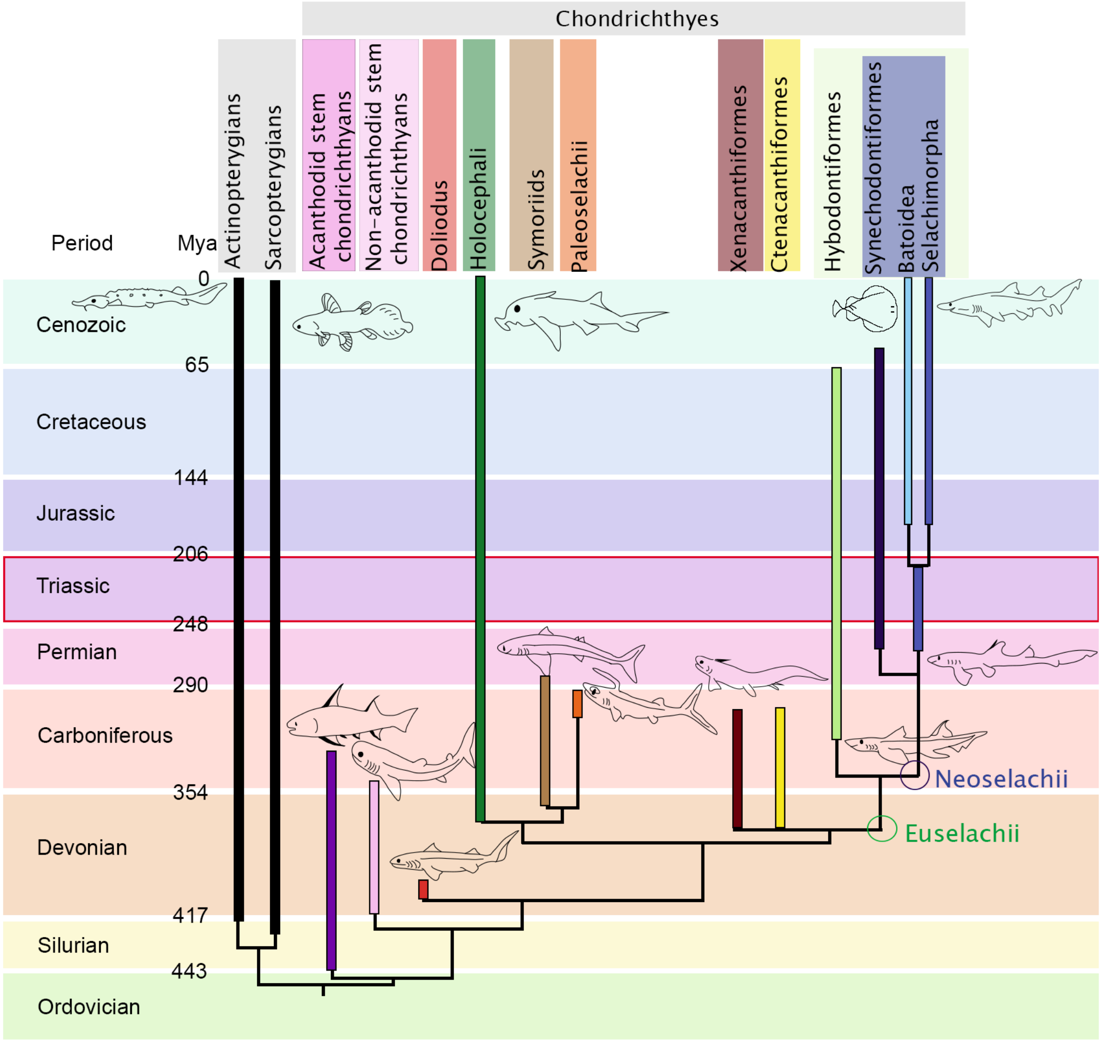

Figure 1. Phylogeny of chondrichthyans with the principals groups and the estimation of their apparition and extinction. Modified from Boisvert *et al.* (2019)

et al., 2010), they reached a maximum diversity peak during the Middle Triassic (Long, 2010), constituting a high diverse group with marine and freshwater representatives (Fischer *et al.*, 2008, 2011; Koot, 2013). Hybodonts are characterised by the lack of calcified vertebrae, well-developed fin spines and small cephalic spines (Capetta, 2012). They are considered the sister group of the neoselachians (batoids and selachimorphs). The phylogeny of hybodont sharks is complex and under current review, but at least four families are recognised for most of the authors: Acrodontidae, Hybodontiidae, Lonchididae and Polyacrondontidae (Rees, 2008; Cappetta, 2012). Their teeth present a wide range of morphologies, from multicuspidated teeth as in *Hybodus* to elongated such as in *Acrodus* (Cappetta, 2012) which indicates the diversity of prey they feed upon.

Late Triassic was a key time for the evolution of chondrichthyans; it was the beginning of the transition from faunas dominated by hybodontiforms and other basal sharks to those dominated by neoselachian (Friedman and Sallan, 2012). From the Late Jurassic onwards hybodonts enter a decline in marine environments and were restricted to freshwater and non-marine environments (Rees, 1998; Rees and Underwood, 2006, 2008; Cuny *et al.*, 2013). By the end of the Cretaceous this group became extinct (Cappetta, 2012).

Although the Hybodontiformes were globally distributed during the Triassic, they appear to be more successful in the Middle Triassic of the Palaeotethys (Koot, 2013). However, this may be due to the excessive fossil screening that has been done in the European localities of Mesozoic age (Underwood, 2009). These localities were dominated by hybodontiforms such as *Hybodus*, *Acrodus*, *Polyacrodus*, *Lissodus*, *Palaeobates*, some neoselachian species (*Mucrovenator*, *Reifia, Synechodus, Paleospinax, Duffinselache, Pseudocetorhinus*) and other genus of unknown relationship such as *Rhomaleodus* and *Pseudodalatias* (Duffin, 1980; Delsate, 1992; Delsate and Duffin, 1999; Cuny *et al.*, 2001; Niedzwiedzki, 2008; Botella *et al.*, 2009b; Andreev and Cuny, 2012; Norden *et al.*, 2015; Korneisel *et al.*, 2015). In the last decades, the focus has swifted to other localities outside Europe such as Middle East, Asia

North and South America (Rieppel *et al*., 2001; Wang *et al*., 2001; Chen *et al*., 2007; Koot *et al*., 2015). In these localities, we can find hybodontiforms from the genus *Hybodus, Acrodus* and *Palaeobates*, but also *Omanoselache*, *Homalodontus, Listracanthus* and *Lonchidion.*

Regarding to the Iberian Peninsula, it has been long time assumed that the chondrichthyan fossil record was very scarce or completely inexistent during the Triassic (Chen *et al*., 2007). This conception started recently to change after the finding of scales and teeth assumed to belong to hybodontiformes (Pla *et al*., 2009) in the Bugarra section of the Iberian Range and the erection of an endemic taxa *Pseudodalatias henarenejensis* (Botella *et al*., 2009b). After those studies, Pla *et al.* (2013) published for the first time, a detailed study of the chondrichthyan remains found in seven sections dated as Longobardian of the Iberian Ranges. These authors found a fossil assemblage composed of seven species of five "non-neoselachian" shark genera (*Palaeobates, Hybodus, Pseudodalatias, Omanoselache* and *Lissodus*). They noted that this assemblage was similar to other European, North America and China, so they suggested that…"*heterogenity of Spanish chondrichthyan fauna can be linked to Ladinian marine transgression (upper Muschelkalk facies, after the severe Anisian/Ladinian regression, 'Keuper type' facies), which allowed for the colonization of the shallow waters of the Iberian Peninsula by fauna coming from both Tethys provinces; the Germanic domain to the north and the Sephardic domain to the south…*"

Taking this work as the starting point for this thesis, the data of the chondrichthyan faunas of two additional Middle-Late Triassic classical basins of the Iberian Peninsula, the Betic range and the Catalan Coastal range, has been investigated. Each chapter corresponds with papers already published.

MIDDLE-LATE TRIASSIC IN THE IBERIAN PENINSULA

The fragmentation of the supercontinent Pangea, which had started during the Permian, became general during the Triassic. In the Middle Triassic the expansion of the Neotethys westward was controlled by the displacement of the microntinent Cimmeria to the north. This expansion led to the development of five transgressive-regressive marine cycles (Anisian, Anisian-latest Ladinian, Canian, late Carnian-Norian and upper Norian; López-Gómez *et al.*, 2002) and changes in the palaeogeography of the western region of the Tethys (Stampfli *et al.*, 2002; Mutoni *et al.*, 2009), which includes the Iberian Peninsula (Figure 2).

The alternation of marine transgression-regression cycles involved frequent environmental changes in the marine zones of the Iberian Plate

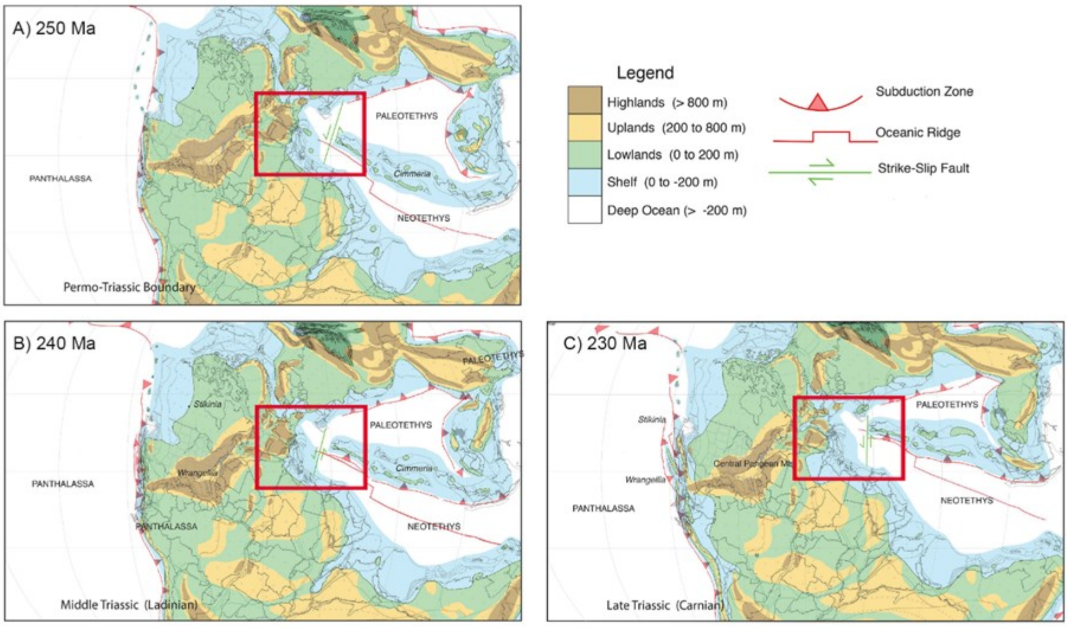

Figure 2. Map of the expansion of the Neotethys during the Triassic and the movements of the microcontinent of Cimmeria. Modified from Scotese (2018)

(Plasencia, 2005). According to several authors this explains the low diversity of different marine organisms, including chondrichthyans (Márquez-Aliaga y Martínez, 1996; Márquez, 2005; Plasencia, 2005; Escudero-Mozo, 2015).

The sections studied in this thesis record the second (Anisian-latest Ladinian) and third cycles (Carnian). As seen in chapter 5 (Manzanares *et al.*, 2020) these cycles affected the composition of the chondrichthyan faunas that were present in the marine epicontinental areas of the Iberian Peninsula. (figure 3)

The studied sections belong to areas from the Betic (Manzanares *et*

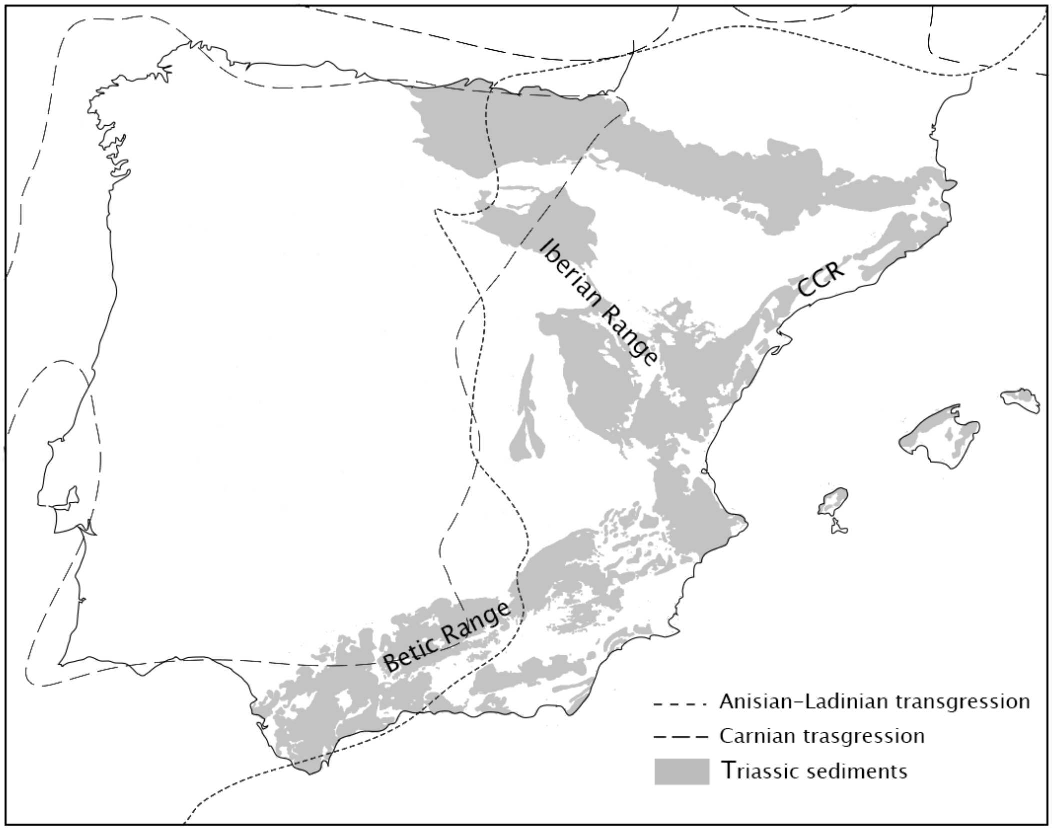

Figure 3. Geological map of the Triassic outcrops of the Iberian Peninsula studied in this thesis. The dash lines indicates the reconstructed coast shores of the Tethys sea in the Anisian-latest Ladinian and Carnian transgression respectively. The map is modified from López-Gómez *et al.* (2002) and the coast shore reconstructions are based on Fortuny *et al.* (2011).

al., 2016; 2018, Chapter 3 and 4) and the Catalan-Coastal Ranges (CCR) (Manzanares *et al.*, 2020; chapter 5). These works complete the study of the chondrichthyan fauna of the Triassic of the Iberian Peninsula started by Pla *et al.* (2013) with the fauna from the Iberian range.

BETIC RANGE

The Betic Range is located in the southeastern part of the Iberian Peninsula, 600 km in west-east direction and 200km from north to south (Pérez-Valera, 2015). It has been traditionally divided in two different domains: the Internal and External zones. In general, they record shallow epicotinental facies (mostly tidal flats, shallow platform and ramp), but in some cases they are continental in origin (see Chapter 4). They show different facies related to differences in subsidence history between and within the Betic External and Internal zones and have been intensely studied.

The Internal zone are composed of four complex most of them consisting of basement and cover rocks, mostly metamorphic, which constitute, during the Mesozoic, a more southern domain (i.e. the Alboran domain) independent from the Southern Iberian Paleomargin (García-Dueñas and Balanyà 1986; Pérez-López 1998; Manzanares *et al.*, 2016, 2017)

All the section studied in this tesis belong to the External zones. Those expose Triassic to lower Miocene sediments deposited in the South Iberian Palaeomargin. Conventionally, the External zones are also divided in two domains spanning in an ENE direction, the Prebetic (Calasparra, Canara and Esperejas sections of this thesis) and Subbetic (Boyar section of this thesis) domains (see Manzanares *et al.* 2016; 2017; chapter 3 and 4).

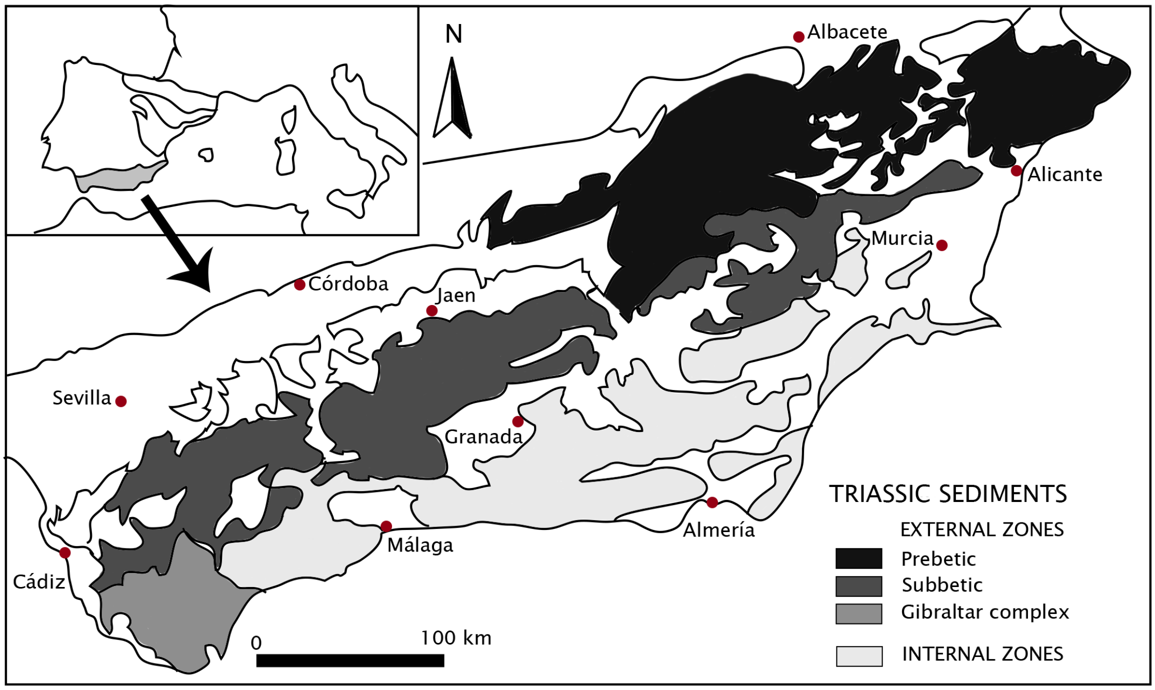

Figure 4. Simplify geological map of the Betic Range. Modified from Pérez-Valera (2015)

CATALAN COASTAL RANGE

The Catalan Coastal Range (CCR) is located in the north-eastern Iberian Peninsula and is composed of two mountain chains that run parallel to the Mediterranean coast in NE-SW direction, from Girona to the Ebro delta (Sopeña and De Vicente; Plasencia, 2005; Escudero-Mozo, 2015).

The general charachteristics of the Middle-Upper Triassic sediments in the Catalonian Range have been described since the 19th and early 20th centuries (De Verneuill and Collomb, 1853; Bauza,1876; Gombau, 1877; Vilaseca, 1920; Bataller, 1933); but it was Virgil (1958) the first who made an extensive study of the stratigraphy, sedimentology, palaeontology

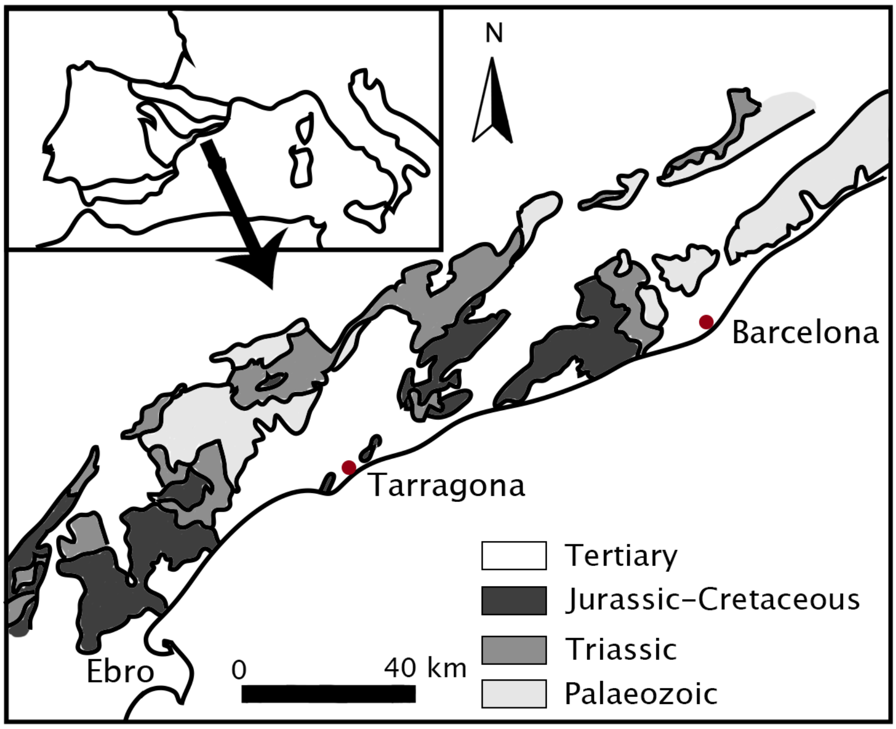

Figure 5. Simplify geological map of the Catalan-Coastal Range. Modified from Plasencia (2005) -

and structure of the Triassic in this range. Afterwards, numerous studies have deepened the knowledge of geology and palaeontological content of this range (see Escudero-Mozo, 2015)

The succession of sediments shows the three typical subdivisions of the Germanic Triassic, and it has been divided in three domains with different subsidence: Gaiá-Montseny; Prades and Priorat-Baix Ebre (Marzo, 1980; Calvet and Ramón,1987; Ramón and Calvet, 1987; Gaspar-Escribano *et al.*, 2004). (Figure5)

This PhD has focused on the study of the remains recovered from the Rasquera-Benifallet and Tivissa sections in the Priorat Baix Ebre domain, and the Ametlla section in the Gaià-Montseny domain (for more details see Manzanares *et al.*, 2020)

EVOLUTION OF ENAMELOID

Elasmobranch teeth are composed of two hipermineralized tissues comprise of an organic matrix and a mineral phase of hydroxyapatite ($Ca_5(PO_4)_3OH$) in the dentine and fluorapatite ($Ca_5(PO_4)_3F$) in the enameloid. The latter is restricted to a relative thin layer on the outside of the tooth-crown, following a layer of dentine (osteodentine or orthodentine) and a central pulp cavity of soft tissue (Kemp and Park, 1974; Cappetta, 2004; Gillis and Donoghue, 2007; Whitenack *et al.*, 2010). These histological features are used, alongside with morphological dental characters, to identify and classify fossil taxa with unclear affinities (Cuny *et al.*, 2017). Although most of the taxonomic studies of fossil shark teeth has focused on the tooth morphology, the micro and ultrastructure of the enameloid layer has been of great importance in order to differenciate teeth of neoselachian (batomorphs and selachimorphs) and in understanding higher-level taxonomic relationships and the evolutionary history of the group (e.g. emergence of new feeding strategies) (Thies, 1993; Cuny *et al.*, 2001; Cuny and Risnes, 2005; Gillis and Donoghue, 2007; Guinot and Cappetta, 2011; Andreev and Cuny, 2012; Enault *et al.*, 2013, 2015; Manzanares *et al.*, 2016, 2017; Cuny *et al.*, 2017).

The study of tooth histology started at the end of the mid-19th century with the works of Agassiz (1833-1843) and Owen (1840-1845). More studies aimed to unravel the histological and microstructural of fossil taxa appeared in the years after, but because of the difficulty to observe accurately the enameloid they were aimed on the dentine instead (Ørvig, 1950, 1967; Peyer, 1968; Enault *et al.*, 2015).

Reif (1973) showed the existence of a triple-layered enameloid in the teeth of neoselachian. This triple layer consist of an external layer, the SLE (Shiny Layer Enameloid), composed of fluorapatite crystals randomly oriented; a middle layer, the PFE (Parallel Fibred Enameloid, renamed as PBE, Parallel Bundled Enameloid *sensu* Enault *et al.* (2015)) forming bundles parallel to each other and parallel to the surface of the teeth; and an internal layer, the TFE (Tangle Fibred Enameloid, TBE (Tangled Bundled Enameloid) *sensu* Enault *et al.* (2015)) in which the bundles are interwoven randomly. The different layers have been associated to the emergence of new trophic strategies and the arise of different forces applied to the teeth due to them (Gilles and Donoghue, 2007). Reif (1977) noted that the enameloid of batomorph was so reduced that made its study difficult and assumed that it was composed by a SCE; although other authors proposed a TBE layer exclusively (Gillis and Donoghue, 2007).

In recent years, several authors have started to study the hybodont enameloid ultrastuctrure, and found the presence of incipient bundles (of Fibers *sensu* Reif 1973, see above) in some of the species (*Rhompahiodon minimus* (Cuny and Risnes, 2005); *Priohybodus* and *Thaiodus* (Duffin and Cuny, 2008)). In view of this findings, Andreev and Cuny (2012) hypothesised that the triple layered enameloid has been adquired gradually in the evolutive history of neoselachian. In stem neoselachian, the teeth were covered by a SCE without any kind of organization (Andreev and Cuny, 2012; Enault *et al.*, 2015). In selachimorpha, the different components of the enameloid layer appeared gradually during their life history (Andreev and Cuny, 2012): first appeared loosely packed bundles, that evolved into a true PBE, and afterwards, at the junction with the dentine apperaed a TBE. However, in the fossil record we find some taxa that seem to defy this scenario. Recently, the study of the enameloid layer of a ctenacanthiform from the Permian, *Neosaivodus flagstaffensis*, has revealed the presence of a PBE (Guinot *et al.*, 2013). This discovery remarks that the presence of a PBE cannot be considered as unique to selachimorpha. This data, however, is unique for ctenacanthiforms and is largely out of the biostratigraphy context of the group, therefore more studies and findings are required to contrast this data (H.B. pers. Commun. 2020)

These assumptions have been questioned in recent years with the study of recent and extinct batomorphs (Cuny *et al.*, 2008; Enault *et al.*, 2013; 2015; Manzanares *et al.*, 2016; 2018). Those studies demonstrated that the diversity of combiantions possible in the enameloid of neoselachian, both stem and derived, is greater than previously assumed.

The aim of the second part of this PhD (Chapters 6-8) has been to comprehend the true extend of an homogeneous SCE in chondrichthyan. For that purpose, I have study the enameloid microstructure in extant batoids and in Archaeobatoidea (the oldest batomorph family Underwood 2006; Cuny *et al.* 2009; Aschilman 2011; Aschliman *et al.* 2012; Cappetta 2012; Enault *et al.* 2013, 2015) as well as the scales and the teeth of the Triassic hybodontid sharks found in the Iberian Peninsula.

For the study of the enameloid microstructure we used the methodology largely described in the literature (see e.g. Gillis and Donoghue, 2007; Botella *et al.*, 2009a). Thus, all the specimens were embedded in a transparent polyester resin and subsequently sectioned along transverse or longitudinal planes, polished, and then etched using different acids (0.1 M HCl; 0,5 orthoposhoric acid or hydrogen peroxide) for period of time varying from 5-10 seconds to three days. Each sample was repolished and etched as many times as necessary to elucidate the enameloid microstructure. Afterwards, all the specimens were photographed in a Hitachi S–4100 scanning electron microscope of the Microscope Service of the University of Valencia. For SEM analysis, teeth were coated with gold and palladium alloy.

The terminology used to describe the enameloid layers follow that of Enault *et al.* (2015). They differentiate two major units in the enameloid layer: the SCE (Single Cristallite Enameloid) which correspond to the SLE described by Reif (1973); and a second unit named BCE (Bundled Crystallite Enameloid) where the crystallites are arranged into bundles of different sizes and orientations. The BCE includes the PFE and the BFE, which are renamed as PBE (Parallel Bundled Enameloid) and TBE (Tangle Bundled Enameloid), and a third layer denominated RBE (Radial Bundled Enameloid). The latter originates in the SCE and is composed of crystallites oriented perpendicular to the surface of the tooth, and generally penetrates the PBE and, sometimes, reaches the TBE.

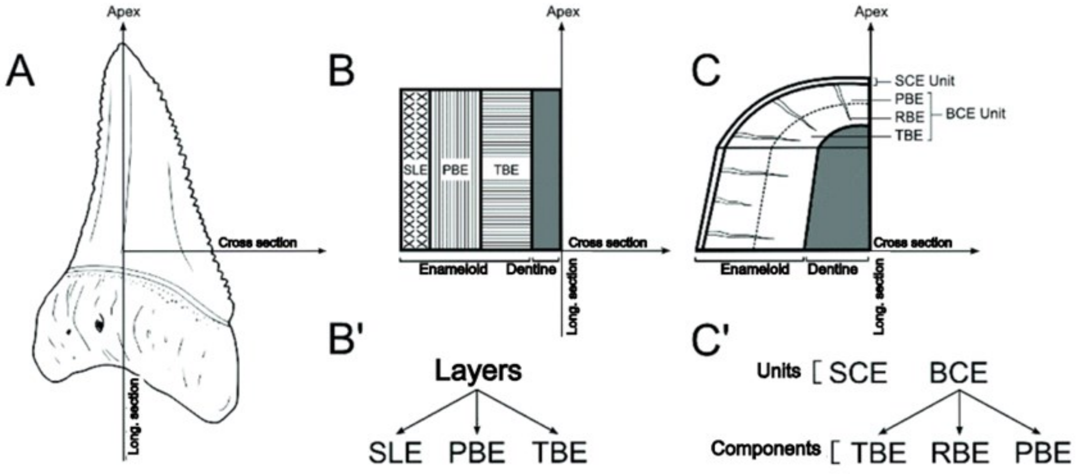

Figure 6. Progression of the understanding of the organization of the enameloid layers in teeth of neosealchian. A) Tooth of *Carcharodon carcharias* ilustrating the sections used. B) Schematic of the tree layer enameloid according to Reif (1973). C) Structure of the enameloid according to Enault *et al*. (2015) From Cuny *et al*., (2018)

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OBJECTIVES

The two main goals of this PhD are to expand the knowledge of the taxonomy of the chondrichthyans communities that habited the coast of the Iberian Peninsula during the Middle-Late Triassic and the evolution of the enameloid layer, more precisely, the SCE in the teeth and scales of hybodonts and batomorphs. The specific objectives to develop this PhD tesis has been dived into two blocks:

Block 1: Systematic and biogeographic study of the Middle Triassic chondrichthyans of the Iberian Peninsula

1.1 Systematic study of the chondrichthyan remains recovered from sections in the Betic Range (Chapters 3 and 4)

1.2 Systematic study of the chondrichthyan remains recovered from the Catalan Coastal Range (Chapter 5)

1.3 Biogeographical study of the remains recovered in the Iberian, Betic and Catalan Coastal Ranges in relationship with the chondrichthyan faunas of the westermost Tethys sea (Chapter 5)

Block2: Study of the SCE layer in chondrichthyan enameloid

2.1 Characterization of the enameloid microstructure in euselachian scales (Chapter 6)

2.2 Characterization of the enameloid in teeth of basal and extant batoids (Chapter 7 and 8)

Block 1

Systematic and biogeographic study of the Middle Triassic chondrichthyans of the Iberian Peninsula

CHAPTER 3
Manzanares *et al.* 2017

CHAPTER 4
Manzanares *et al.* 2017

CHAPTER 5
Manzanares *et al.* 2020

CHAPTER 3

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RESEARCH PAPER

Middle-Late Triassic chondrichthyans remains from the Betic Range (Spain)

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Purpose In the present study, we described, for first time, the chondrichthyan fauna from several Middle-Late Triassic sections in the Betic Domain and compare it with other recent described coeval faunas from the Iberian Ranges.

Methods Specimens were retrieved after the dissolution (with 10% acetic acid) of carbonate rocks.

Results The assemblage comprises of seven species belonging to six genera (*Hybodus plicatilis, Omanoselache bucheri* comb. nov.*, Omanoselache contrarius* comb. nov.*, Lonchidion derenzii, Lissodus* aff. *L. lepagei*, *Pseudodalatias henarejensis* and cf. *Rhomaleodus budurovi*), most of them non-nesoselachian. Chondrichthyans remains occur in levels dating from Ladinian to Carnian according with bivalves, ammonoids and conodonts.

Conclusions The findings are comparable, in taxonomical terms, to the chondrichthyan fauna from the Ladinian of the Iberian Range that was recently described, although chondrichthyans seems noticeably less abundant in the Betic Domain. Most of the species found occur also in the Iberian Range, with the exception of *Lonchidion derenzii* and cf *Rhomaleodus budurovi*, which occur in the Boyar Section, dated as Carnian. The small size of all teeth recovered, belonging probably to young specimens, suggest that the very shallow epicontinental environments recorded in Middle-Upper Triassic rocks from the Betic Domain could be used as nursery areas.

1. INTRODUCTION

A number of recent studies in the Middle Triassic of the Iberian Range (Spain) have evidenced the presence of a rich and diverse chondrichthyan fauna (Botella *et al.* 2009; Pla *et al.* 2009, 2013; Ferrón *et al.* 2014), changing the inaccurate previous perception that chondrichthyans are rare, or even completely absent, in the Triassic marine sediments of the Iberian Peninsula (e.g. Chrzastek 2008; Fortuny *et al.* 2011). These works revealed the presence of a paleobiogeographically heterogeneous chondrichthyan association, which includes components of Middle Triassic faunas of northern Europe, together with species only previously known in North America and China with some additional "Iberian" endemic taxa.

In order to increase our understanding of the chondrichthyan diversity in the epicontinental shallow marine environments of eastern Iberia during Triassic times, we have extended our investigations to other "classical" Middle-Late Triassic basins of the Iberian Peninsula (see López-Gómez *et al.* 2002).

The present study focuses on the Middle-Late Triassic chondrichthyan remains recovered in four sections of the Betic Domain, located on the South-Eastern parts of the Iberian Peninsula (Fig. 1a). As in the Iberian Range, Middle-Upper Triassic rocks of the Betic Domain have been extensively studied, providing an excellent stratigraphic and palaeoenvironmental framework for the study of the shark fauna.

Chondrichthyan microremains, that are studied in this paper, were derived from conodont residues supplied by Dr. Pablo Plasencia (Plasencia 2009) with more microvertebrate material recovered from resampling of selected levels.

2. GEOGRAPHICAL AND GEOLOGICAL SETTING

Middle-Upper Triassic rocks are well preserved in the south of the Iberian Peninsula and show different facies related to different transgressive-regressive cycles during the Triassic times (López *et al*. 2002). In the Betic Range, two tectonic domains have been differentiated: the External Zones and the Internal Zones. The External Zone contains sediments deposited in the continental margin in the south of the Iberian Peninsula, whereas the Internal Zone is restricted to the Alborán domain (García-Dueñas and Balanyà 1986). All the sections studied here, Espejeras, Calasparra, Canara and Boyar, belong to the External Zones of the Betic Range (Fig. 1a). Rocks from these zones record tidal flats and shallow platforms of an epicontiental sea, but in some cases, they are continental in origin (see Boyar section below) (López-Gómez *et al*. 2002).

Palaeontological studies have been intensively carried out in the Betic Range so, there exist a great amount of literature about fossils groups such as bivalves (López-Gómez *et al.* 1994; Márquez-Aliaga *et al*. 1999; Márquez-Aliaga and Márquez 2000), ammonoids (Goy and Pérez-López 1996; Goy *et al*. 1996), foraminifera (Pérez-López *et al*. 2003, 2005) and conodonts (Plasencia 2009 and references therein). Chondricthyan remains have been named in the conodont study of the Betic Range but, even so, the chondrichthyan fauna has not been studied in detail.

2.1. Espejeras Section (Fig. 1 b)

This section is located near the city of Elda (0º 39’ 10” N 38º 23’ 55” O). This column has a total of 144.5 m. The section has yielded fossils of bivalves and foraminifera, which dated the section as Ladinian (López-

Gómez *et al.* 1994).

2.2. Calasparra Section (Fig. 1 b)

This section is in the province of Murcia near the city of Calasparra (38º 12' 30" N 1º 38' 10"O). The section was divided in five units, it is the richest of all the studied upper Ladinian based on the ammonoids found in the section (Goy 1986, 1995; Goy and Martínez 1996; Goy *et al.* 1996; Goy and Pérez -López 1996).

2.4. Canara section (Fig. 1 b)

This section is located between the cities of Canara and Cehegín (in the province of Murcia) (38º 07' 49"N

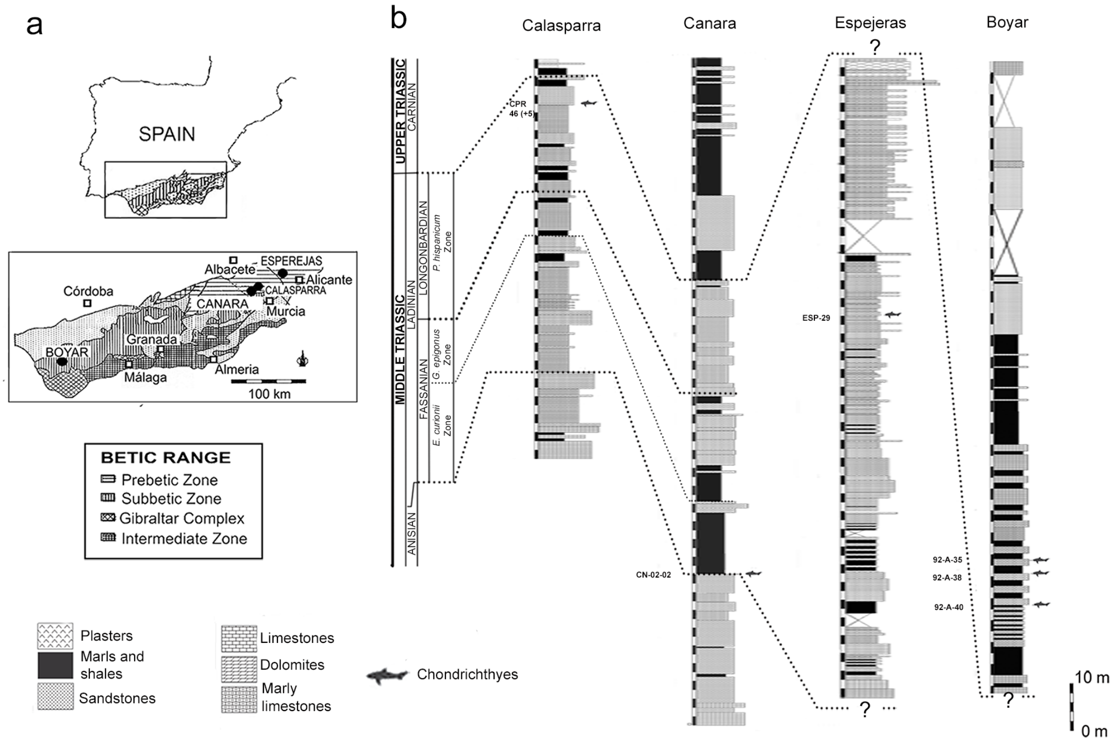

Fig. 1 a Geologic setting and location of the Betic Range. b Stratigraphic section of Espejeras, Canara, Calasparra and Boyar. The levels from where the microremains were recovered are indicated by the shark outlines. Modified from Plasencia (2009) and Pé rez-Valera (2016)

1º 46’ 28”O). This column has a total of 150 meters, it is divided into four units and it has been dated as Anisian to upper Ladinian (Pérez-Valera 2005), although the top of the section belongs to the Keuper facies.

2.3. Boyar Section (Fig. 1 b)

It is situated near the cities of Ubrique and Grazalema, in the province of Cádiz, southern Spain. The section is located in the southwest part of the Betic Ranges (36º 44’ 49” N 5º 25’ 12” O). The Boyar Section is subdivided into 4 main units comprising strata belonging to the upper Muschelkalk and Keuper facies (Fig. 1B), and it has been dated as Carnian (Late Triassic) in age on the basis of the contained bivalve, conodont and pollen assemblages (Martín-Algarra *et al*. 1995).

3. SYSTEMATIC PALEONTOLOGY

The carbonate rocks from the four sections were dissolved using 10 per cent acetic acid and screened with sieves meshes of 2, 0.125 and 0.063 mm, respectively. Subsequently, the microremains were picked up under a binocular microscope. The photographs were taken with the HITACHI 4100 Electronic Microscope from SCSIE from the University of Valencia. The achieved specimens are mainly isolated teeth and fragments, housed in the Geological Museum of the University of Valencia (MGUV), Spain.

Class Chondrichthyes Huxley 1880

Subclass Elasmobranchii Bonaparte 1838

Cohort Euselachii Hay 1920

Order Hybodontoformes Zangerl 1981

Superfamily Hybodontoidea Owen 1846
Family Hybodontidae Owen 1846

Genus *Hybodus* Agassiz 1837

Type Species—*Hybodus reticulatus*

Agassiz 1837.

Hybodus plicatilis Agassiz 1843 (Fig. 2a–c)

Material Two almost complete tooth and more than 20 incomplete teeth from Calasparra section, level CPR-46(?5 m) (MGUV-35896); Canara section, level CNI-02-2(MGUV-35897, MGUV-35898); Espejeras section, level ESP-29 (MGUV-35899); and Boyar section, level 92-A-40 (MGUV-35900).

Description Most of the material consists of broken cusps. Only two of the teeth are almost complete. Their sizes vary from 2.816 mm in mesiodistal length and 2.421 mm in height in the smaller specimen (Fig. 2c, d), to 4.518 mm in length and 2.031 mm in height in the biggest specimen (Fig. 2a, b). The central cusp is flanked by up to two pairs of cusplets (Fig. 2a–d), all aligned and ornamented with rectilinear ridges. The base presents the random foramina characteristic of hybodonts. In the biggest specimen (Fig. 2a, b) is straighter than the base of the smaller specimen, which has a more arched shape (Fig. 2c, d).

Remarks It is widely accepted that the genus *Hybodus*, as used actually, is broadly polyphyletic (Rees 1998; Underwood and Rees 2002; Rees and Underwood 2008; Ginter 2010; Cappetta 2012) and do not correspond to a biological group. A taxonomical revision is required, which surely will lead to the division of the species currently included in *Hybodus* into different genera. This taxonomical revision is beyond the aim of this work, but all the morphological and vascular characteristics shared with teeth of the *Hybodus* type species, *H. reticulatus*, (e.g. high and slender cusp, with a circular cross-section and accessories lateral cusplets orates with vertical ridges) recommend maintaining, for the meantime, the generic epithet for *H. plicatilis* until hybodont taxonomy will be resolve. Teeth of *Hybodus plicatilis* are the largest teeth recovered from the Betic Range, as happens in the Iberain Range (Pla *et al.* 2013).

Ocurrence Muschelkalk, Middle Triassic of Schwenningen, Ger- many (Agassiz 1843); Muschelkalk, Middle Triassic of Monte Giorgio, Switzerland (von Meyer 1849; Rieppel 1981); Muschelkalk, Middle Triassic of Luxemburg (Delsate 1992, 1993); Middle Triassic of the Iberian Range, Spain (Pla *et al.* 2013), Middle-Late Triassic of the Betic Range.

Family Lonchidiidae

Genus *Lissodus* Brough 1935

Type specie—*Hybodus africanus* Broom 1909

Lissodus aff. *L. lepagei* (Figure 2 e–g)

Material Two complete crowns from the Calasparra section, level CPR-46 (?5 m) (MGUV-35901, MGUV-35902).

Description The tooth crowns found in the material from the Betic Range have a characteristic ''boomerang'' shape with the labial side larger than the lingual (Fig. 2g–h). A well-marked occlusal crenulated crest separates both faces. A main low central cusp is flanked by two cusplests on each mesial and distal side. In both specimens, the cusplets show signs of wear (Fig. 2e, g). They present a faint labial peg with a poorly developed cusplet near the crown shoulder and under the central cusp. No bases are preserved, but when observed in basal view all teeth indicated the presence of a sulcus in the crown/base junction.

Remarks Betic specimens present no significant morphological differences, when compared with the teeth described by Pla *et al.* (2013) as Lissodus aff. *L. lepagei* from the Iberian Range. Thus, we suggest that they belong to the same species; however, the scarcity of the material does not allow for the formal description of a new species, so it is left as *Lissodus* aff. L*. lepagei.*

Ocurrence Middle Triassic of the Iberian Range, Spain (Pla *et al.*

2013), Middle Triassic of the Betic Range.

Lonchidion Estes 1964

Type Species—*Lonchidion selachos* Estes 1964, Maastritchian, Lance Formation, Eastern Wyoming, USA.

Lonchidion derenzii Manzanares, Pla, Martínez-Pérez, Ferrón and Botella 2016 (Fig. 2i–l)

Material 10 teeth from the Boyar section, level 92-A-40, Spain. (MGUV-27.744 to MGUV-27.748)

Description These minuscule teeth are elongated and gracile, measuring 0.5–0.4 mm mesiodistally, 0.3 –0.2 mm apicobasally, and 0.3–0.2 mm labiolingually. The crown has a very distinctive ‘‘whale tail’’-shape in labial view (Fig. 2j, l). The main central cusp is small, rounded to triangular in shape and labially inclined; flanked by 2–3 pairs of lateral cusplets (Fig. 2k, l). The main cusps and the lateral cusplets appear very abraded. A very prominent labial peg with one small accessory cusplet is situated above the crown-root junction (Fig. 2i–l). The crown/root junction is very constricted and only in one specimen half of the base is preserved (Fig. 2j).

Remarks These specimens were recovered only from the level 92-A-40 of the Boyar section. The presence of pollen in this level has been interpreted as evidence in favour of the entire sequence being deposited in very shallow waters in close proximity to continental areas.

Occurrence Late Triassic of the Betic Range.7

Order incertae sedis

Family Homalodontidae Mutter, De Blanger and Neuman 2008

Genus *Omanoselache* Koot, Cuny, Tintori and Twitchett 2013

Remarks Genus *Omanoselache* Koot, Cuny, Tintori and Twitchett, 2013 was erected by Koot *et al.*

(2013) to include several new forms (*Omanoselache hendersoni, Omanoselache angiolinii*) found in the Middle Permian of central eastern Oman. In parallel Pla *et al.* (2013) described new material of ''*Polyacrodus*'' *contrarius* Johns, Barnes and Orchard, 1997 and '*P.*' *bucheri* Cuny, Rieppel and Sander, 2001 from the upper Ladinian of the Iberian Range, Spain, and erected the new genus *Prolatodon* Pla, Márquez-Aliaga and Botella, 2013 for the reaccommodation of this two species (see also Mutter *et al.* 2007). Recently Koot *et al*. (2015) have suggested that *Omanoselache* and *Prolatodon* are synonyms genus, and, in fact, the diagnostic characteristics defined for both genus match to a large degree. Although both taxa were erected in 2013, *Omanoselache* (March) takes priority over *Prolatodon* (Juny). Thus, Koot *et al*. (2015) refers *Prolatodon contrarius* and *Prolatodon bucheri* to Omanoselache.

Here we follow the taxonomical proposal of Koot *et al*. (2015). The Spanish material refered to ''*Prolatodon*'' fits perfectly within *Omanoselache*, and *Prolatodon* is accepeted as a junior synonym of *Omanoselache*.

Type specie—*Omanoselache henedrsoni* Koot, Cuny, Tintori and Twitchett 2013

Omanoselache bucheri comb. nov. (Cuny, Rieppel, Sander 2001) (Fig. 2m–r)

Material Three complete and more than 20 incomplete teeth from the Boyar section, level 92-A-40 (MGUV- 35903, MGUV-35904, MGUV-35916); Espejeras section, level Esp-29 (MGUV-35905, MGUV-35917); and Calasparra section, level CPR-46(?5 m) (MGUV-35906).

Description Most of the teeth are broken, specially near the main cusp. Teeth are labiolingually compressed and elongated mesiodistally; measuring between 2.322 and 1.811

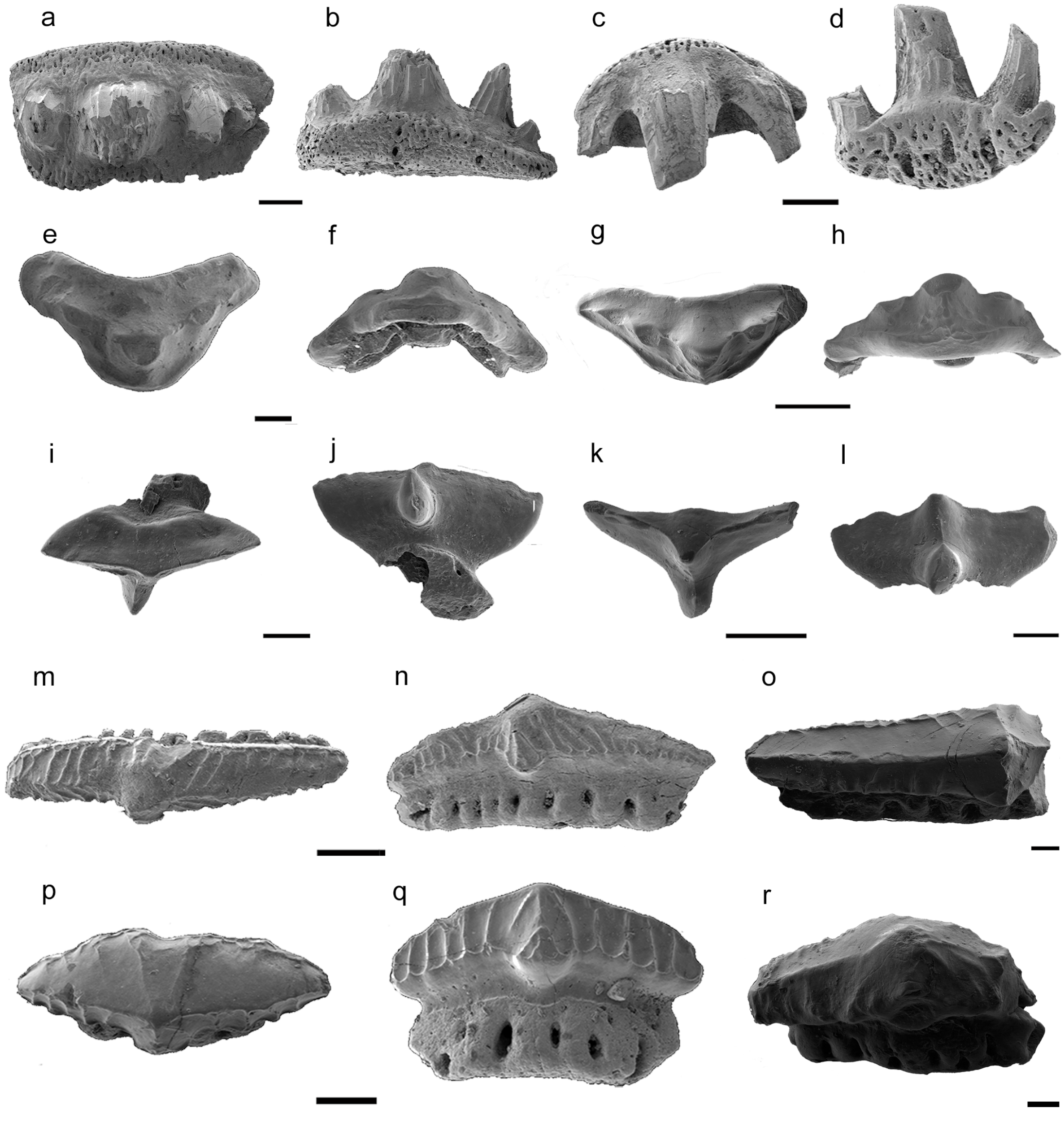

Fig. 2 a–d *Hybodus plicatilis*, scale bar 600 μm; a, b MGUV- 35896, c, d 35897; e–h *Lissodus* aff. *lepagei*, scale bar 200 μm, e,f MGUV-35901, g, h MGUV- 35902; i–l *Lonchidion derenzii*, modified from Manzanares *et al*. (2016), i, j MGUV-27744, k, l MGUV 27745; m–r *Omanoselache bucheri* comb. nov., m, n scale bar 400 μm, MGUV-35904; o scale bar 100 μm, MGUV-35916, p, q scale bar 200 μm, MGUV-35903; r scale bar 100 μm, MGUV- 35917 mm mesiodistally, 0.696–0.606 mm in height and 0.957–0.682 mm labiolingually. All teeth present only a main pyramidal cusp with an occlusal crest that covers the crown teeth mesiodistally with undulated vertical ornamentation ridges appearing on labial and lingual sides. Two of the teeth (Fig. 2p, q) has a clear pyramidal shape and shows a bigger size than the other specimens, which are more elongated and compressed labiolingually (Fig. 2m–o). A well-developed sulcus is located in the crown/base junction. A lingual peg is easily recognizable and placed under the main cusp. The base has a size comparable to the crown and it is also labiolingually compressed. Vascularization consists of a row of well-developed foramina in both labial and lingual walls.

Remarks Teeth assigned to *''P.'' bucheri* from the Iberian Range (about 170 specimens) show a great variation in morphology and shape that has been interpreted as evi- dence of (at least) monognatic heterodonty (Pla *et al*. 2013). Thus, minute specimens were assigned to symphyseal positions (Pla *et al*. fig: 3a, b); possible messial teeth present one main cusp and are arched, which gives them a ''boomerang''-shape (Pa *et al*. fig: 3c–f); and latero-distal teeth are blunt and display a reduced main cusp (Pla *et al*. fig: 3g). The small number of teeth recovered in the Betic Range (around 25 partially complete specimens) does not reflect all the morphological variation present in the specie. Almost all of the elements recovered belong probably to latero-distal teeth (Fig. 2m–o), whereas two complete teeth (and few fragment) (Fig. 2p–r), which have a more pyramidal shape and a more evident central cusp, could probably occupied symphyseal positions. the Betic Range.

Omanoselache contrarius comb. nov. (Cuny, Rieppel, Sander 2001) (Fig. 3a –e)

Material 4 complete teeth and more than 10 incomplete teeth from the Boyar section, level 92-A-40 and 92-A-35E (MGUV-35907, MGUV-35908, MGUV-35909); and

Description The found specimens are labiolingually compressed, measuring 1.092 mm mesiodistally, 0.474 in height and 0.293 mm labiolingually (Fig. 3a–e). A crenulated crest extends across the whole tooth from the distal to the mesial side, in most of the specimens this crest is worn (Fig. 3c–e). A main pyramidal cusp placed in the center of the tooth is flanked by a few pairs of cusplets (Fig. 3a, b). Vertical sinuous ridges, which originated radially from the apex of the main cusp, ornate the crown of the teeth. This is more evident in those specimens that present less wear (Fig. 3a, b). *O. contrarius* presents labial and lingual pegs, both at the same level of the tooth and flanking the main cusp.

Occurrence Middle Triassic of British Columbia, Canada (Johns *et al.* 1997); Middle–Late Triassic of Yang Liu Jing, China (Chen *et al.* 2007); Middle Triassic from the Iberian Range of Spain (Pla *et al.* 2013), Middle-Late Triassic of the Betic Range.

Order incertae sedis

Family Pseudodalatiidae Reif 1978

Genus *Pseudodalatia*s Reif 1978

Type Species—*Pseudodalatias barnstonensis* Sykes 1971.

Pseudodalatias henarejensis Botella, Plasencia, Márquez-Aliaga, Cuny and Dorka 2009 (Fig. 3f, g)

Material Four incomplete crowns from the Calasparra section, level CPR-46(?5 m) (MGUV-35914, MGUV- 35915).

Description All the crown teeth are broken and there are no bases. The lower jaw teeth present the typical spearhead-like, sharp and slightly net towards the apex; with a few coarse denticles that are directed upwards present in the cutting edges, most of them damaged in our material (Fig. 3f, g).

Remarks Although the teeth of *P. henarejensis* can be clearly separated into two different morphologies, assuming dignathic heterodonty (Botella *et al*. 2009) only the teeth from the lower jaws are recovered in the Betic Range.

Occurrence Middle Triassic from the Iberian Range, Spain (Pla *et al*. 2013), Middle Triassic of the Betic Range.

Subcohort Neoselachii Compagno 1977

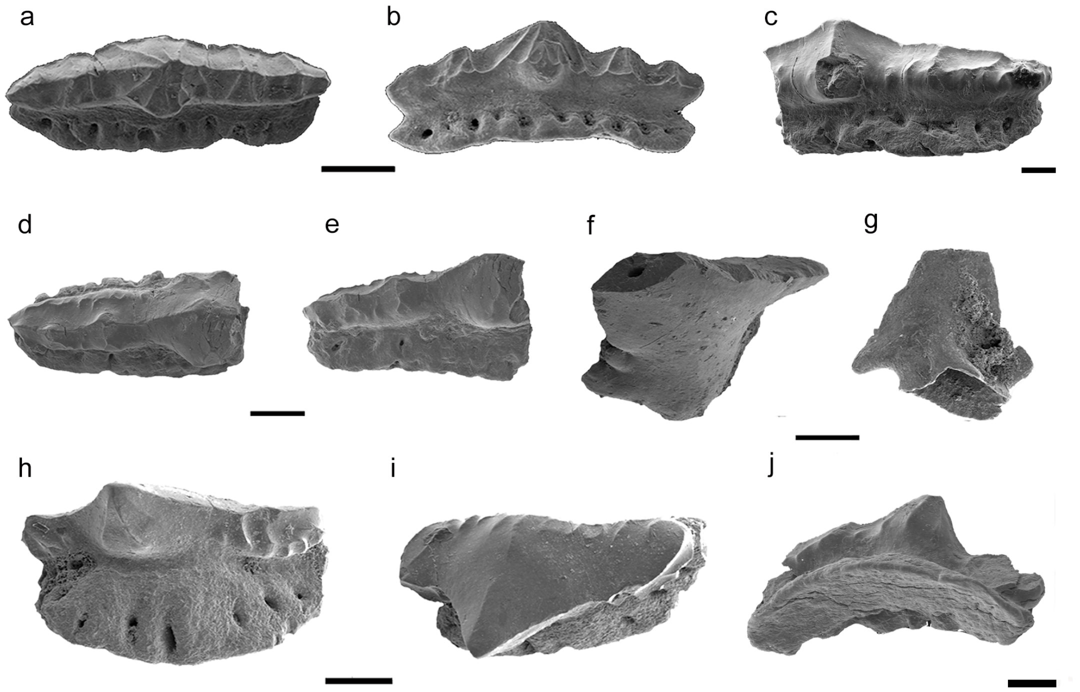

Fig. 3 a–e *Omanoselache contrarius*, scale bar 200 µm, a, b MGUV-35905; c scale bar 100 lµm, MGUV-35918; d, e MGUV- 35908; f, g *Pseudodalatias henarejensis*, scale bar 200 µm; MGUV-35914; h–j cf. *Rhomaleodus budurovi*, h, i scale bar 200 µm, MGUV-35909; j scale bar 100 µm, MGUV-35919

Superorder Selachimorpha Nelson 1984

Order incertae sedis

Family incertae sedis

Genus *Rhomaleodus* Andreev and Cuny 2012

Type specie—*Rhomaleodus budurovi* Andreev and Cuny 2012

cf. *Rhomaleodus budurovi* (Fig. 3h–j)

Material 3 complete and several incomplete teeth from Boyar section; levels 92-A-40 (MGUV-35911, MGUV-35912, MGUV-35919) and 92-A-38 (MGUV-35913).

Description Teeth of small sizes and very well preserved. They present a massive triangular main cusp flanked by a pair of lateral cusplets (Fig. 3h, i). All the cusp and cusplets are lingually inclined, and it is possible to see the vertical ridges that descend from the apex of each cusp in the lingual side (Fig. 3h–j). The root is compact and has a trapezoidal outline in basal view (Fig. 3j), with a prominent lingual torus penetrated by a single row of large foramina (Fig. 3h).

Remarks The specimens show clear morphological similarities with teeth form the Middle and Upper Triassic of Bulgaria assigned to *Rhomaleodus budurovi* (Andreev and Cuny, 2012), including the presence of a prominent lingual torus penetrated by a single row of large foramina and the triangular main cusps flanked by one pair of smaller cusplets, all of them inclined lingually. *Rhomaleodus budurovi* has been reported to have three different morphologies (A, B and C) by Andreev and Cuny (2012). By the moment, all the teeth found in the Betic Range belong to the type B. Andrew and Cuny (2012) realized a surface study of the enamaloid layer of *Rhomaleodus budurovi* and found the presence of a Parallel Bundle Enameloid (PBE) layer, which led them to include this species as a basal selachimorpha. We also have realized ground sections of one of the specimens found in the Betic Range, but the study has not yield any satisfatory results. More histological studies of the enameloid layer would clarify the enameloid layer of this species.

Occurrence Middle and Upper Triassic of Bulgaria (Andreev and Cuny 2012); Late Triassic of the Betic Range.

4. DISCUSSION

This paper report a diverse shark fauna from the Betic Range, confirming the presence of chondrichthyans as an important component in the shallow marine environments of the Iberian Peninsula during Middle-Late Triassic times.

The assemblage found in the Betic Range comprises of six genera and seven species belonging to five families, all of them belonging to non-nesoselachian, with the only probable exception of cf. *Rhomaleodus budurovi*. This assemblage is taxonomically comparable to that described by Pla *et al*. (2013) from the Ladinian of the Iberian Range, although chondrichthyans become visibly less abundant in the Betic Domain. Five of the seven species found in the Betic basin (i.e. *Hybodus plicatilis*, *Omanoselachae bucheri* comb. nov., *Omanoselache contrarius* comb.nov., *Pseudodalatias henarejensis* and *Lissodus* aff*. L. lepagei*) occur as well in the Iberian Ranges. Among these, *Pseudodalatias henarejensis* and *Lissodus* aff. *L. lepagei* are endemic of the Middle Triassic of the Iberian Peninsula. Two species, *Lonchidion derenzii* and cf. *R. budurovi* appear in the Betic Range but not in the Iberian Range.

The chondrichthyan assemblage from the Iberian Range, studied in Pla *et al*. (2013), was dated as Longonbardian (late Ladinian) based on the presence of the conodonts *Sephardiella mungoensis* and *Pseudofurnishius murcianus* (see Pla *et al*. 2013, Fig. 1). Sections studied in the Betic Range, however, exposed sediments from Anisian to Carnian. The species *Pseudodalatias henarejensis* and *Lissodus* aff. *L. lepagei* appear only in level CPR-46(? 5 m) of the Calasparra section, which can be dated as Longonbardian due to the presence of the same conodont taxa, already mentioned above (Plasencia, 2009). In the same sense *Lonchidion derenzii* and cf. *Rhomaleodus budurovi*, only appear in levels of the Boyar section, dated as Carnian by Martín-Algarra *et al.* (1995). In contrast, *Hybodus plicatilis*, *Omanoselache bucheri* comb. nov. and *Omanoselache contrarius* comb. nov. appear in all the sampled sections, including the Boyar section, so their stratigraphic record in the Betic Domain is from the Ladininan to Carnian. With this new data, the upper stratigraphic range of the species *H. plicatilis* and *Omanoselache bucheri* comb.nov. extends to the Upper Triassic. In addition, the occurrence of *H. plicatilis*, *O. bucheri* comb. nov. and *O. contrarius* comb.nov., *L. derenzii* and cf. *Rhomaleodus budurovi* in levels of the Boyar section represents the first record of late Triassic direct vertebrate remains in the South–East of the Iberian Peninsula (see Fortuny *et al.* 2011).

Based on the tooth morphology, most of the taxa found in this study present grasping-crushing dentitions, adapted, potentially, to moderate durophagous diets containing by example, crustaceans, ostracods and even small bivalves and gasteropods, which are abundant in the shallow near-coastal facies of the Betic Domain. According to López-Gómez *et al.* (2002, and references therein), carbonate rocks of the Betic Domain were deposited in the shallow platform and tidal flats of an epicontinental sea connected to the Tethys. It would offer a number of restricted and protected areas from open seas, which could have been a perfect place for development and growth of juvenile sharks. It is known that females of extant sharks migrate to these kinds of environments to deposit their eggs or give birth and the juvenile specimens live there until they reach maturity (Castro 1993; Grubbs 2010; Matich and Heithaus 2015).

An exception to this grasping-crushing pattern is found in *Pseudodalatias henarejensis.* This species, exhibit a peculiar clutching-cutting dentition analogous to that present in some recent Dalatiidae, which feed excising bites of flesh from larger animals (i.e. marine mammals, other sharks, etc.). In this sense, remains of large reptiles and bony fishes have been discovered in Ladinian rocks on nearby sites of the Betic Range deposited in a marine setting near the shoreline (Niemeyer 2002; Fortuny *et al.* 2011).

As said above, chondrichthyans remains seem considerably less abundant in the Betic Domain than in the Iberian Range. To be exact, in most of the studied sections in the Iberian Ranges by Pla *et al.* (2013, see their Fig. 1), the chondrichthyans are abundant in several levels dated as Longobardian. In contrast, in the Longobardian units of Espejeras, Canara and Calasparra from the Betic Range shark remains only appear in two punctual levels (CPR 46 (?5) and ESP-29, Fig. 1) despite some of the studied sections were sampled in detail for conodont studies (Plasencia 2009). In fact, a large number of samples were processed, being that a great majority of them is sterile for chondrichthyans (Fig. 1). Moreover, some other Triassic sections from the Betic Domain (i.e. Arroyo Hurtado, Salmerón, Valdepeñas de Jaén and Cabo Cope sections) were sampled in detail in that study but do not yield shark remains, and they have not been considered in the present study (but see Plasencia 2009 for more information).

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CHAPTER 4

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Lonchidion derenzii, sp. nov., a new lonchidiid shark (Chondrichthyes, Hybodontiforms) from the Upper Triassic of Spain, with remarks on lonchidiid enameloid

Esther Manzanares, Cristina Pla, Carlos Martínez-Pérez, Humberto Ferrón & Héctor Botella

Lonchidiidae Herman, 1977, represents one of the most diverse and controversial families of Hybodontiformes, the sister group of Neoselachii (i.e., modern sharks, skates, and rays). It was initially erected as a monogeneric family including only *Lonchidion* Estes, 1964, a genus of small euryhaline hybodonts from the Mesozoic. Recently, Cappetta (2012) recognized up to eight genera within the family: *Baharyodon*, *Diplolonchidion*, *Vectiselachos*, *Hylaeobatis*, *Isanodus*, *Parvodus*, *Lissodus*, and *Lonchidion*, although the content of the family is still under discussion (see, e.g., Rees, 2008; Khamha et al., 2016). Major discrepancies concern the phylogenetic relationships between *Lonchidion* and *Lissodus* and the taxonomic status of the latter. Thus, based on the general similarity of their teeth, Duffin (1985, 2001) considered *Lonchidion* as a junior synonym of *Lissodus*. Subsequently, Rees and Underwood (2002) restored *Lonchidion* as a valid genus, closely related to *Lissodus*, within the family Lonchidiidae (together with *Vectiselachos*, *Parvodus*, and *Hylaeobatis*). This interpretation has been followed by several authors (e.g., Fischer, 2008; Cappetta, 2012; Johns et al., 2014). In contrast, Rees (2008) considered *Lonchidion* and *Lissodus* not so closely related to each other, excluding *Lissodus* from *Lonchidiidae*.

The majority of *Lonchidion* species has been described on the basis of disarticulated teeth, and complete or partial articulated skeletons have been known only recently from juvenile specimens, assigned to *Lonchidion* sp., from the inland lacustrine Konservat-Lagerstätten outcrop of Las Hoyas (Lower Cretaceous, Spain) (Soler-Gijón et al., 2016). Currently, the stratigraphic distribution of the ranges from the Middle–Upper Triassic (Fischer et al., 2011; Johns et al., 2014) to the Upper Cretaceous (Estes, 1964).

In the present study, we describe a new species assigned to Lonchidiidae, *Lonchidion derenzii*, sp. nov., based on distinctive isolated teeth from the Upper Triassic (Carnian) of Spain, representing the earliest well-documented occurrence of the genus in Europe.

1. INTRODUCTION

Lonchidiidae Herman, 1977, represents one of the most diverse and controversial families of Hybodontiformes, the sister group of Neoselachii (i.e., modern sharks, skates, and rays). It was initially erected as a monogeneric family including only *Lonchidion* Estes, 1964, a genus of small euryhaline hybodonts from the Mesozoic. Recently, Cappetta (2012) recognized up to eight genera within the family: *Baharyodon, Diplolonchidion, Vectiselachos, Hylaeobatis, Isanodus, Parvodus, Lissodus*, and *Lonchidion*, although the content of the family is still under discussion (see, e.g., Rees, 2008; Khamha *et al.*, 2016). Major discrepancies concern the phylogenetic relationships between *Lonchidion* and *Lissodus* and the taxonomic status of the latter. Thus, based on the general similarity of their teeth, Duffin (1985, 2001) considered *Lonchidion* as a junior synonym of *Lissodus*. Subsequently, Rees and Underwood (2002) restored *Lonchidion* as a valid genus, closely related to *Lissodus*, within the family Lonchidiidae (together with *Vectiselachos, Parvodus*, and *Hylaeobatis*). This interpretation has been followed by several authors (e.g., Fischer, 2008; Cappetta, 2012; Johns *et al.,* 2014). In contrast, Rees (2008) considered *Lonchidion* and *Lissodus* not so closely related to each other, excluding *Lissodus* from Lonchidiidae.

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Institutional Abbreviation—MGUV, Museum of Geology at the University of Valencia, Valencia, Spain

2. GEOLOGIC SETTING AND AGE

The material studied here was collected from the Boyar Section near the cities of Ubrique and Grazalema, in the province of Cádiz, southern Spain (Fig. 1A). The section is located in the southwest part of the Betic Ranges (36º 44’49” N, 5º25’12” W; see Martín Algarra *et al.*, 1995, for a more detailed geographic and geologic setting). The Boyar Section is subdivided into four main units comprising strata belonging to the upper Muschelkalk and Keuper facies (Fig. 1B), which has been dated as Carnian (Upper Triassic) in age on the basis of the contained bivalve, conodont, and pollen assemblages (Martín-Algarra *et al.*, 1995).

All teeth were recovered as isolated elements after dissolution in 5–10% formic acid of carbonate rocks (samples around 10 kg) from the middle part of the lower unit (Muschelkalk facies). After dissolution, the residues were screened with sieve meshes of 2, 0.125, and 0.063 mm, respectively. Apart from the teeth of *Lonchidion*, conodonts and teeth and scales of other chondrichthyans and actinopterigians were also recovered. This middle part is characterized by platy limestone interbedded with gray marls and some sporadic dolomitic levels. The presence of pollen in the marly levels has been interpreted as evidence in favor that the entire sequence being deposited in very shallow waters in close proximity to continental areas. The recovered teeth were photographed using a scanning electron microscope at the University of Valencia, Spain.

In order to study tooth histology, and following the methodology described in the literature (Gillis and Donoghue, 2007; Botella *et al.*, 2009a; Manzanares *et al.*, 2014), several specimens were embedded in a transparent polyester resin and subsequently sectioned along transverse or longitudinal planes, polished, and then etched using 0.1 M HCl for 5–10 s. Each

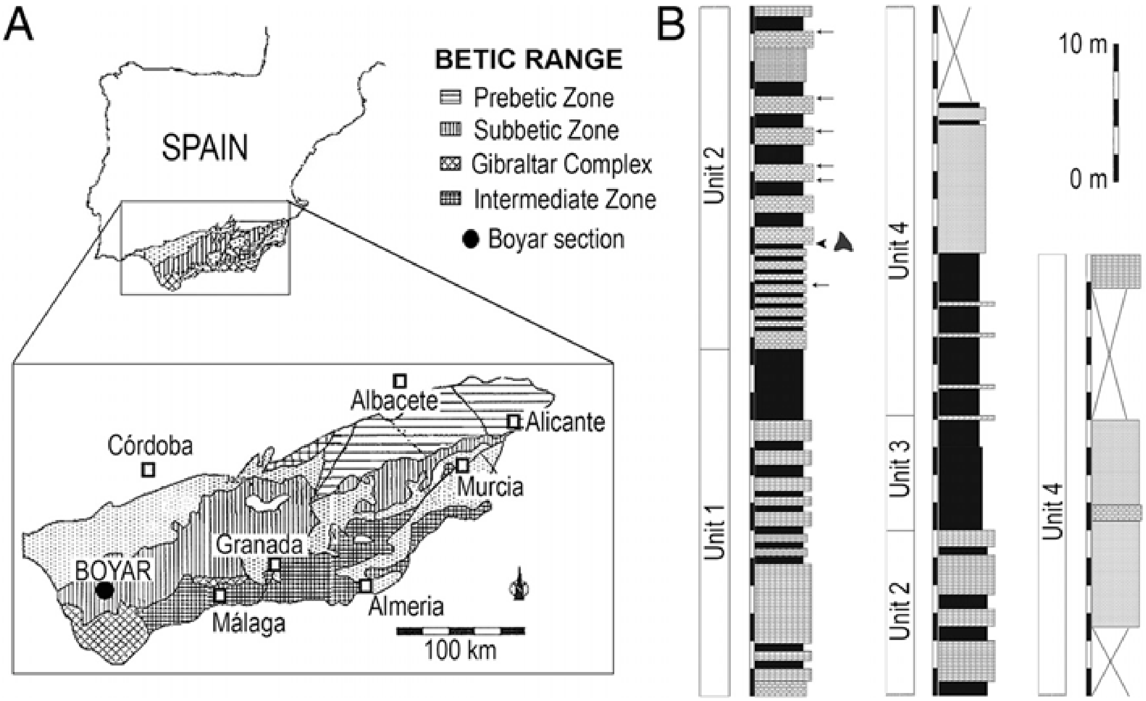

FIGURE 1. A, map of the Iberian Peninsula showing the location of the studied Boyar Section in the Betic Ranges. B, biostratigraphic column of the Boyar Section, with indication of levels sampled (thin arrows) and the level that yielded the material described in this work (black head arrow). Modified from Plasencia (2009).

sample was repolished and etched as many times as necessary to elucidate the enameloid microstructure.

3.SYSTEMATIC PALEONTOLOGY

Class Chondrichthyes Huxley, 1880

Subclass Elasmobranchii Bonaparte, 1838

Order Euselachii Hay, 1902

Superfamily Hybondotoidea Owen, 1846

Family Lonchidididadae Herman, 1977

Lonchidion Estes, 1964

Type Species *Lonchidion selachos* Estes, 1964, Maastritchian, Lance Formation, eastern Wyoming, U.S.A.

Lonchidion derenzii, sp. nov. (Figs. 2, 3A–C)

Etymology—Named after Emeritus Professor Miquel de Renzi from the Universitat de València, Spain, for his contribution to the development of paleobiology in Spain.

Type Locality—Boyar Section, near the cities of Ubrique and Grazalema, in the province of C´adiz, Spain.

Holotype—MGUV-27744, a tooth (Fig. 2A–C).

Additional Material—MGUV-27745 (Fig. 2D–F), MGUV- 27746 (Fig. 2G–I), MGUV-27747 (Fig. 2J–L), MGUV-29994 (Fig. 3A–C), and six additional teeth cataloged as MGUV-27748.

Occurrence—Middle part (level 92-A-40) of the lower unit (Muschelkalk facies) of the Boyar Section, dated as Carnian (Upper Triassic).

Diagnosis—A species based on isolated teeth. One parallel-sided to slightly triangular protruding labial peg at the crown shoulder; peg ornamented by a small cusplet and a well-developed labial crest that reaches the principal cusp; crown-root junction very constricted, representing half the width of the crown.

Description—Elongated and gracile teeth, measuring 0,4 to 0,5 mm mesiodistally, 0,2 to 0,3 mm apicobasally, and 0,2 to 0,3 mm labiolingually, with a low crown profile and presence of very low lateral cusplets. In occlusal view, some teeth have a slight 'V' shape (Fig. 2E, H), with the main cusp situated in the center of the apex of the V, whereas others show a straighter shape (Fig. 2B). Main central cusp small, rounded to triangular in shape, and labially inclined (Fig. 2E, F, H). Commonly two to three pairs of lateral cusplets, which appear very abraded in our specimens (Fig. 2D–L), with the most distal cusplets of a heigh similar to the principal cusp, giving the crown a very distinctive 'whale tail' shape in labial view (Fig. 2A, G). Labial peg very prominent and narrow, developed above the crown-root junction, parallel-sided in occlusal view, with one small accessory cusplet on the labial crest (Fig. 2A–F, J–L). Occlusal crest well developed, mesiodistally expanded, reaching the last lateral cusplet and descending from the principal cusplet to the labial peg. Lingual face slightly convex below the principal cusp. Crown-root junction very constricted, half the width of the crown, with all the bases absent except in one partial specimen (Fig. 2A). The low number of specimens does not allow us to differentiate clearly between different morphotypes or position in the jaw.

Section etched in 10% HCl for a few seconds revealed a layer of single crystallite enameloid (SCE), where individual crystallites are well discernible (Fig. 3A–C). Crystallites are around 2 mm in length and randomly arranged near the enameloid-dentine junction, whereas in the rest of the enameloid they appear more compacted and preferenctially oriented perpendicular to the crown surface.

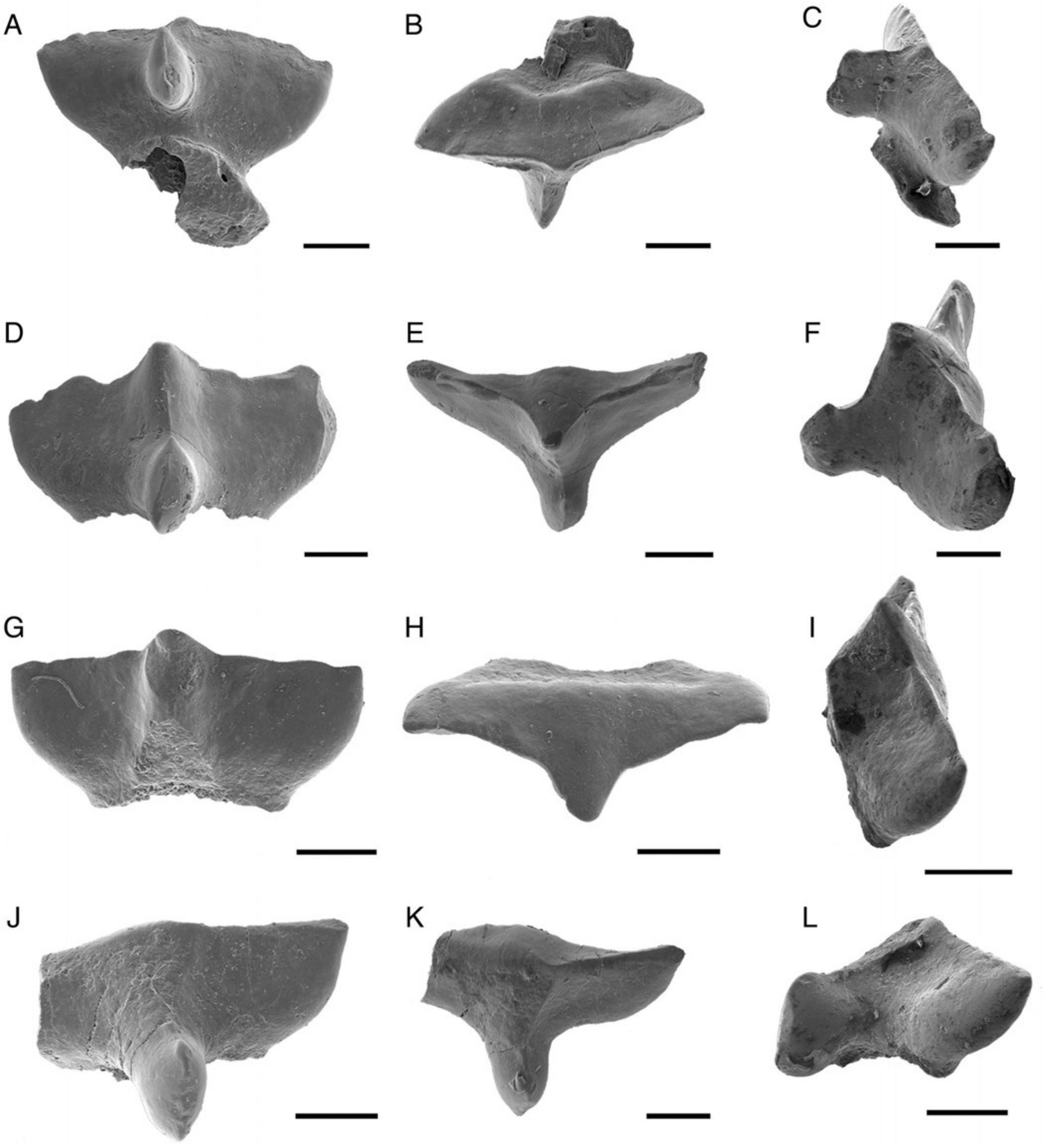

FIGURE 2. *Lonchidion derenzii*, sp. nov. A–C, MGUV-27744, holotype, in labial, occlusal, and lateral views. D–F, MGUV-27745, paratype, in labial, occlusal, and lateral views. G–I, MGUV-27746, paratype in labial, occlusal, and lateral views. J–L, MGUV-27747, paratype, in labial, occlusal, and lateral views. All scale bars equal 100 mm.

Comparision—The gracile labiolingually narrow crown of our teeth, along with the well-developed peg, clearly identify them as Lonchidion. Apart from those features, the minimal coronal ornamentation characteristic of this morphologically very conservative genus makes the differentiation among *Lonchidion* species difficult (Rees and Underwood, 2002)

Notwithstanding the combination of a very prominent and narrow peg with an accessory cusplet and a ridge that reaches the principal cusp, along with the severe constriction of the crown-root junction, sets *Lonchidion derenzii* apart from other contemporary Middle-Upper Triassic species of the genus. *Lonchidion. derenzii*, sp. nov., teeth differ from those of *L. ferganensis* (Middle-Upper Triassic of Central Asia, Fischer *et al.,* 2011) by the lack of the characteristicnodes at shoulder height labially. In addition, *Lonchidion derenzii*, sp. nov., does not show the vertical striation and crown shoulder nodes that commonly ornament *L. estesi* teeth from the Late Triassic of India (Prasad *et al*., 2008). On the other hand, notwithstanding that the simple crown of *L. derenzii*, sp. nov., resembles those of *L. paramillonensis* from the Middle–Upper Triassic of Argentina (Johns *et al.,* 2014) the latter shows a more triangular shape in labial view and the number of accessory cusplets is higher than in *L. derenzii*, sp. nov., In addition, L. *derenzii*, sp. nov., has a strongly developed labial peg, and the constriction of the crown-root junction is more severe than in *L. paramillonensis*. A well developed labial peg and the severe constriction of the crown-root junction also appear in *L. humblei* from the Upper Triassic of North America (Heckert *et al*., 2007), but this species lacks the accessory cusplets surmounting the labial peg as well as the lateral cusplets that are present in L. *derenzii,* sp. nov.

4. DISCUSSION

The lack of detailed studies on chondrichthyan faunas from the Triassic of the Iberian Peninsula has led paleontologists, for some time, to the mistaken perception that chondrichthyans were rare or absent in the region (e.g., Chrzastek, 2008). Nevertheless, during recent years, several works have indicated the presence of a rich and diverse chondrichthyan fauna from differ- ent localities in the Iberian ranges (Botella *et al*., 2009b; Pla *et al*., 2013). The present report of *Lonchidion derenzii*, sp. nov., shows that Triassic chondrichthyan remains are common not only in sediments of the Iberian ranges but also in other Triassic outcroups of the Iberian Peninsula. *Lonchidion derenzii*, sp. nov., represents the earliest record of the genus in Spain, considering its Carnian age (Upper Triassic) according to the bivalve, conodont, and pollen assemblages (Martín-Algarra *et al*., 1995).

Moreover, although Patterson (1966) in his description of the Early Cretaceous taxon *Lonchidion breve breve* from England (United Kingdom) mentioned the presence of teeth from the Muschelkalk (Ladinian) of Craislheim in Germany "which are almost indistinguishable from *Lonchidion breve breve*" (1966:331), neither a description nor figures of these teeth were provided. Therefore, *Lonchidion derenzii*, sp. nov., can also be considered as the oldest unequivocal record of the genus in Europe.

Lonchidion has been proposed as an euryhaline genus living preferentially in freshwater or brackish environments (Rees and Underwood, 2002; Heckert *et al*., 2007; Fischer *et al*., 2011; Johns *et al*., 2014). Although the Boyar Section represents marine environments, and other Triassic marine sharks have been found in several levels through the whole section (pers. observ.), the record of *Lonchidion derenzii*, sp. nov., is limited to a particular level that represents a very shallow marine platform with close continental influence (Martín-Algarra *et al*., 1995).

Other earliest (Middle–Late Triassic) records of the genus occur in freshwater facies from geographically widely separated localities (i.e., *L. paramillonensis* from South America; *L. ferganensis*, *L. estesi*, and L*. incumbens* from Asia; and *L. humblei* from North America). As noted by Johns *et al*. (2014), this requires a dispersion based on a pattern of coastal migrations, but, in our opinion, it also necessarily indicates a more ancient origin of *Lonchidion*.

Lonchidiidae Enameloid Microstructure

The enameloid of chondrichthyan teeth consists of elongated fluorapatite ($Ca_5(PO_4)_3F$) crystallites embedded in an organic matrix, which contains mainly collagen and amelogenin-like proteins (see Gillis and Donoghue, 2007; Enax *et al*., 2014; Manzanares *et al*., 2016, and references therein). Although in neoselachian sharks (Reif, 1973) and some batoids (Enault *et al*., 2015; Manzanares *et al*., 2016) crystallites appear organized in bundles (or 'fibers' sensu Reif, 1977), in all major stem-chondrichthyan groups, crystallites are individualized (SCE), usually randomly oriented, and lack any degree of higher microstruc-tural differentiation (Gillis and Donoghue, 2007; Botella *et al*., 2009a). However, in Hybodontiforms, although many species present a homogeneous layer of SCE (e.g., Reif, 1973; Gillis and Donoghue, 2007; Cuny *et al*., 2009; Pla *et al*., 2013; Enault *et al*., 2015), some Mesozoic taxa with crushing dentitions developed a distinct two-layered enameloid consisting of an outer compact single-crystallite layer and an inner layer with some crystallites organized into short, loosely defined bundles (Cuny *et al*., 2001; Pla *et al*., 2013; Enault *et al*., 2015).

Johns *et al*. (2014) described an enameloid showing fibrous structure in *Lonchidion paramillonensis.* The supporting images, however, clearly show a layer of randomly oriented individual crystallites without any superior microstructural differentiation, i.e., bundles or fibers (Johns *et al*., 2014:fig. 9). Our analysis of *Lonchid-*

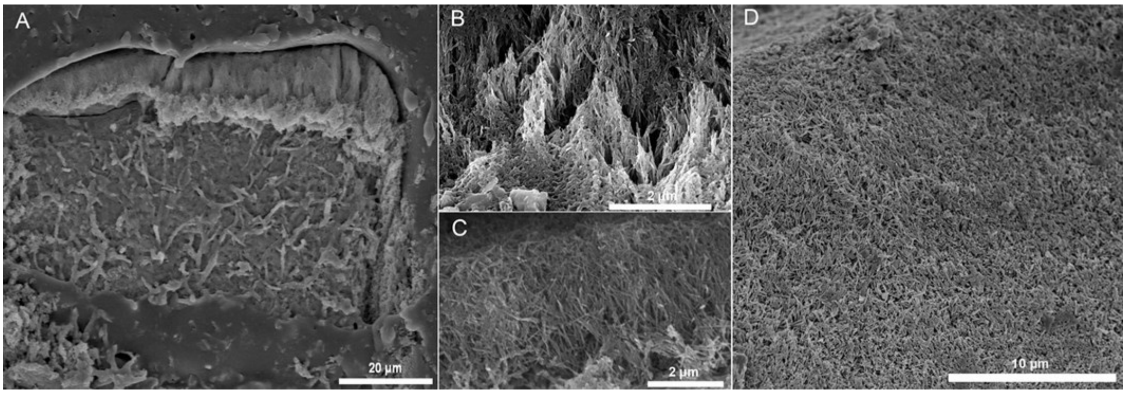

FIGURE 3. A–C, scanning electron micrograph of MGUV-29994, *Lonchidion derenzii*, nov. sp., enameloid, etched with 10% HCl for 5 s. A, overview of tooth in longitudinal section showing a well-defined enameloid layer with an irregular enameloid-dentine junction. B, detail of the general aspect of the inner part of the enameloid layer, close to the enameloid-dentine junction. C, detail of the individualized crystallites in the outer part of the enameloid layer. D, general aspect of the whole enameloid layer of *Lissodus* aff. *lepagei*, MGUV-25863 (from the Jaraf-3 Section in the Iberian Range, Spain).

ion derenzii, sp. nov., enameloid demonstrates the pres- ence of SCE (Fig. 3A–C). Individualized crystallites appear randomly arranged near the enameloid-dentine junction (Fig. 3B), whereas in the rest of the enameloid, the crystallites seem to be more preferably oriented perpendicular to the crown surface (Fig. 3C).

Previous studies on the enameloid microstructure of other lonchidiid taxa also have reported the presence of a homogeneous SCE layer in *Lissodus angulatus* (Blaz_ ejowski, 2004), *Lissodus minimus* (Cuny and Risnes, 2005), and *Lissodus* aff. *L. lepagei* (Pla *et al.*, 2013; Fig. 3D). Therefore, a single crystallite enameloid without any kind of arrangement into fibers (or bundles) is the widespread condition among Lonchidiidae.

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CHAPTER 5

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Middle Triassic sharks from the Catalan Coastal ranges (NE Spain) and faunal colonization patterns during the westward transgression of Tethys

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Palaeogeographic changes that occurred during the Middle Triassic in the westernmost Tethyan domain were governed by a westward marine transgression of the Tethys Ocean. The transgression flooded wide areas of the eastern part of Iberia, forming new epicontinental shallow-marine environments, which were subsequently colonized by diverse faunas, including chondrichthyans. The transgression is recorded by two successive transgressive–regressive cycles: (1) middle-late Anisian and (2) late Anisian–early Carnian. Here, we describe the chondrichthyan fauna recovered from several Middle Triassic stratigraphic sections (Pelsonian-Longobardian) located at the Catalan Coastal Basin (western-most Tethys). The assemblage consists of isolated teeth of the species *Hybodus plicatlilis*, *Omanoselache bucheri*, *O. contrarius* and *Pseudodalatias henarejensis*. Our data complement a series of recent studies on chondrichthyan faunas from Middle-Late Triassic marine basins of the Iberian Peninsula, allowing us to evaluate patterns of faunal colonization. Sharks that reached East Iberia during the first transgressive pulse (Anisian) show strong affinities with the Sephardic bioprovince (related with the Neotethys Ocean) whereas a later increase in chondrichthyan diversity (during the Ladinian transgressive pulse) is due to the arrival of taxa with affinities to European faunas (Palaeotethys). This contrasts with recently evaluated colonization patterns of invertebrates and conodonts, where Middle-Upper Anisian assemblages are composed of taxa with affinities to the Alpine/Germanic bioprovince whereas faunas from the Sephardic bioprovince only reached the shallow waters of the Iberian peninsula during the second (Ladinian) transgressive pulse. The differences between chondrichthyans *vs.* invertebrate and conodonts colonization patterns are explained here on the basis of differing dispersal strategies. Our finding suggests the existence, during the Anisian, of connections between Palaeo- and Neotethys through the western Cimmerian microcontinent, which served as pathways for the entrance of chondrichthyans from the Sephardic bioprovince.

1. INTRODUCTION

The Triassic is a key period to understand the evolutionary history of chondrichthyans, with the decline or extinction of several major Palaeozoic groups (such as xenacanthiforms, symmoriiforms, ctenacanthiformes and non-chimaeroid holocephalans), the increasing dominance of hybodontiforms, and the emergence of the modern sharks (Elasmobranchii). The Triassic was also an important period for the Iberian Peninsula in terms of palaeogeography. During these times, the western Tethys domain was characterised by important palaeogeographic changes and the subsequent advance of the broad westward marine transgression of the Tethys Sea that reached East Iberia during the Middle Triassic (Anisian) (Escudero-Mozo *et al.*, 2015). This transgression, for the first time during the Mesozoic, flooded wide areas of the eastern part of Iberia, connecting some internal basins (i.e. Iberian and Catalan), which until this moment had been independent (e.g. Arche *et al.*, 2004; Galán-Abellán *et al.*, 2013; Escudero-Mozo *et al.*, 2015), causing the demise of continental sedimentation and the consequent deposition of marine carbonates and mixed coastal facies (Muschelkalk).

In East Iberia, this general transgression is recorded by two transgressive-regressive cycles that show important palaeogeographic and fossil content changes (Escudero-Mozo *et al.*, 2015). The first one (middle–late Anisian) took place from the NE to the Catalan Coastal Basin reaching the Iberian Basin through a narrow corridor bounded by palaeo-highs. The second transgressive-regressive cycle (late Anisian–early Carnian) was characterised by an extensive transgression that covered a wide area of E Iberia including some of the previous topographic highs. After that, during the early Carnian, this area is characterised by the development of an extensive siliciclastic-evaporitic setting of the Keuper. These transgressive events resulted in the evolution of a diversity of epicontinental shallow coastal environments and associated colonization by marine faunas, including chondrichthyans. Accordingly, Middle Triassic faunas from the Iberian Peninsula reflect the transformation from continental settings to a wide variety of coastal and marine depositional environments owing to the Muschelkalk transgression, which occurred in the middle Anisian (Escudero-Mozo *et al.*, 2015).

In this paper, we document for the first time the presence of chondrichthyans in the Middle Triassic rocks of the Catalan Coastal ranges (CCR). This paper is complementary to Pla *et al.*'s (2013) and Manzanares *et al.*'s (2018) studies on Middle–Late Triassic chondrichthyans from other areas of the Iberian Peninsula, i.e. the Iberian (IR) and Betic ranges (BR), respectively. Together, these three works give the first general overview of the chondrichthyan communities that inhabited the shallow epicontinental seas of the westernmost Tethys during the Middle to Late Triassic. This allows us to evaluate colonization patterns of the new shallow epicontinental seas of the western Tethys followed by coastal sharks and to compare it with invertebrate faunas. Findings require some adjustments of previous palaeogeographic reconstructions of the westernmost Tethyan realm for the Middle Triassic.

2. GEOLOGRAPHICAL AND GEOLOGICAL SETTINGS

The Catalonian Coastal range is located in the north-eastern Iberian Peninsula (Fig. 1A) and includes two mountain chains that run parallel to the Mediterranean Coast in a NE–SW direction, from the Gerona region toward the Ebro delta, separated by the Prelitoral Depression (Sopeña and de Vicente, 2004; Escudero-Mozo *et al.*, 2015).

The Catalonian Basin constituted an intracratonic basin that was mainly developed during Mesozoic times when it experienced some extensional periods with several *syn*-rift and post-rift phases (Vargas *et al.*, 2009; De Vicente *et al.*, 2009). It is dominated by longitudinal NE–SW faults and to a lesser extent by a conjugated NW–SE fault system (Vegas, 1975); the latter divided this basin into three domains with different subsidence (from SW to NE): Priorat-Baix Ebre, Prades, and Gaiá-Montseny (Marzo, 1980; Calvet and Ramon, 1987; Ramon and Calvet, 1987; Gaspar-Escribano *et al.*, 2004). During the Cenozoic, Alpine compressional tectonics gave rise to the present-day Iberian range and Catalan Coastal range.

In the Catalonian Basin, the succession shows the three typical subdivisions of the Germanic Triassic, namely Buntsandstein, Muschelkalk and Keuper facies. In the Catalan Coastal range, the Muschelkalk facies is represented by two carbonate units (“Lower” and “Upper” Muschelkalk) that record the deposition of shallow to outer carbonate ramps, separated by a mixed evaporite-siliciclastic unit (“Middle Muschelkalk”). This facies, combined with the uppermost part of the Buntsandstein (or Röt) facies and the lower part of the Keuper facies, records two major transgressive–regressive cycles, respectively of middle–late Anisian and late Anisian–early Carnian age (Escudero- Mozo *et al.*, 2014, 2015), each one related to the development of a wide carbonate ramp. The studied sections are constituted from materials of the “Lower Muschelkalk” (L'Ametlla section) from the Gaia–Montseny domain, and "Upper Muschelkalk" (Rasquera-Benifallet and Tivissa sections) from the Priorat-Baix-Ebre (Fig. 1B).

In the Gaia–Montseny domain, the Lower Muschelkalk shows an average thickness of 120 m, showing a sharp lower contact with lutite, carbonate and evaporite units (Röt facies) and a transitional upper contact with the Middle Muschelkalk. The unit was divided by Calvet and Ramon (1987) and Calvet *et al*. (1990) into four members, which correspond to El Brul (shallow subtidal to supratidal deposits), Olesa (lagoonal bioclastic carbonates), Vilella Baixa (open marine burrowed deposits) and Colldejou (white peritidal dolomite facies). Based on ammonoids, foraminifera, pollen and brachiopods, the Lower Mu-schelkalk unit is dated as middle–upper Anisian (Calvet and Marzo, 1994; Goy, 1995; López-Gómez *et al*., 1998; Márquez-Aliaga *et al*., 2000). Chondrichthyan remains are concentrated in different levels of the Olesa unit.

The L'Ametlla section is located close to the village of L'Ametlla del Vallès in the province of Barcelona, Catalonia, 35 km northeast of Barcelona City (coordinates at the base; 41° 43′ 18″ N and 2° 15′ 26″ W; Fig. 1A). The section and its fossil record have been studied previously by Márquez-Aliaga *et al*. (2000) and Plasencia (2009, and references therein).

In the Baix-Ebre Priorat domain, the Upper Muschelkak carbonates show a gradual to sharp lower contact with the "Middle" Muschelkalk and a gradual upper contact with the Keuper, and show an average thickness of 140 m. In this area, the carbonate unit was divided by Calvet *et al*. (1987) into several members from base to top that are: 1) Rojals Member (shallow subtidal to supratidal oolitic limestone and dolostone); 2) Benifallet Member (low energy subtidal bioturbated limestone and dolostone); 3) Rasquera Member (outer to middle ramp limestone, dolostone and shales with Daonella); 4) Tivissa Member (outer to inner ramp car-

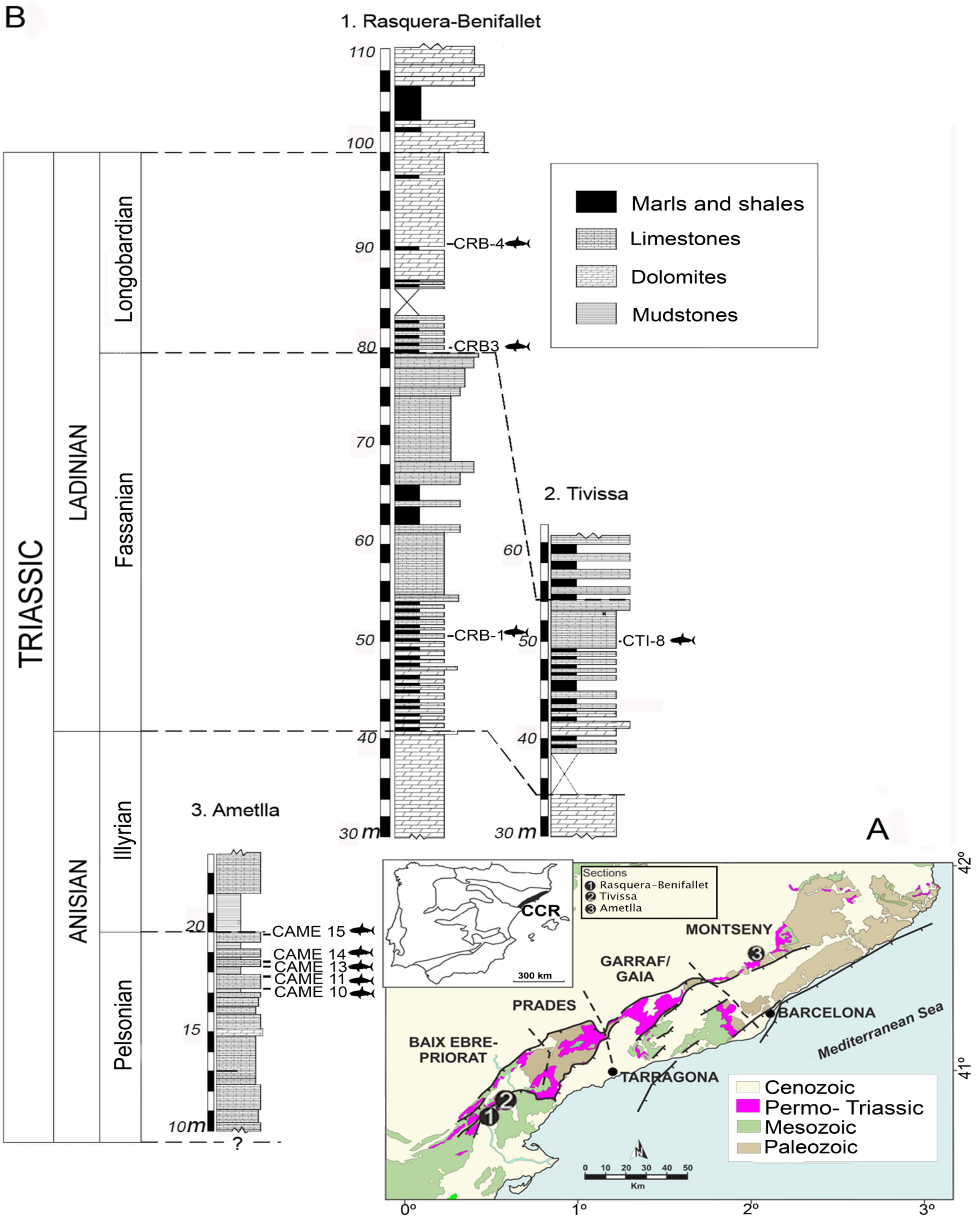


Fig. 1. Geographical and geological settings. A) Geographical setting of Catalonian Coastal Range (CCR) with location of sections studied here, modified from Escudero-Mozo (2015); B) Stratigraphic columns of the sections studied. Only levels where chondrichthyans remains were recovered are marked in the columns; for complete columns see Plasencia (2009)

bonates); and 5) Capafons Member (peritidal dolomites, marls and shales). Based on the ammonoids yielded by the Rasquera and Tivissa members and the sequence stratigraphic analysis, the Upper Muschelkalk has been dated as late Illyrian–late Longobardian (Goy, 1995; Escudero-Mozo *et al.*, 2015). Conodonts from the same samples confirm a Ladinan age (Plasencia, 2009).

Both the Rasquera–Benifallet and Tivissa sections expose sediments of the five members as defined by Calvet *et al.* (1987). These sections are located in the province of Tarragona, Catalonia (Fig. 1A). The Rasquera–Benifallet section is along the road of Rasquera to Benifallet villages (coordinates at the base 40° 59′ 38″ N and 0° 32′ 22″ W), and the Tivissa section is close to Tivissa village (coordinates at the base 41° 02′ 33″ N and 0° 44′ 48″ W). Samples that have yielded chondrichthyan remains came from both the Rasquera and Tivissa members (Fig. 1B). Conodonts from the same samples confirm a Ladinan age (Plasencia, 2009).

3. METHODOLOGY

The specimens studied here were obtained by acid etching in 5–10% acetic acid, of more than 60 Kg of limestones (in samples of around1.5 kg each) from the Rasquera–Benifatell, Tivissa and Ametlla sections. After dilution residues were sieved with sieves meshes of 2, 0.125 and 0.063 mm respectively, which created three fractions refer as the “total sample”. Fig. 1B shows the stratigraphic position of all samples that have yielded chondrichthyan remains. Fossils were picked under a bi -nocular microscope and photographed with an HITACHII S-4800 Electronic Microscope hosted at the Microscopy Services of the University of Valencia. The archived specimens are mainly isolated teeth and scales, housed in the Museum of Natural History of the University of Valencia (former Geological Museum of Valencia University), Spain (MGUV-36111 to MGUV-36113; and MGUV-36117 to MGUV-36131)

4. RESULTS

The chondrichthyan assemblage found in the Middle Triassic of the Catalan Basin includes four species belonging to three different genera; i.e. *Pseudodalatias henarejensis, Omanoselache bucheri, O. con*trarius and *Hybodus plicatilis* (Fig. 2). A detailed Systematic Palaeontology section is provided as Supplementary material.

5. RESULTS

5.1.Middle Triassic sharks from the Catalan Basin in the context of the Iberian Peninsula

This association is similar to, but less diverse than, the chondrichthyan faunas recently described by Pla *et al*. (2013) and Manzanares *et al*. (2018) from other Middle-Late Triassic basins of the Iberian Peninsula (see also Botella *et al*., 2009; Ferrón *et al*., 2014; Manzanares *et al*., 2017). In fact, *P. henarejensis*, *O. bucheri*, *O. contrarius* and *H. plicatilis* are a typical selection of Middle Triassic forms from Iberia being also present in the Ladinian of both the Iberian and Betic Ranges (Fig. 3 B).

In general, the Middle Triassic shark communities from the epicontinental seas of the westernmost margin of the Tethys ocean (i.e. east of the actual Iberian Peninsula) were largely dominated, in terms of diversity of species and abundance, by durophagous sharks with crushing dentitions (i.e. *O. bucheri*, *O. contrarius, Lissodus* aff. *L. lepagei, Lonchidion derenzii*), well adapted for grabbing and crushing prey with a hardened exoskeleton (e.g. crustaceans and mollusks). The teeth in general are small, suggesting that these sharks were also of small size, possibly in turn subsidiary prey of some of the larger aquatic

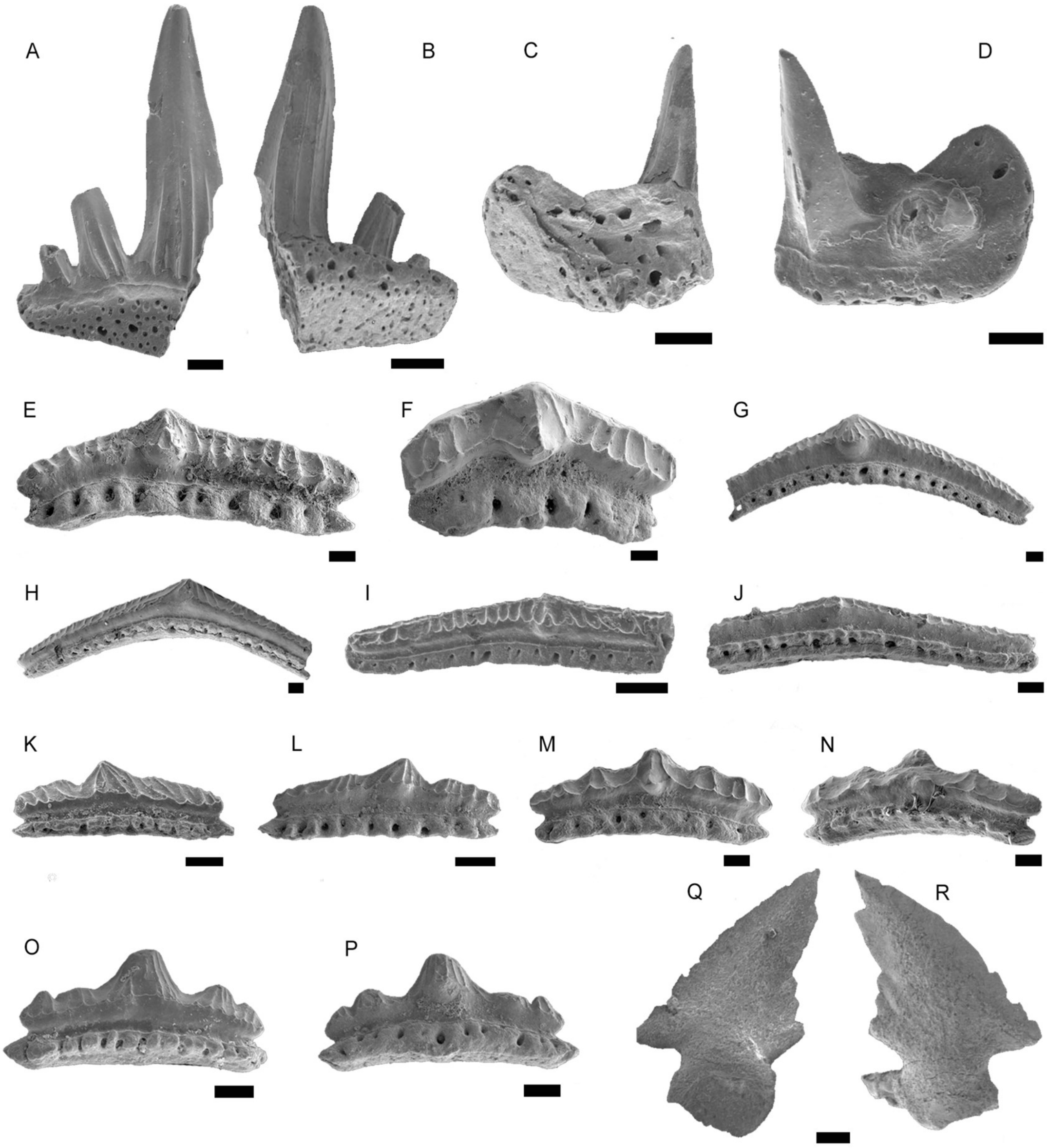


Fig. 2. Chondrichthyan teeth from the Middle Triassic of the Catalan Coastal Ranges, Spain. A–D) *Hybodus plicatilis*; A–B) labial and lingual view, MGUV-36111; C–D) basal and occlusal view, MGUV-36112; E-J) *Omanoselache bucheri* E) labial view, MGUV-36121; F) labial view, MGUV-36122; G–H) labial and lingual view, MGUV- 36123; I–J) labial and lingual view, MGUV-36127; K–P) *Omanoselache contrarius*; K–L) labial and lingual view, MGUV-36127; M–N) labial and lingual view, MGUV-36120; O–P) MGUV-36118. Q–R) *Pseudodalatias henarejensis* in labial and lingual view, respectively, MGUV-36113. All scale bars equal 100 µm.

reptiles, e.g. pachypleurosaurs, nothosaurs or ichthyosaurs, that were common in the same areas (Fortuny *et al.*, 2011; Márquez-Aliaga *et al.*, 2000), being these (most probably) the apex predators of these shallow marine environments.

The stratigraphic distribution of taxa in East Iberia (i.e. Catalonian, Iberian and Betic basins) is summarized in Fig. 3 A. Fig. 3B, providing synthetic information on the specific abundance. Teeth of O*. bucheri, O. contrarius* and *H. plicatilis* from samples of the Olesa Unit in section L'Ametlla represent the first report of Anisian (Pelsonian–lower Illyrian) sharks of the Iberian Peninsula showing that chondrichthyans reached the epicontinental shallow waters of NE Iberia during the first (middle–late Anisian) Triassic transgression. The stratigraphic record of all these three species extends up into the Carnian (Late Triassic) in the Iberian Peninsula, being also the most representative and abundant taxa.

On the other hand, an important increase of the chondrichthyan diversity in the Triassic Iberian basins occurs in the Longobardian, during the second transgressive–regressive cycle, with the appearance of several taxa, such as *Palaeobates angustissimus, Lissodus* aff. *L. lepagei*, *Rhomaleodus*, or Pseudodalatias *henarejensis* (Fig. 3A).

5.2. Biogeographical affinities of chondrichthyan *vs.* invertebrate faunas from East Iberia during the Triassic westward transgressions of the Tethys Sea

Recent evaluations on the evolution of invertebrate faunas from the Middle Triassic carbonate platforms in eastern Iberia reflect a drastic palaeobiogeographic change during the Upper Anisian. Thus, fossil associations (mainly bivalves, foraminifers, brachiopods and conodonts) from the Landete Formation (Iberian

Range) and lower Muschelkalk (Catalan Coastal Range), both dated as Middle–Upper Anisian (Pelsonian–early Illyrian), are almost exclusively composed by taxa with affinities to the Alpine/Germanic bioprovince, related with the Palaeotethys Ocean. This has been interpreted as the Iberian and Catalonian Basins acting as a palaeogeographic gulf opening northwards, only connected with the Palaeotethys. The presence of geographical barriers should have prevented the entrance of faunas from the Sephardy bioprovince, related with the Neotethys (Escudero-Mozo *et al*., 2015; see fig. 19). In contrast, fossil associations of invertebrates and conodonts from the upper Anisian to upper Ladinian (late Illyrian–Longobardian; i.e. Cañete Fm., Iberian Range and upper Muschelkalk, Catalan Coastal Range) show a mixed origin, where, along with endemic species, typical species of both the Alpine/ Germanic and Sephardic bioprovinces appear. This palaeobiogeographical heterogeneity of faunas has been linked to the second and most severe, transgressive–regressive cycle (late Anisian–early Carnian), which allowed for the colonization of shallow waters of the Iberian Peninsula by faunas coming from both Tethys provinces: the Germanic/ Alpine to the north and the Sephardic to the south.

Noticeably, the stratigraphic distribution of Middle–Upper Triassic chondrichthyans in the Iberian Peninsula differs from this pattern of colonization. Thus, taxa that reached the new shallow-water environments of NE Iberia during the middle Anisian transgression (i.e. *Hybodus plicatilis* , *O. bucheri* and *O. contrarius*) do not show the clear Alpine/Germanic affinities reflected in invertebrate and conodont faunas. Only *Hybodus plicatilis* has been reported from other localities in Europe (see supplementary information) including Poland (Niedzwiedzki, 2008); Luxembourg (Delsate and Duffin, 1999); Bul-garia (Stefanov, 1966); Germany and UK

(Korneisel *et al.*, 2015). However, this taxon might be a cosmopolitan species from the Tethys realm as teeth assigned to *Hybodus* sp. *Aff. Hybodus plicatilis* have been reported from the Middle Triassic of Saudí Arabia (Vickers *et al.*, 1999). In European localities, *H. plicatilis* occurs in association with other “*Hybodus*”, *Acrodus*, *Polyacrodus, Lissodus* and some neoselachian species (Niedzwiedzki, 2008; Delsate and Duffin, 1999; Korneisel *et al.*, 2015), which are absent in the Anisian of the Iberian Peninsula. In the same sense, the genus *Omanoselache* has never been reported from the other Triassic localities in Europe apart from the Iberian Peninsula occurrences. *O. contrarius* oc-

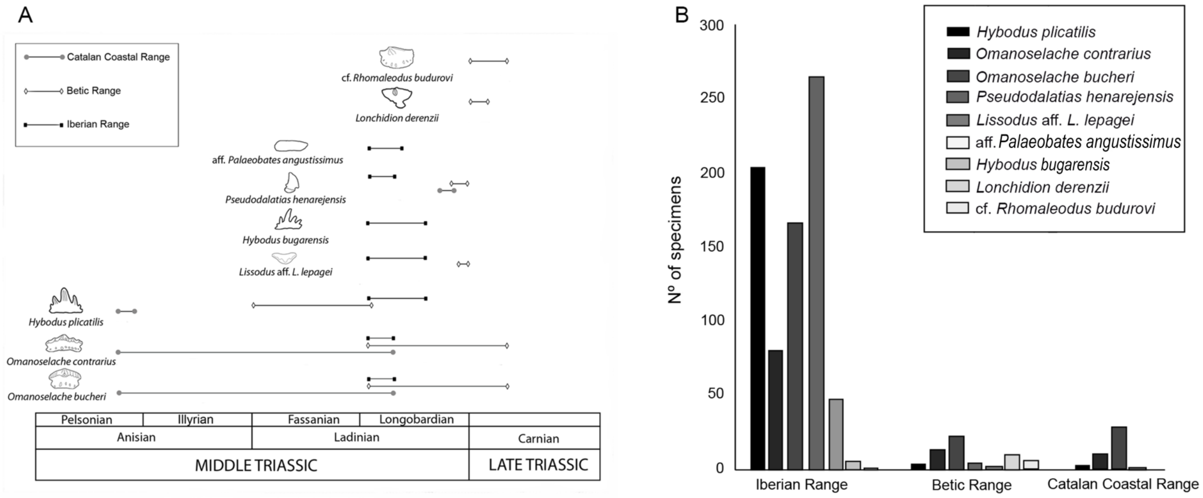


Fig. 3. Stratigraphic distribution and abundance of chondricthyans in Middle-Late Triassic of the Iberian Peninsula A) Stratigraphic distribution of taxa *H. plicatilis*, *O. bucheri*, *O. contrarius* and *P. henarejensis* are the typical fauna that can be found in any Middle Triassic basin of the Iberian Peninsula. *Lissodus* aff. *L lepagei* appears only in the Iberian Ranges and Betic Ranges, whereas *Hybodus bugarensis* and *Palaeobates angustissimus* have been only found in the Iberian Ranges. *Lonchidion derenzii* and cf. *Rhomaleodus budurovi* are only present at the Betic Ranges. B) Histograms showing the number of chondrichthyan teeth recovered (for each species) from in the three basins of the Iberian Peninsula considered in this study (the Iberian Range, the Betic Range and the Catalan-Coastal Range).

curs in the Middle Triassic of Canada (Johns *et al*., 1997) and in the Middle–Late Triassic of Gualing, China (Chen *et al*., 2007), whereas *O. bucheri* is known from the Middle Triassic of Nevada, U.S.A. (Rieppel *et al*., 1996; Cuny *et al*., 2001). Pla *et al*. (2013) suggested that this species might have reached the Iberian Peninsula through the Sephardic province (i.e., the southern margin of the Tethys Ocean). The recent discoveries of several new *Omanoselache* species from the Middle Permian (Koot *et al*., 2013) and Lower Triassic (Koot *et al*., 2015) of Oman confirm the Sephardic affinities of the genus. Then again, the increase of the chondrichthyan diversity occurred during the second transgressive–regressive cycle (Longobardian, Fig. 3A) is due to the appearance of several taxa with clear affinities with Alpine/Germanic faunas, such as *Palaeobates angustissimus, Lissodus, Rhomaleodus* or *Pseudodalatias* (Fig. 3).

5.3. Dispersal strategies, oceanic palaeocurrents and readjustment of palaeogeographic reconstructions; an overview

Oceanic currents therefore act as distinctive unidirectional corridors for passive dispersion of organisms with a microscopic pelagic life-stage (Zinsmeister and Emerson, 1979; Jablonski and Lutz, 1983). Consequently, palaeontologists have recognized for a long time that the distribution of some marine fossils are useful for reconstructing ancient marine current patterns. As Jablonski and Lutz (1983) pointed out, low (or high) faunal similarity between regions does not necessarily imply large (or small) geographic disjunctions, but rather it can reflect the prevailing current system.

In this sense, dispersion of bivalves, ammonites and foraminifers was largely determined by the dominant systems of marine currents. Most marine invertebrates include a microscopic, free-living, pelagic life history stage—the larval form. Larvae feed in the plankton (except for lecithotrophs) exhibiting long pelagic durations and high dispersal capacity. Distribution of these taxa is largely determined by ocean currents transporting larvae between distant patches of suitable habitat for adults, often over great distances (Scheltema, 1986; Jablonski and Lutz, 1983; Pechenik, 1999; Kinlan *et al.*, 2005; Trakhtenbrot *et al.*, 2005; Treml *et al.*, 2008; Wangensteen *et al.*, 2017). On the other hand, ammonites underwent a direct development lacking a distinct larval stage (as other, extant cephalopods: nautiluses, cuttlefishes, squids and octopuses) consisting of four ontogenetic stages (embryonic/egg, neanic, juvenile, and adult; see e.g. Westermann, 1996; Klug *et al.*, 2015; Zell and Stinnesbeck, 2016; Tajika *et al.*, 2018). Nevertheless, most authors consider that ammonite hatchlings – the neanic stage – were planktic, with ocean currents enhancing their dispersion. In fact, the patchy yet widespread palaeogeographic distribution of numerous ammonite species suggests that extensive planktic dispersal with ocean currents played an important role in their dispersion patterns (Zell and Stinnesbeck, 2016). The same is valid for foraminifers. Thus, for benthic attached species, such as those fossils found in NE Iberia (L. Marquez pers. comm. (2018), but see also the list of species in Escudero-Mozo *et al.*, 2015), the most common ways of dispersion were, based on living forms (1) release of gametes, zygotes, or of embryonic agamonts/gamonts to the water column, and (2) adaption to a meroplanktonic juvenile stage with passive spread by currents. Moreover, shallow-water taxa can also be transported long distances by floating objects, larger animals and/or by suspended particles (Myers, 1936; Murray, 2006; Nomura *et al.*, 2010; Alve, 1999).

In contrast, chondrichthyans lack

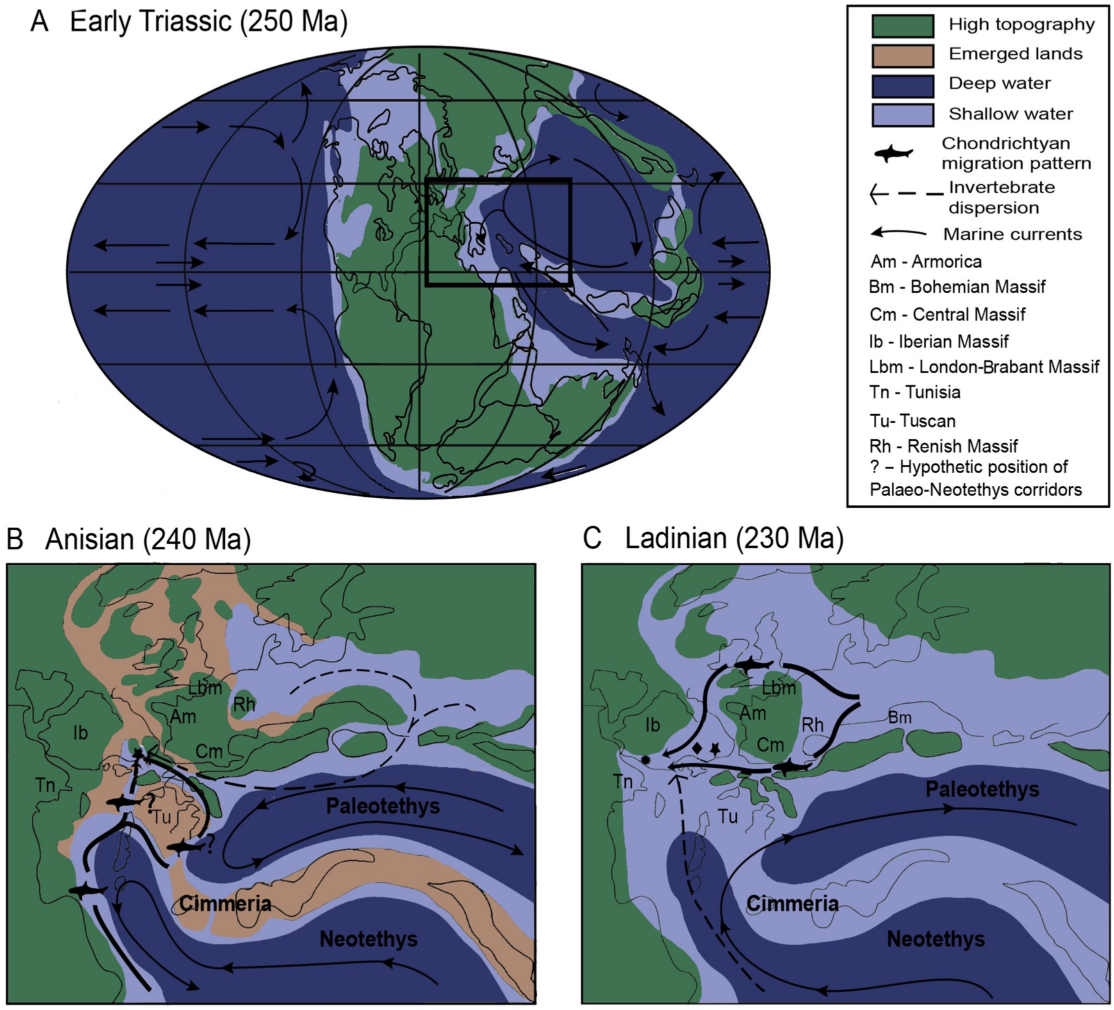
any planktic stage but undergo a direct development after internal fertilization. They possess large eggs with a high amount of yolk and produce ovoviviparous or oviparous juveniles (there are some viviparous species). In any case, the distribution of the chondrichthyan species largely depends on the dispersal abilities of adults and much less on marine currents. In this sense, it is well known that the vagility of sharks is directly proportional to body size (e.g. Musick *et al.*, 2004). As a whole, small-bodied coastal sharks present a very low vagility and short forage distances, with oceanic distances, deep channels or strong regional currents acting as dispersal barriers (Musick *et al.*, 2004; Munroe *et al.*, 2015; Whitney *et al.*, 2012). Thus, dispersion of coastal species usually requires continuous appropriate habitats across regionally small geographical scales.

5.4. Palaeogeography and dominant marine currents at the westernmost Tethys

To consider the different dispersal capability of organisms is important in order to evaluate the timing and nature of palaeobiogeographical barriers. Taxa with planktic stages can provide considerable information for reconstructing marine palaeocurrent systems while taxa lacking these stages (or with low-dispersal larvae) can be used to infer continuity among continental shelves, both groups being affected differentially by palaeobiogeographic barriers (see e.g. Shuto, 1974; Jablonski and Lutz, 1983). Following these general rules, the existing differences in the colonizing patterns of invertebrate vs chondrichthyan faunas in the Triassic shallow waters of E Iberia can be reasonably well explained. However, it requires some adjustments of previous palaeogeographic reconstructions of the westernmost Tethyan realm (see Escudero-Mozo *et al.*, 2015; their fig. 19 and references therein).

We suggest that the colonization by bivalves, ammonites and foraminifers, during both the late Pelsonian–early Illyrian and the late Illyrian–Longobardian marine transgressions, was largely determined (or enhanced) by the prevailing marine currents in the westernmost Tethys and by the transgression of the sea itself (Fig. 4).

Thus, in late Pelsonian–early Illyrian times, marine currents from the Palaeotethys enhanced the arrival of planktic organisms to the new Catalan and Iberian marine basins. At this time, the Cimmerian microcontinent, which controlled the regional palaeogeography in the westernmost Tethys (Yin and Song, 2013; Escudero-Mozo *et al.*, 2015), would have acted as a barrier for marine currents (if any) of the incipient Neotethys. However, the occurrence of chondrichthyans from the Sephardic domain indicates the formation of narrow, probably temporary connections (acting as alternative routes for the entrance of sharks) between Palaeo- and Neotethys during the northward displacement of the Cimmerian microcontinent (Fig. 4). During the next greater and more generalized sea level rise (Illyrian–Longobardian transgression), the Tethys sea flooded vast areas of the westernmost Cimmerian microcontinent, such as Alboran, Majorca, Minorca, Sardinia, Corsica, Tuscany, Apulia, Mani (Escudero-Mozo, 2015: fig. 19). Subsequently, the Palaeotethys and Neotethys became broadly con- nected, allowing for the entrance of planktonic organisms (i.e. bivalve larvae, neatic ammonites and foraminifers, among others) into E Iberia, which were transported by marine currents from the Neotethys (Fig. 4). Notably, during both colonization events, conodonts follow the same dispersion patterns as invertebrates and not those of chon- drichthyans. Conodonts are considered by some authors a basal group of vertebrates (e.g. Donoghue *et al.*, 2000) although their placement in this group is seriously questioned by other authors (e.g. Turner *et al.*, 2010). Little



A Early Triassic (250 Ma)

B Anisian (240 Ma)

C Ladinian (230 Ma)

Fig. 4. Faunal colonization patterns of East Iberia during the Anisian and Ladinian westward transgression of the Tethys ocean. A) Global palaeogeographic map, Early Triassic (Induan), with schematic surface-current circulation. B–C) detailed palaeogeographic reconstructions of the westernmost Tethyan realm for the Anisian(B) and Ladinian (C) with interpreted colonization routes for chondrichthyans and invertebrates. Palaeogeographic maps modified from Scotese (1994) (A), Stampfli _and Borel (2002) and Escudero-Mozo *et al.* (2015) (B); oceanic currents interpreted according with Yan and Zhao (2001); Crasquin *et al.* (2010) and Martindale *et al.* (2019). The black star marks the position of the Catalan Coastal Range, the black circle marks the Betic Range, the black diamond represents the Iberian Range.

is known about their reproductive strategies (with the finding of putative conodont eggs, von Bitter and Pohl, 2009), although it has been suggested, based on their crown growth and function (see Martínez-Pérez *et al.*, 2014), that conodonts could had developed a larval stage (Shirley *et al.*, 2018). Data here discussed are compatible with conodonts having a passive dispersive planktic stage. Planktic larval stages (or eggs) are present in several osteichthyan (bony fishes; common in teleosteans) but unknown in other fishes. A larval stage is present in agnathan lampreys (but not in mixines) and consists of a large sedentary burrowed larva in freshwater streams (e.g. Kelly and King, 2001), and could to be present in other extinct Palaeozoic agnathan fishes (Ferrón *et al.*, 2018). The cosmopolitanism and high dispersal of Palaeozoic conodonts might also agree with a planktic dispersion drifted by ocean currents; however, further and more extensive studies on this topic are necessary to support this idea.

It should further be noted that, despite that our interpretation are well supported by data from Pla *et al.*, (2013), Manzanares *et al.* (2018) and here, the number of specimens and taxa in the Iberian Peninsula is relatively low. Moreover, Triassic shark faunas are still poorly known in general. Therefore, new findings could change (or refiene) this interpretation in the future.

6. CONCLUSIONS

This work completes a series of studies on the chondrichthyan faunas from Middle–Late Triassic of Iberia which has changed the previously inaccurate perception that chondrichthyans were rare, or completely absent, in the Iberian Peninsula. These studies provide now a detailed overview of the evolution of chondrichthyan communities in the new shallow-water environments developed in E Iberia (western- most Tethys) during the Triassic westward transgression of the Tethys Sea. Chondrichthyan communities were largely dominated by small durophagous coastal sharks with crushing dentitions well adapted for preying on crustaceans and mollusks. Sharks that reach NE Iberia during the first transgressive pulse (middle–late Amirian) of the Tethys Sea show clear affinities with taxa from the Sephardic bioprovince, related to the Neotethys Ocean (i.e. *Omanoselache*). The notable increase in chondrichthyan diversity that occurs in E Iberia during the second transgressive pulse (Ladinian) is due to the arrival of new taxa with clear affinities with European faunas from the Palaeotethys Ocean (i.e. *Paleobates angustissimus*, *Lissodus*, *Hybodus*, *Rhomaleodus*, or *Pseudodalatias*). This colonization pattern is somewhat inverse to those recently evaluated for other fossil groups Thus, Anisian bivalves, foraminifers, brachiopods and conodont faunas from E Iberia are almost exclusively compose of taxa from the Alpine/ Germanic bioprovince. In contrast invertebrate and conodonts from the Sephardic bioprovince reach the shallow waters of the Iberian Peninsula for first time during the second (Ladinian) transgressive pulse. The existing differences between the colonization patterns of sharks and other groups are explained on the basis of their dissimilar disper- sion strategies: dispersion of bivalves, ammonites and foraminifers, is largely determined by oceanic currents whereas dispersion of coastal sharks depends of adult vagility (very low) and requires continuous appropriate habitats. Based on this, we propose some adjustments of previous palaeogeographic reconstructions including: (1) the existence of narrow, probably short-term, shallow-water corridors between Palaeo- and Neo-Tethys during the Anisian; (2) the Cimmerian micro-continent would have acted as a barrier for the oceanic currents in the incipient Neotethys, while currents from the Palaeotethys transported planktic organisms to the new Catalan and Iberian Marine Basins, and (3) during the next sea-level rise (Ladinian), the Tethys sea onlapped vast areas of the westernmost Cimmerian microcontinent allowing marine currents from the Neotethys to extend toward the Palaeotethys reaching E Iberian epicontinental seas.

DECLARATION OF COMPETING INTEREST

We confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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SUPPLEMENTARY MATERIAL

Sistematic Palaeontology

Class CHONDRICHTHYES Huxley, 1880
Subclass ELASMOBRANCHII Bonaparte, 1838
Order EUSELACHII Hay, 1902
Superfamily HYBODONTOIDEA Owen, 1846
Family HYBODONTIDAE Owen, 1846
Genus *HYBODUS* Agassiz, 1837

Type species—*Hybodus reticulatus* Agassiz, 1837

Occurrence— Lower Jurassic, Lias of Lyme Regis, England (Woodward, 1916).

HYBODUS PLICATILIS Agassiz, 1843

Figure 2, A–D

Material—Two incomplete teeth from the Rasquera–Benifallet section (CAME-14 level: MGUV-36111/-36112).

Description—Most of our material is broken, with only almost complete two multicuspid teeth. They show a central cusp flanked by up to two pairs of cusplets, all of them lingually inclined (Fig. 2 A–D). Ornamentation ridges run from the tooth shoulder towards the tip of the cusp and cusplets. In the main cusp, the ridges do not reach the apical part; they cannot be seen in the lateral cusplets due to their damage (Fig. 2A-B). The vascularization is of the anaulacorhize type (Fig. 2 A-B).

Remarks—Several recent authors agree that the genus *Hybodus* is a polyphyletic group and that it does not correspond to a natural group (Rees, 1999; Underwood and Rees, 2002; Rees and Underwood, 2008; Ginter *et al.*, 2010; Cappetta, 2012, Leuzinger *et al.*, 2017), a statement with which we fully agree. A large number of isolated teeth described in the literature have been assigned to *Hybodus,* although a revision of this material is required to verify, which of them fit completely the original diagnosis of the genus. However, this is beyond the scope of the present work; nevertheless, our material shows characteristics of the type species *Hybodus reticulatus*: well-defined and well-ornamented conical cusps; lateral cusplets decreasing in height, frequently inclined lingually; and a fairly shallow base (see Maisey, 1987; Duffin, 1993; Ginter *et al*., 2010). Thus, we choose to maintain the name of the genus *Hybodus* until a revision of the genus is done.

Occurrence—Muschelkalk, Middle Triassic of Schwenningen, Germany (Agassiz, 1843); Muschelkalk, Middle Triassic of Monte Giorgio, Switzerland (von Meyer, 1849; Rieppel, 1981); Muschelkalk, Middle Triassic of Luxemburg (Delsate, 1992, 1993); Middle Triassic, Iberian Ranges, Spain (Pla *et al*., 2013), Middle–Late Triassic of the Betic Ranges, Spain (Manzanares *et al*., 2018); Middle Triassic, Catalan Coastal Ranges, Spain.

Order INCERTAE SEDIS

Family HOMALODONTIDAE Mutter, De Blanger and Neuman, 2008

Genus *OMANOSELACHE* Koot, Cuny, Tintori and Twitchett, 2013

Type species—*Omanoselache hendersoni* Koot, Cuny, Tintori and Twitchett, 2013

Occurrence—Middle Peermian, Wordian from the Khuff Formation, Oman (Koot *et al*., 2013)

OMANOSELACHE BUCHERI (Cuny, Rieppel and Sander, 2001) Figure 2, E-J

Material—Eight complete teeth and more than 20 fragments from the Rasquera–Benifallet section (CRB-1 level: MGUV-36121/-36122/-36126/-361627/-36128; and CRB-3 levels MGUV-MGUV-361216/36128; MGUV/-36123/-36130/-36131), l’Ametlla section (CAME-12: MGUV-36124; and CAME-13 levels; MGUV-36125) and the Tivissa section (CT-8 level: MGUV-36129).

Description—Teeth are labio-lingually compressed and elongated mesiodistally. All display a well-developed occlusal crest and a pyramidal main cusp (Fig. 2 E-H). Undulated vertical ornamentation ridges originate on the occlusal crest and appear on the labial and lingual sides (Fig. 2 E-F, I). A rounded lingual peg, characteristic of this species, is located under the main cusp (Fig. 2 E-G, I). Vascularisation is anaulacorhize with a row of well-developed foramina opening on the labial and lingual sides (Fig. 2 E, G).

Remarks—*Omanoselache bucheri* teeth are the most widespread, abundant and well-preserved chondrichthyan remains from the Iberian Peninsula (Pla *et al.*, 2013; Manzanares *et al.*, 2017). Pla *et al.* (2013), described three different morphotypes related to the different position they occupied in the jaws: symphyseal, mesial, or latero-distal teeth. Most of our material can be classified as their mesial teeth. These teeth exhibit one main cusp and are arched, showing a 'boomerang' appearance (Fig. 2 G-H). A few show a blunt and reduced main cusp and an overall more rectilinear appearance (Fig. 2 I-J), which are the characteristics of latero-distal teeth. The presence of a lingual peg, but no labial one, and the rounded edges (Fig. 2E-F, G, I) permit us to differentiate them from *Omanoselache contrarius* (Johns, Barnes and Orchard, 1997).

Occurrence—Middle Triassic, North-West of Nevada (Rieppel *et al.*, 1996; Cuny *et al.*, 2001); Middle Triassic, Iberian Ranges, Spain (Pla *et al.*, 2013); Middle–Late Triassic from the Betic Ranges of Spain (Manzanares *et al.*, 2018); Middle Triassic, Catalan Coastal Ranges, Spain.

OMANOSELACHE CONTRARIUS
(Johns, Barnes and Orchard, 1997)
Figure 2 K-P

Material—Four complete teeth and several fragments from the Rasquera–Benifallet section (CRB-1 level: MGUV-36117; and CRB-3 levels: MGUV-3619/-36120) and l'Ametlla section (CAME-12 level: MGUV-36118).

Description—Teeth elongated mesiodistally and compressed labiolingually. All of them show one main cusp flanked by up to two lateral cusplets (Fig. 2 K-P). Ornamentation consists of vertical ridges that originate from the apex of the cusps and extend to the tooth shoulder. The teeth present two pegs (labial and lingual) under the main cusp (Fig. 2 M-N). As in O. bucheri, the vascularization is anaulacorhize (Fig. 2 K-P).

Remarks—All the teeth recovered of this species present evident wear signs on the cusps.

Occurrence—Middle Triassic of British Columbia, Canada (Johns *et al*., 1997); Middle–Late Triassic of Yang Liu Jing, China (Chen *et al*., 2007); Middle Triassic, Iberian Ranges, Spain (Pla *et al*., 2013); Middle–Late Triassic, Betic Ranges, Spain (Manzanares *et al*., 2018); Middle Triassic, Catalan Coastal Ranges, Spain.

Order INCERTAE SEDIS

Family PSEUDODALATIIDAE Reif, 1978

Genus *PSEUDODALATIAS* Reif, 1978

Type species—*Pseudodalatias bartonensis* (Sykes, 1971)

Occurrence— Upper Triassic, Rhaetian of Barnstone, England (Skyes, 1971)

PSEUDODALATIAS HENAREJENSIS Botella, Plasencia, Márquez-Aliaga, Cuny and Dorka, 2009 Figure 2 Q-R

Material—One tooth from the Tivissa section (CT-8 level: MGUV-36113).

Description—The tooth is almost complete, but poorly preserved with the base missing (Fig. 2Q-R). It shows the characteristic features of *Pseudodalatias henarejensis*: a serrated-triangular morphology, with three (damaged) denticles on the edges and often lacking the base (Botella *et al*., 2009). The cusp is also damaged and the whole tooth surface is eroded.

Remarks—*Pseudodalatias henarejensis* is an endemic species from the Iberian Peninsula, which shows a dignathic heterodonty, as described in Pla *et al.* (2013). The tooth found in Tivissa belongs to the lower jaw.

Occurrence—Middle Triassic from the Iberian Chain of Spain (Botella *et al.*, 2009; Pla *et al.*, 2013); Middle–Late Triassic of the Betic Ranges (Manzanares *et al.*, 2018); Middle Triassic of the Catalan Coastal Ranges, Spain.

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Block 2

Study of the SCE layer in Chondrichthyan enameloid

CHAPTER 6
Manzanares *et al*. 2014

CHAPTER 7
Manzanares *et al*. 2016

CHAPTER 8
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CHAPTER 6

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The Enameloid Microstructure of Euselachian (Chondrichthyes) Scales[1]

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The enameloid microstucture of chondrichthyan teeth has been studied for decades and it has proven to be a useful taxonomic tool. Changes in enameloid organization have been related to the emergence of new trophic strategies and Mesozoic radiation of the neoselachian crown group. However, in contrast to the abundance of these data on tooth enameloid, descriptions of chondrichthyan scale enameloid are almost nonexistent. The topology and microstructure of scale enameloid in particular euselachian groups: fossil Mesozoic Hybodontiformes and living neoselachians, including batoids and sharks, are described. It is shown that a thick layer of single crystallite enameloid (SCE) covers all studied scales. Although the enameloid of scales clearly does not reach high levels of microstructural differentiation present in the dental enameloid of some neoselachians, we found some degree of organization, such as oriented crystallites, differentiation into sublayers, and the presence of poorly structured sets of densely arranged parallel crystallites. As scales lack feeding functions of teeth, we suggest that the emergence of microstructural organization/differentiation of chondrichthyan enameloid can be understood as consequence of a self-organizing process rather than adaptive pressure.

1. INTRODUCTION

Enameloid is a hypermineralized tissue that forms a thin layer covering the outer tooth surface of Chondrichthyes, Actinopterygii, larval stages of some caudate amphibians, and other extraoral elements of the integumentary skeleton of chondrichthyans and several lineages of extinct jawless vertebrates (see Janvier, 1996; Donoghue *et al.*, 2006; Sire *et al.*, 2009).

The enameloid microstructure of chondrichthyan teeth has repeatedly been studied since the early work of Reif (1973), focusing principally on euselachian sharks (Gillis and Donoghue, 2007 and references therein), and emerged as a useful tool for taxonomic studies of the group (e.g., Reif, 1977, 1978; Duffin, 1980; Cuny *et al.*, 1998; Cuny and Risnes, 2005; Botella *et al.*, 2009a; Guinot and Cappetta, 2011). In fact, changes in the organization of tooth enameloid can be related to the evolutionary history of Chondrichthyes. Thus, the multicuspidate teeth of many Paleozoic chondrichthyans adapted exclusively for grasping and swallowing predation, i.e., *Leonodus*, Xenacanthiformes, Phoebodontiformes, Cladoselachiformes, Ctenacanthiformes, and Symmoriiformes, possess a homogeneous layer of single crystallite enameloid (SCE) lacking any microstructural differentiation within the layer. Individual hydroxy (fluor-) apatite crystallites are generally discernable and arranged randomly with no preferred orientation relative to the outer enameloid surface (Gillis and Donoghue, 2007; Botella *et al.*, 2009b). In contrast, teeth of all nonbatoid neoselachians, i.e., modern sharks, show a triple-layered enameloid, consisting of an inner layer of tangled-bundled enameloid (TBE), intermediate layer of parallel-bundled enameloid (PBE), and the outermost shiny-layered enameloid (SLE) with crystallites not arranged in bundles (Reif, 1973, 1977; see also Cuny and Risnes, 2005). This microstructural differentiation has been linked to the emergence of new trophic strategies, especially of the cutting and gouging strategy, increasing tensile strength, and resistance to compressive forces that arise from these feeding habits (Preuschoft *et al.*, 1974; Reif, 1978,1979; Thies and Reif, 1985; Cappetta, 1986). Some of these "novel" feeding strategies characteristic of neoselachians also evolved independently in a number of non-neoselachian lineages, which lack a triple-layered enameloid, although, interestingly, they show some degree of organization in their SCE. Thus, the crushing teeth of the hybodontiforms *Acrodus* and *Polyacrodus* have a two-layered SCE, with a compact outer layer and a bundled inner layer (Cuny *et al.*, 2001). On the other hand, the cutting dentitions of *Carcharopsis*, *Priohybodus*, *Thaiodus*, and *Pseudodalatias* exhibit a very compact single-layered SCE (Reif, 1978; Duffin and Cuny, 2008; Botella *et al*, 2009a). This compaction of crystallites building up the enameloid has been linked with the necessity of increasing resistance to the tensile stresses induced by these modes of feeding (Duffin and Cuny, 2008).

In contrast to available information regarding the tooth enameloid microstructure, there are virtually no data on the organization of enameloid in chondrichthyan scales. The scale enameloid microstructure in chondrichthyans has never been described in detail and, although it is generally assumed that a thin layer of enameloid covers the scales of euselachians and that of all other groups of extinct chondrichthyans (see Janvier, 1996; Donoghue *et al.*, 2006), this has not been confirmed systematically.

In this study, we attempt to complete this lack of information concerning the microstructure and organization of enameloid in the dermal skeleton elements of euselachians. We describe the scale enameloid layer in certain euselachians: some fossil Mesozoic Hybodontiformes and living neoselachians, including both batoids and sharks in terms of topology and microstructure. As the scale enameloid microstructure is not subject to the same selective pressure due to mechanical stresses derived from new feeding practices of neoselachians, our data can be compared independently with those available for euselachian tooth enameloid. The aims of our analysis are (i) to confirm the presence of enameloid layer in the scales of the examined chondrichthyan taxa and (ii) if so, to describe its microstructure and organization.

2. MATERIAL AND METHODS

For this purpose polished sections of scales were prepared. Scales were embedded in a transparent polyester resin at 120°C for two hours prior to polishing with a mix of carborundum (800 and 1200 μm) and water until the desired part of the fossil was reached. Afterwards, the sections were etched for 5 to 10 s in 10% HCl or for 1 to 5 minutes in 0.5% orthophosphoric acid. Each sample was repolished and etched as many times as necessary to elucidate the enameloid microstructure. Furthermore, some scales were broken for direct observation of a fresh fracture. The broken surfaces were etched for 2 to 5 s in 10% HCl. Analysis and photography of ground sections were done on a Hitachi S-4100 scanning electron microscope of the Microscope Service of the University of València. Before SEM analysis, scales were coated with gold and palladium alloy. The scales of hybodontiform sharks were collected in the Bugarra section, Ladinian (Middle Triassic), located in the Iberian Ranges, Spain (see Plá *et al*., 2009, 2011). Plá *et al*. (2011) distinguished 13 different morphotypes, following the classification proposed by Johns *et al*. (1997). For the present work, we studied the ultrastructure of six most abundant morphotypes (*Lobaticorona*, *Undulaticorona*, *Parvidiabolus*, *Coniunctio*, *Glabrisubcorona*, and *Parviscapha* morphotypes sensu Plá *et al*., 2011). Scales of the living neoselachians *Raja clavata* (Rajiformes) and *Scyliorhinus canicula* (Carcharhiniformes) were removed from the skin of specimens captured by commercial fisheries at the Mediterranean coast of Spain. All the material studied is deposited in the Museum of Geology in University of València. Referred materials: scales MGUV-26.092 to MGUV-26.100, MGUV-27.191, and MGUV-27.192.

3. RESULTS

Hybodontiformes

As a rule, a thick (25 to 40 μm) capping enameloid layer, with a well-defined enameloid–dentin junction and many dentine tubules that cross the junction and extend into the capping tissue, is present in all different morphologies of Hybodontiformes scales studied (Fig. 1). Individual hydroxyapatite crystallites are discernible, elongate in shape, varying from 0.5 to 1 μm in length (Figs. 1b, 1f, 1j, 1m). Crystallites usually lack any kind of orientation (e.g., Fig. 1g); although in some areas of the scale morphotypes *Lobaticorona* and *Glabrisubcorona*, crystallites seem to be arranged in a preferred orientation perpendicular to their surfaces (e.g., Figs. 1c, 1j). The parts of the scales where crystallites show this preferred orientation correspond to inter-ridges, whereas in the ridges, the crystallites are randomly oriented (Figs. 1k, 1m). Finally, in the scales of the *Coniunctio* morphotype (Figs. 1h, 1j), crystallites of the inner sublayer are densely arranged in incipient sets and perpendicular to the enameloid–dentine junction (Figs. 1i, 1j).

Remarks. According to Reif (1978), the hybodontid morphogenetic scale type compensates for the growth of the animal by both addition of new scales in the skin and growth of some scales by addition of consecutive odontodes (sensu Ørvig, 1967; see also Karatajüte-Talimaa, 1998). Thus, the squamation of hybodontids contains nongrowing (single odontode) scales and compound growing scales (following terminology of Reif, 1978; see also Karatajute-Talimaa, 1992,1998). Both of them have been found in the material studied. In addition, Reif (1978, text- figs. 2D, 3D) has described scales that represent an intermediate situation between growing and nongrowing ones, in which a pair of odontodes was formed almost at the same time and connected by a neck canal. These types of scales are also present in our material (Fig. 1h).

Although scales from Bugarra are clearly of hybodontid type; they cannot be identified at the generic or familial level. Isolated teeth of the genera *Paleobates*, *Hybodus*, *Lissodus*, "*Polyacrodus*" (order Hybodontiformes), and *Pseudodalatias* (order *incertae sedis*) occur along with scales (Plá *et al.*, 2013) hampering definite generic identification of scales. However, based upon similar stratigraphical occurrence and comparison with articulated specimens, Plá *et al.* (2011) suggested association of scales of *Lobaticorona* morphotype with teeth of *Lissodus* and the *Parvidi-abolus* morphotype with teeth of *Hybodus*.

Neoselachian

Raja clavata (Rajiformes)

Scales of Raja clavata (Figs. 2a–2f) were selected to represent Batoidea. The morphology of *Raja clavata* scales varies depending on the position they occupy in the body. Scales from the anterior and dorsal parts of the body are analyzed here. In both cases, an enameloid cap of around 15 μm thick is present on top of each scale, where crystallites show a different arrangement between the outer and inner parts of the enameloid layer (Figs. 2b, 2c). In all scales, the crystallites of the outer part of

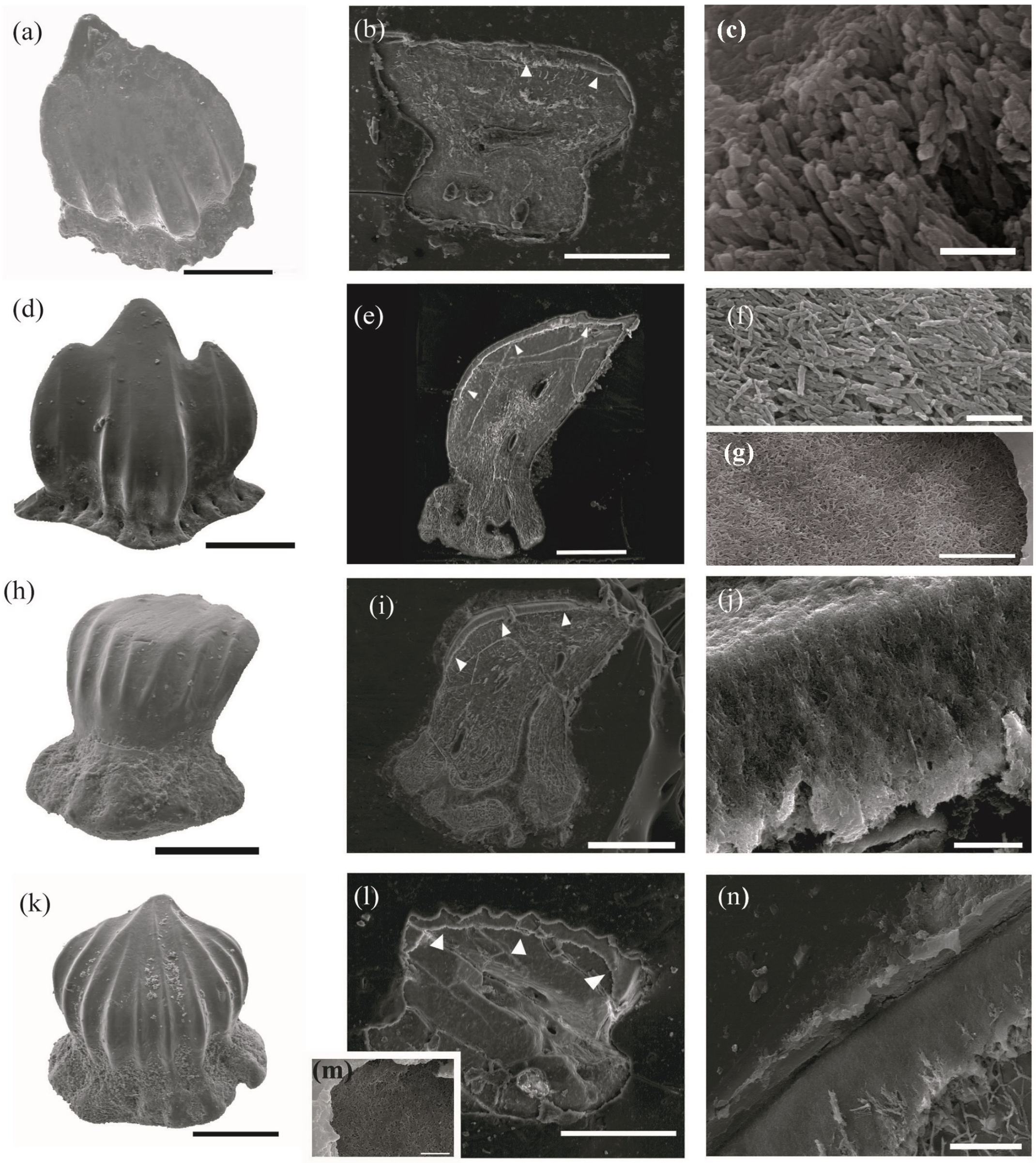

Fig. 1. Different morphologies of hybodontiform scales from the Middle Triassic of Spain: (a) *Glabrisubcorona* type scale (MGUV-26.092); scale bar, 200 µm. (b) Overview of the embedded scale MGUV-26.092 in longitudinal section where the enameloid layer is discernible; etched 5 s in HCl 10%; scale bar, 200 µm. (c) Detail of the SCE layer; etched 5 s in HCl 10%; scale bar 3 µm. (d) *Parvidiabolus* type scale (MGUV-26.093); scale bar, 700 µm. (e) General view of the embedded scale MGUV-26.093 with an enameloid layer that covers the crown surface; etched 5s in HCl 10%; scale bar, 200 µm. (f) High resolution picture of the enameloid crystallites in surface view; etched 5 s in HCl 10%; scale bar 800 nm. (g) Detail of the whole length of the monolayer of SCE randomly arranged; etched 5 s in HCl 10%; scale bar, 6 µm. (h) *Co-*

the layer are randomly oriented but very compact. In the inner part of the enameloid layer, the hydroxyapatite crystallites are densely arranged and mainly perpendicular to the surface (Figs. 2b, 2c, 2f). The compaction of crystallites is more evident in some areas of scales, where interwoven incipient “sets” of crystallites appear with a high level of compaction (Fig. 2f). Crystallites of the same set are arranged in parallel, showing the same orientation (Figs. 2e, 2f). Close to the enameloid–dentine junction, the organization of the hydroxyapatite crystallites is less evident and they seem to be randomly oriented (Fig. 2b). In all scales, the hydroxyapatite crystallites are at most 1 μm long; and they are elongated (Figs. 2c, 2e, 2f).

Scilorihinus canicula
(Carchariniformes)

Scyliorhinus canicula scales (Figs. 2g–2i) bear an enameloid layer approximately 15 μm thick on the crown surface. Its microstructural organization is somewhat similar to that of *Raja scales*. Two “sublayers” are found in the scales of this carcharhiniform shark; the inner sublayer, with crystals showing a preferred orientation perpendicular to the enameloid–dentine junction, and a thin outer sublayer, with more compact crystallites (Figs. 2h, 2i). In some areas of the scales, crystallites appear densely arranged. As in *Raja clavata*, the hydroxyapatite crystallites are 1 μm long and they are elongate.

niunctio type scale (MGUV-26.094); scale bar, 200 μm. (i) Overview of the embedded scale MGUV-26.094 in which it is possible to distinguish clearly the enameloid across the crown surface and the underlying dentine core; 5 s in HCl 10%; scale bar, 200 μm. (j) Detail of the SCE with an inner part where crystallites are grouped into packets perpendicular to the crown surface; etched 5 s in HCl 10%; scale bar, 7 μm. (k) *Undulaticorona* type scale (MGUV-26.095); scale bar, 200 μm. (1) General view of the embedded scale MGUV-26.095 showing the same pattern as the other morphologies: a cap of enameloid on the crown surface with a dentine layer below; 5 s in HCl 10%; scale bar, 200 μm. (m) Detail of one cusplet in which the SCE are randomly arranged; etched 5 s in HCl 10%; scale bar, 3 μm. (n) Detail of the enameloid-dentine junction with dentine tubules that penetrate into the enameloid layer; etched 5 s in HCl 10%; scale bar, 20 μm. The arrows show the enameloid-dentine junction

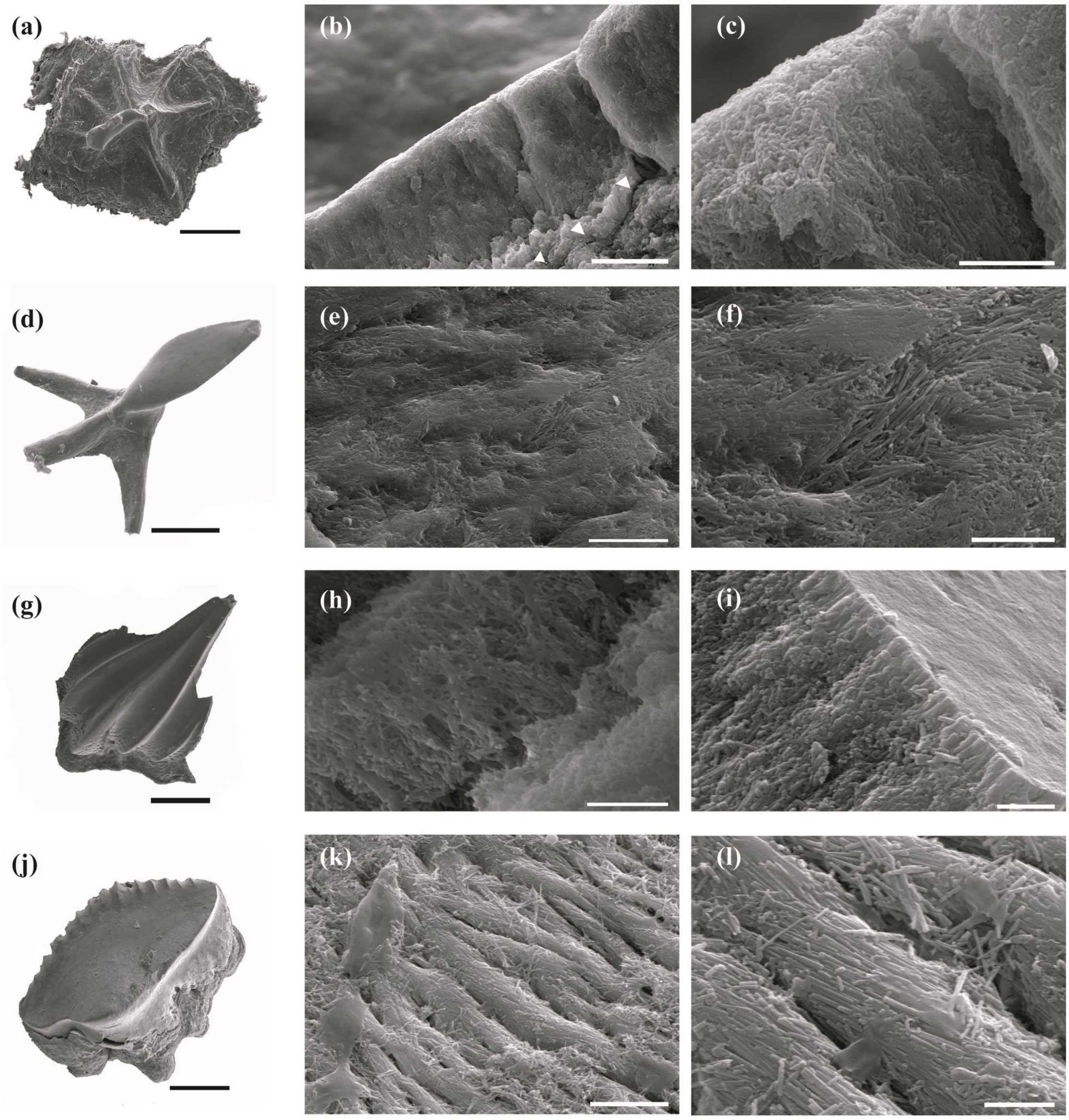

Fig. 2. (a) Body scale of *Raja clavata* (MGUV-26.096); scale bar, 500 µm. (b) Detail of the enameloid layer in a fresh fracture on the crown surface of the scale. The arrows show the enameloid-dentine junction; etched in 0.5% orthophosphoric acid for 2 min; scale bar, 8 µm. (c) SCE with crystallites arranged into two sublayers: the inner one with crystallites aligned perpendicular to the crown surface and the outer one with crystallites arranged more parallel to the crown surface; etched in 0.5% orthophosphoric acid for 2 min; scale bar, 2 µm. (d) Snout scale of *Raja clavata* (MGUV-26.097); scale bar, 200 µm. (e) Detail of the surface in MGUV-26.097, showing the densely arranged crystallites; etched in 0.5% orthophosphoric acid 2 for min; scale bar, 5 µm. (f) Detail of the embedded scale MGUV-27.191 showing the densely arranged crystallites perpendicular to the surface of the scale; etched 5s in HCl 10%;

Remarks. According to Reif (1978), all neoselachians possess the *Heterodontus* morphogenetic type. Their “placoid” scales, i.e., simple nongrowing scales containing only a single wide pulp cavity, do not grow and are continuously replaced. The growth of the animal is compensated by addition of new scales and by the increasing size of replacement scales. As a result, the number of scales increases throughout ontogeny.

4. DISCUSSION

Our study demonstrates the existence of a layer of hypermineralized tissue, different from the dentine layer, covering all scales. In all specimens, this layer is composed of individually discernible single elongate crystallites. The location of this hypermineralized layer in the outer surface, the presence of a distinct border between this layer and the underlying dentine, the presence of numerous dentine tubules extending across this junction zone, and the presence of individual hydroxyapatite crystallites suggest its interpretation as single crystallite enameloid (SCE).

Although euselachian scales clearly do not reach the high degree of microstructural differentiation present in the tooth enameloid of some neoselachians with distinct individual bundles, they exhibit some microstructural organization of the crystallites, such as different organization in two “sublayers”, preferred orientation and occurrence of poorly

scale bar, 1 µm. (g) Scale of *Scyliorhinus canicula* (MGUV-26.098); scale bar, 200 µm. (h) Embedded and etched scale (MGUV-26.099) showing the enameloid layer (arrows show the enameloid-dentine junction); etched 10s in HCl 10%; scale bar 4 µm. (i) Detail of a fresh fracture in MGUV-27.192, in which, two sublayers are discernible: the thick inner one with the individual crystallites perpendicular to the surface and the thin outer one with high compacted crystallites; etched 10s in HCl 10%; scale bar 1 µm. (j) Tooth of *Mustelus mustelus* (MGUV-26.100); scale bar 300 µm. (k) Detail of the etched tooth surface where the crystallites forming the fibers of the parallel fibered enameloid (PFE) layers are still clearly discernible; etched 10s in HCl 10%; scale bar 7 µm. (1) Detail of the (k); etched 10 s in HCl 10%; scale bar 1 µm.

structured sets of densely arranged parallel crystallites. Thus, enameloid found in several scales of hybodontiforms and neosela- chians demonstrates the presence of an outer part of compact SCE and the inner part of SCE, with some densely arranged crystallites parallel to the scale surface (Figs. 1j, 2i). The tightly compacted enameloid with parallel crystallites aligned perpendicular to the enameloid–dentine junction is comparable to that described in various non-neoselachian shark teeth (Duffin and Cuny, 2008). Finally, the arrangement of crystallites within groups of parallel, more densely arranged crystallites found in scales of rajiformes (Figs. 2e, 2f) resemble in some way the assembly of crystallites in the loosened enameloid bundles of stem selachiomorphs (Johns *et al.*, 1997; Andreev and Cuny, 2012), although it is clearly different from the highly structured enameloid of crown selachiomorphs (compare with Figs. 2k, 2l, where preparation allows identification of crystallites forming the bundles of the PBE of a neoselachian tooth).

As noted above, the emergence of microstructural differentiation in the enameloid of chondrichthyan teeth has usually been interpreted as an adaptational process, preventing crack propagation and increasing resistance to tensile forces (Preuschoft *et al.*, 1974) related to the emergence of new feeding strategies in the group (see Gillis and Donoghue, 2007; Duffin and Cuny, 2008). However, we show here that the enameloid of scales exhibits some degree of microstructural organization. Taking into account that scales lack feeding functions of teeth (and, hence, there is no need to assemble enameloid crystallites in order to resist any compressive or tensile force during feeding), the occurrence of incipient microstructural organization/differentiation in scale enameloid (i.e., preferred orientation of crystallites, or "sublayers") cannot be linked with any obvious adaptive function and it could be understood as a consequence of self-organizing processes. Self-assembly of hydroxyapatite crystallites into fibers (or enamel prismlike structures) has been reproduced "in vitro" (e.g., Chen *et al.*, 2005; Wang *et al.*, 2008) and self-organizing processes of growing crystallites, along with interactions with amelogenins, have been proposed to play a major role in establishing structural patterns in amniote enamel (Sander, 2000; Margolis *et al*, 2006 and references therein). It is possible that the self-organization process of enameloid crystallites and proteins also occurred in the evolution of chondrichthyan teeth irrespective of adaptive pressure. Emerging mechanical properties of the microstructural organization/ differentiation of the enameloid would make possible its subsequent co-option for new purposes (i.e., new feeding strategies). This supports: (1) the convergent evolution of cutting dentitions in several Paleozoic and Early Mesozoic non-neoselachian chondrichthyans that demonstrate an SCE with crystallites that are densely packed and oriented perpendicular to the tooth surface (for example, in Paleozoic *Carcharopsis*, or Mesozoic *Priohybodus*, *Thaiodus* (hybodonts), and *Pseudodalatias*: Duffin and Cuny, 2008; Botella *et al.*, 2009a); and (2) the fact that microstructural differentiation of the triple-layered enameloid in neoselachians phylogenetically precedes the emergence of cutting and gouging feed- ing strategies in the group (see Gillis and Donoghue, 2007). Thus, we suggest that an initial incipient micro- structural organization due to self-organizing processes of crystallites could provide an appropriate "substrate" for selective pressure, which led to acquisition of a triple-layered organization in neoselachian teeth. The recognition of several levels of increasing architectural complexity in the enameloid crystallite bundles of stem selachiomorphs (Andreev and Cuny, 2012) suggest that the acquisition of the triple-layered enameloid progressed gradually, step-by-step through several phases, from a plesiomorphic single crystallite state.

A plausible scenario for the increased complexity of the enameloid in neoselachian sharks was proposed by Gillis and Donoghue (2007). The ancestral teeth of gnathostomes, evolutionarily derived from placoid scales, would have had a capping layer of SCE, resulting from a late differentiation of ameloblasts (ectodermal origin) during odontogenesis. Then, the enameloid matrix would be rich in ameloblastic cell secretions and deficient in odontoblast (ectomesenchimal origin) cell products. The acquisition of the triple-layered enameloid in neoselachian (non batoid) lineage would be concordant with heterochronic processes in ameloblast differentiation during odontogenesis with an increase in odontoblast-derived tubular vesicles in the enameloid matrix considering that a mixed matrix composed of both ameloblast cell secretions and odontoblast-derived tubular vesicles is critical for the development of higher order enameloid structures (Gillis and Donoghue, 2007). This underlies the idea that thin layer of SCE overlying the PBE of neoselachians (i.e., SLE produced in complete isolation from odontoblast secretion due to its outer enameloid position and, therefore, derived almost exclusively from ameloblast products: Reif, 1979 in Gillis and Donoghue, 2007) is reminiscent of the plesiomorphic SCE. However, other authors suggested that the SLE (being of ectodermal origin, see above) of neoselachians is a late addition during the evolution of enameloid in Elasmobranchii and that primitive chondrichthyan enameloid would have been a pure mesodermal prod- uct (Cuny and Risnes, 2005). Nevertheless, more studies are required before one of these scenarios (or others) can be definitively accepted.

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CHAPTER 7

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New insights into the enameloid microstructure of batoid fishes (Chondrichthyes)

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Chondrichthyan teeth are capped with a hypermineralized tissue known as enameloid. Its microstructure displays a hierarchical organization that has increased in structural complexity from a homogenous singlecrystallite enameloid (SCE) in early Chondricthyans to the complex multilayered enameloid found in modern sharks (consisting of bundles of crystallites arranged in intriguing patterns). Recent analyses of the enameloid microstructure in batoid fishes, focused on Myliobatiformes and fossil taxa, point to the presence of a bundled (or fibred) multilayered enameloid, a condition proposed as plesiomorphic for Batoidea. In this work, we provide further enameloid analysis for a selection of taxa covering the phylogeny of batoids. Our SEM analysis shows a superficial layer of SCE, where individualized crystallites are clearly discernable, capping the teeth in most of the species studied. A bundled double-layered enameloid was found only in a Rhinoidei, *Rhina ancylostoma* Bloch & Schneider, 1801. We conclude that the most widespread condition among extant batoids is a monolayer SCE lacking microstructural differentiation, probably plesiomorphic at least for crown batoidea. We suggest that the complex bundled enameloid present in other batoids is a convergent character that has appeared repeatedly during the evolution of batoids, probably as a mechanical adaptation towards moderate durophagous diets.

1. INTRODUCTION

Chondrichthyes teeth are covered by a thin layer of hypermineralized tissue known as enameloid. In contrast to the 'true' enamel of tetrapod and sarcopterigian teeth, produced solely by ameloblasts of ectodermal origin, the enameloid is produced by a combination of ameloblasts and odontoblasts of ectomesenchymal origin (Gillis & Donoghue, 2007). The enameloid is mostly composed by hydroxy (fluor-)apatite crystallites and an organic matrix. The latter contains mainly collagen, synthesized by odontoblasts, and amelogenin-like proteins, produced by ameloblasts (Sasagawa, 2002; Sasagawa *et al.,* 2009; Sire, Donoghue & Vickaryous, 2009, and references therein).

The structure of the mineral phase of the enameloid in Chondrichthyes has an intricate hierarchical organization. The smallest repeating structural units are individualized elongated fluoroapatite crystallites $[Ca_5(PO_4)_3F]$, each formed by a number of hexagonal fluoroapatite unit cells. In a higher level of structural complexity, crystallites are arranged in parallel, forming tightly packed bundles, each with an envelope of organic matrix. Bundles with different orientations make assemblies that can be found in different layers, forming the whole enameloid Layer of the teeth (Enax *et al.*, 2014).

The acquisition of these hierarchical structural levels has been progressive along the phylogeny of Chondrichthyans and traditionally has been related to the emergence of new feeding strategies in the group (Thies & Reif, 1985; Gillis & Donoghue, 2007; but see remarks in

Enault *et al.,* 2015). Thus multicuspidate teeth of the grasping and swallowing dentitions of early chondricthyans that dominate Palaeozoic marine communities (such as *Leonodus* Mader, 1986; Xenacanthiforms, Ctenacanthiforms, Phoebodontiforms, and Symmoriiformes), possess an enameloid capping layer consisting of randomly oriented individual crystallites (single-crystallite enameloid, SCE), lacking any degree of higher microstructural differentiation (Gillis & Donoghue, 2007; Botella, Donoghue & Martínez-Pérez, 2009a). Exceptions to this 'general' pattern are found in certain Xenacanthiforms, which lack an enameloid cap (Hampe & Long, 1999; Gillis & Donoghue, 2007), and in the ctenacanth *Neosaivodus flagstaffensis* Hodnett, Elliott, Olson, Wittke, 2012, which shows crystallites associated into bundles parallel with the crown surface (PBE, see below; Guinot *et al.*, 2013). In many Hybodonts, a homogeneous layer of SCE is present (Reif, 1973; Gillis & Donoghue, 2007; Pla, Márquez-Aliaga & Botella, 2013; Enault *et al.*, 2015); however, some Mesozoic taxa with crushing dentitions developed a distinct two- layered enameloid consisting of an outer compact single-crystallite layer and an inner layer of SCE, with some crystallites organized into short, loosely defined bundles, usually perpendicular to the enameloid dentine junction (Cuny, Rieppel & Sander, 2001; Pla *et al.,* 2013; Enault *et al.*, 2015).

In contrast, modern sharks (Selachimorpha) – i.e. non-batoid neoselachians – show a triple-layered enameloid consisting of: an inner layer of tangled bundled enameloid (TBE), with bundles of crystallites randomly oriented and adjacent to the dentine layer; an intermediate layer of parallel-bundled enameloid (PBE), with bundles of crystallites oriented parallel with the surface; and an outermost shiny layer enameloid (SLE), with single crystallites not arranged into bundles (Reif, 1973, 1977; for terminology, see also Cuny *et al.*, 2001). This complex microstructural differentiation has been considered as a pre-adaptation for the evolution of novel trophic strategies in this group (e.g. cutting, clutching, and grinding), increasing resistance to compressive forces and tensile strength that arise from these new feeding habits (Gillis & Donoghue, 2007; see also Preuschoft, Reif & Müller, 1974; Reif, 1978, 1979; Thies & Reif, 1985; Cappetta, 1986). According to Andreev & Cuny (2012), the acquisition of the triple-layered enameloid organization during the evolution of Selachimorphii occurred in consecutive steps from an ancestral single crystallite: beginning with the appearance of PBE (through several phases), followed by that of TBE, and considering the SLE as a remnant of the primitive SCE. In addition, the triple -layered enameloid of some derived shark teeth can either become more complex, adding some additional structures (reviewed in Enault *et al.*, 2015), or become simpler, by lacking specific components (Reif, 1973).

Noticeably, the organization of the enameloid in batoids, which represent more than half of all the extant neoselachians, has been poorly studied. Early studies on batoid enameloid have shown: (1) the lack of the triple-layered enameloid characteristic of Selachimorpha; and (2) the presence of great microstructural diversity in the group (Reif, 1977; Thies, 1982, 1983). Thus, Reif (1977) reported the presence of a thick cap of tangled fibred enameloid (TFE = TBE, after Cuny *et al.*, 2001) in *Rhina ancylostoma* Bloch & Schneider, 1801, whereas Thies (1983) found a SCE in some Early Jurassic *Spathobatis;* however, these findings remained partially neglected. As a result, it has been assumed that batoid enameloid was exclusively composed of a single layer of TBE (Gillis & Donoghue, 2007) or of SCE (Cuny *et al.,* 2009). A further debate emerged about whether this ‘simplifted’ enameloid is a derived or a retained ancestral character for the group. These opposed interpretations have been influenced by both the placement of the batoidea in the chondrichthyan phylogeny, which has changed as the knowledge of this group has increased, and the different interpretations of the enameloid structure of some primitive batoids. Thus Underwood (2006), supported by the consideration that two putative Early Jurassic batoids (*Jurobatos* and *Doliobatis*) present a multi-layered enameloid (Thies, 1983; Delsate, 2003; respectively), suggests that the single-layered enameloid of batoids is a derived character, reverted from the neoselachian triple-layered enameloid. This suggestion would agree with a view of batoids as a derived group of saw sharks and angel sharks (Hypnosqualea hypothesis, Compagno, 1977; de Carvalho, 1996; de Carvalho & Maisey, 1996; Shirai, 1992b, 1996); however, the placement of batoidea not as a derived group but as the sister group of all living sharks has been posteriorly well supported by both morphological and molecular data (Douday *et al.,* 2003; McEachran & Aschliman, 2004; Aschliman *et al.,* 2012). Additionally, the report of a multi- layered enameloid in *Doliobatis* has been questioned; for example, Cuny *et al.* (2009) argued that the tis- sue interpreted as a TBE by Delsate (2003) is the dentine, and that *Jurobatos cappettai* Thies, 1993 is most likely not a batoid but a Galeomorpha (Thies, 1993). Therefore, Cuny *et al.* (2009) considered that only a SCE is present among basal batoids, and proposed that it is a retained plesiomorphic condition, as the SCE is also present in the successive sister groups of Neoselachii. The amalgamation of individual crystals into bundles would mark the appearance of Selachimorphii (Andreev & Cuny, 2012).

In contrast, several recent studies focusing on batoid enameloid oppose this perspective, claiming that a large number of batoid taxa present a layered bundled enameloid .Thus, in a study focused on the enameloid of stingrays, Enault, Cappetta & Adnet, 2013 (see also Cappetta, 2012) identifted a double-layered enameloid in most Myliobatiformes, whereas a reduced single-layered enameloid was present only in some gymnurids and in the derived filter feeder mobulids. These facts, along with the identification of a double-layered enameloid in two non-myliobatoids taxa (*Raja* and *Rhynchobatus*), prompted Enault *et al.* (2013) to suggest that this is the plesiomorphic condition for batoids. Nevertheless, Enault *et al.* (2015) have later shown that, in fact, there are not two distinct layers in the enameloid of Myliobatiformes. These authors, however, still consider a complex enameloid as the ancestral batoid condition, based on the presence of a bundled multi-layered enameloid in several Mesozoic batoids (see Discussion).

In this work, we present a systematic analysis of the enameloid microstructure in current batoid fishes, broadening the span of the phylogeny of the group and focusing on non-myliobatiform taxa. The new data obtained in our analysis contrast with the recent interpretations of the plesiomorphic condition for batoidea (Enault *et al.,* 2013, 2015), which will be discussed here in a phylogenetic context.

2. MATERIAL AND METHODS

2.1. TAXA EXAMINED

Taxa examined in this study were selected to have a wide representation of the phylogeny of batoids (following that from Aschliman *et al.,* 2012). Thus, nine species belonging to the five major groups of batoids were chosen to analyse their enameloid microstructure. Skates (Rajoidei) are represented by *Raja clavata* Linnaeus, 1758; *Atlantoraja platana* Günther, 1880; *Sympterygia acuta* Garman, 1877 and *Rioraja agassizii* Müller & Henle, 1841. *Torpedo marmonata* Risso, 1810 is chosen to represent the electric rays (Torpedinoidei), *Plathyrinoidis triseriata* Jordan & Gilbert, 1880 exemplifies the thornbacks (Platyrhinoidei), *Dasyatis americana* Hildebrand & Schroeder, 1928 represents the stingrays (Myliobatoidei), and polyphyletic ‘guitarfishes’ are represented by *Rhinobatos producuts* Ayres, 1854 (Rhinobatoidei) and *Rhyna ancylosotma* (Rhinoidei). In the case of species with sexual dimorphism we have studied both male and female teeth when they were available, as indicated for each taxa.

All the teeth were loaned by the museum El Cau del Tauró (L’Arboc, Tarragona, Spain). Teeth were removed from prepared jaws, selecting those placed at intermediate positions of the dental family. All the teeth studied are deposited in the Museum of Geology at the University of Valencia: MGUV 27749–60.

2.2. Analysis of enameloid structure

For the study of enameloid microstructure, teeth were embedded in Canada balsam at 120° C for 2 h prior to being polished with a mix of carborundum (800 and 1200 μm) and water, following the chosen plane of section (longitudinal or transversal). Afterwards, the sections were etched. Two protocols were tested for the etching of the teeth. The first is the most commonly used for the study of fossil sharks (e.g. Cuny & Risnes, 2005; Gillis & Donoghue, 2007; Botella *et al.*, 2009a), where teeth were etched in HCl 10% for 5–10 s. The second treatment seems more accurate for removing the organic matrix present in recent material (Enault *et al.*, 2013), and teeth were etched in hydrogen peroxide for a time period ranging from a few hours up to 3 days. Both treatments were used separately in a number of specimens used as controls (including triple-layered selachimorphy teeth). In general, satisfactory results can be obtained for both methods because they do not show significant differences; nevertheless, the clearest images were visualized with hydrogen peroxide, so this treatment was mostly used in our study. Each sample was re-polished and etched as many times as necessary to expose the enameloid microstructure. Additionally, some teeth were broken for the direct observation of a fresh fracture. The broken surfaces were etched as described above. The analysis and photography of ground sections were performed on a Hitachi S-4100 scanning electron microscope of the Microscope Service of the University of València. For SEM analysis, teeth were coated with gold and palladium alloy.

3. RESULTS

3.1. Skates

Atlantoraja platana Günther, 1880 (Fig. 1A–D)

The dentition of this species displays sexual heterodoncy. In females, the teeth are of the crushing type, with flat crowns and little pronounced rounded cusps, whereas in mature males dignathic heterodoncy is present in addition with monocuspidate teeth (with long and sharp cusps) in the central region and teeth with flattened crowns in the distal regions (Rangel *et al.,* 2014). In both sexes, teeth of *Atlantoraja* are covered by a thin homogeneous monolayer of SCE that visibly differs from the underlying dentine core No microstructural differentiation is observed across the enameloid layer (Fig. 1B, C). At higher magniftcation, individual crystallites are clearly discernable. They are elongate, ~1 μm in length, and appear to be randomly oriented (Fig. 1B, D).

Raja clavata Linnaeus, 1758 (Fig. 1E–K)

As in *A. platana*, both sexual and dignathic heterodoncy is present in this species. In the pointed clutching-type teeth of male upper jaws, the enameloid layer is wider in the apex of the cusp and becomes thinner towards the base (Fig. 1E). In the rounded, crushing-type teeth of female lower jaws, the enameloid layer is homogeneous all over the superior part of the crown surface, but is considerably thinner in the laterals parts

(Fig. 1I). In all teeth studied the enameloid consists of SCE. Single crystallites are compacted and arranged perpendicular to the enameloid–dentine junction (EDJ) in almost the complete layer (Fig. 1F, G, H, J, K), although in the outermost surface the orientation of crystallites changes, appearing parallel with the tooth surface (Fig. 1H, J). The enameloid crystallites are elongate and measuring~2 μm in length.

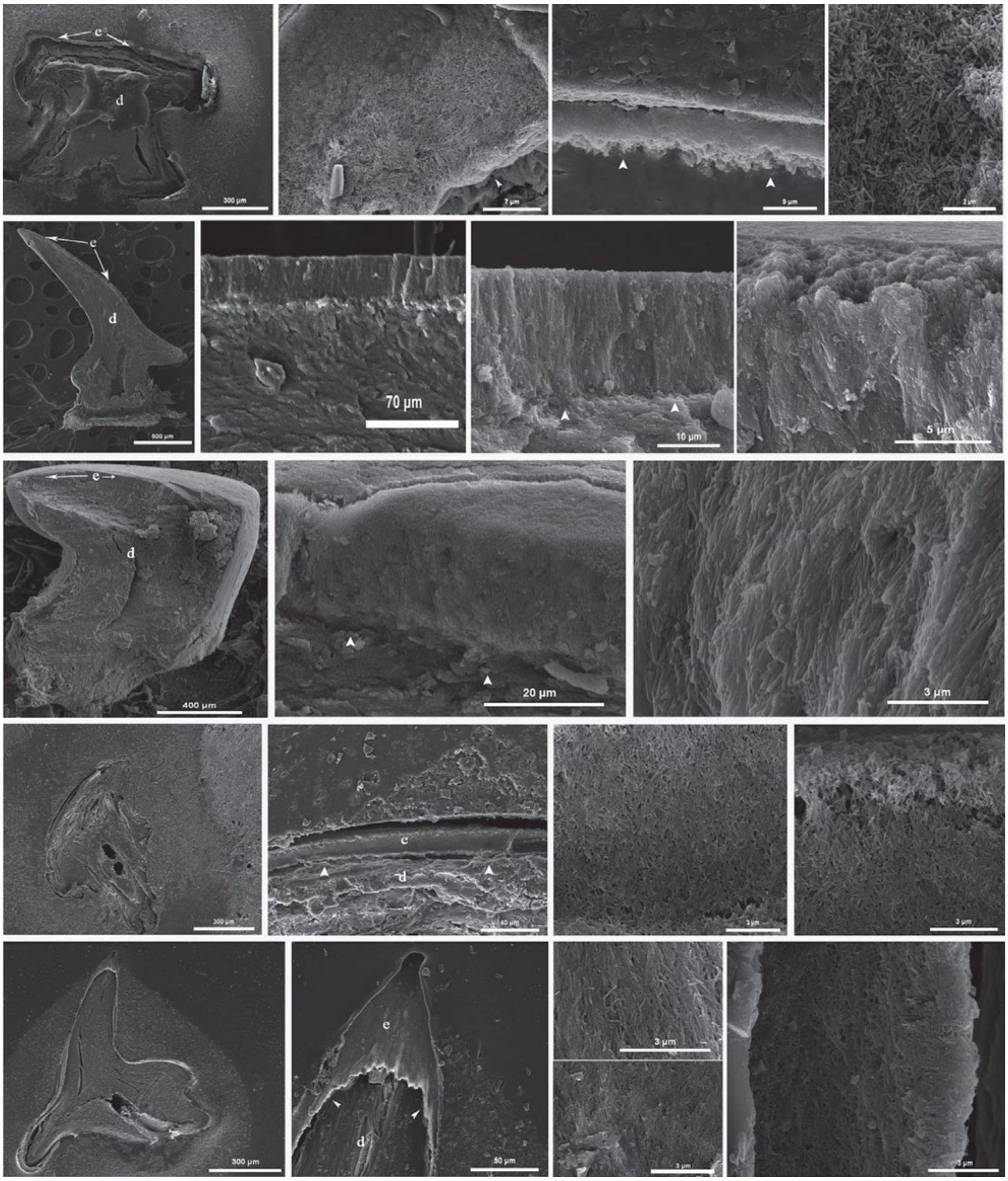
Rioraja agassizii Müller & Henle, 1841 (Fig. 1L–O)

Sexual dimorphism present. Only female teeth were accessible: they are of the grasping type, with rounded crowns (Fig. 1L). The enameloid is very similar to that of *Atlantoraja*. The SCE is homogeneously composed of randomly oriented enameloid crystallites of ~1 μm in length, and they are elongate with rounded ends (Fig. 1N, O). A sharp EDJ is apparent, and the enameloid crystallites seem to be less compacted near this junction (Fig. 1M).

Sympterygia acuta Garman, 1877 (Fig. 1P–T)

Sexual dimorphism is present. Only teeth of males were accessible for our study. They are cuspidate with a single pointed cusp (Fig. 1P). A SCE layer caps the crown of the teeth. The width of the layer changes

Figure 1. Scanning electron micrograph of batoid enameloid: Rajoidei. A–D, Atlantoraja platana, tooth of a female, MGUV 27749, etched for 3 h in H2O2. A, overview of tooth in longitudinal section; B, enameloid layer at the tooth edge; C, aspect of the enameloid layer in the centre of the crown – a well-defined but irregular enameloid–dentine junction (EDJ) is present; D, detail of the randomly organized individual crystallites. E–H, Raja clavata, tooth of a male, etched for 1 day in H2O2, MGUV 27750. E, general view of the tooth, showing the enameloid capping layer, the dentine core, and a central pulp cavity in a fresh fracture; F, detail of the enameloid layer and the underlying dentine tissue; G, H enameloid crystallites are highly compacted and arranged perpendicular to the EDJ. I–K, tooth of a female Raja clavata, etched for 1 day in H2O2, MGUV 27751. I, overview of the fresh fracture of the tooth showing the enameloid layer and the dentine core; J, general aspect of the enameloid layer; K, detail of the enameloid crystallites, highly compacted and arranged perpendicular to the crown surface. L–O, tooth of a male Rioraja agassizii, etched for 3 h in H2O2, MGUV 27753. L, complete tooth, showing the enameloid layer and the dentine core; M, detail of the whole layer of single-crystallite enameloid; N, detail of the individual fluorapatite crystallites randomly oriented in the inner part of thelayer; O, N, detail of the individual fluorapatite crystallites randomly oriented in the outermost part of the layer. P–T, tooth of a male Sympterigia acuta, etched for 3 days in H2O2, MGUV 27754. P, overview of an embedded tooth; Q, enameloid cap at the cusp of the tooth, showing the pre-



sence of a sharp enameloid-dentine junction EDJ; R, detail of the enameloid crystallites, highly compacted and predominantly oriented perpendicularly to the enameloid-dentine junction; S, detail of the crystallites near the apex surface; T, detail of the enameloid layer on the lateral part of the cusp with the enameloid crystallites randomly oriented in the cusps. Arrowheads point to the EDJ; d, dentine; e, enameloid. Scale bars: A, L, P, 300 lm; B, 7 lm; C, 9 lm; D, 2 lm; E, 500 lm; F, 70 lm; G, 10 lm; H, 5 lm; I, 400 lm; J, 20 lm; K, N, O, R, S, T, 3 lm; M, 40 lm; Q, 50 lm.

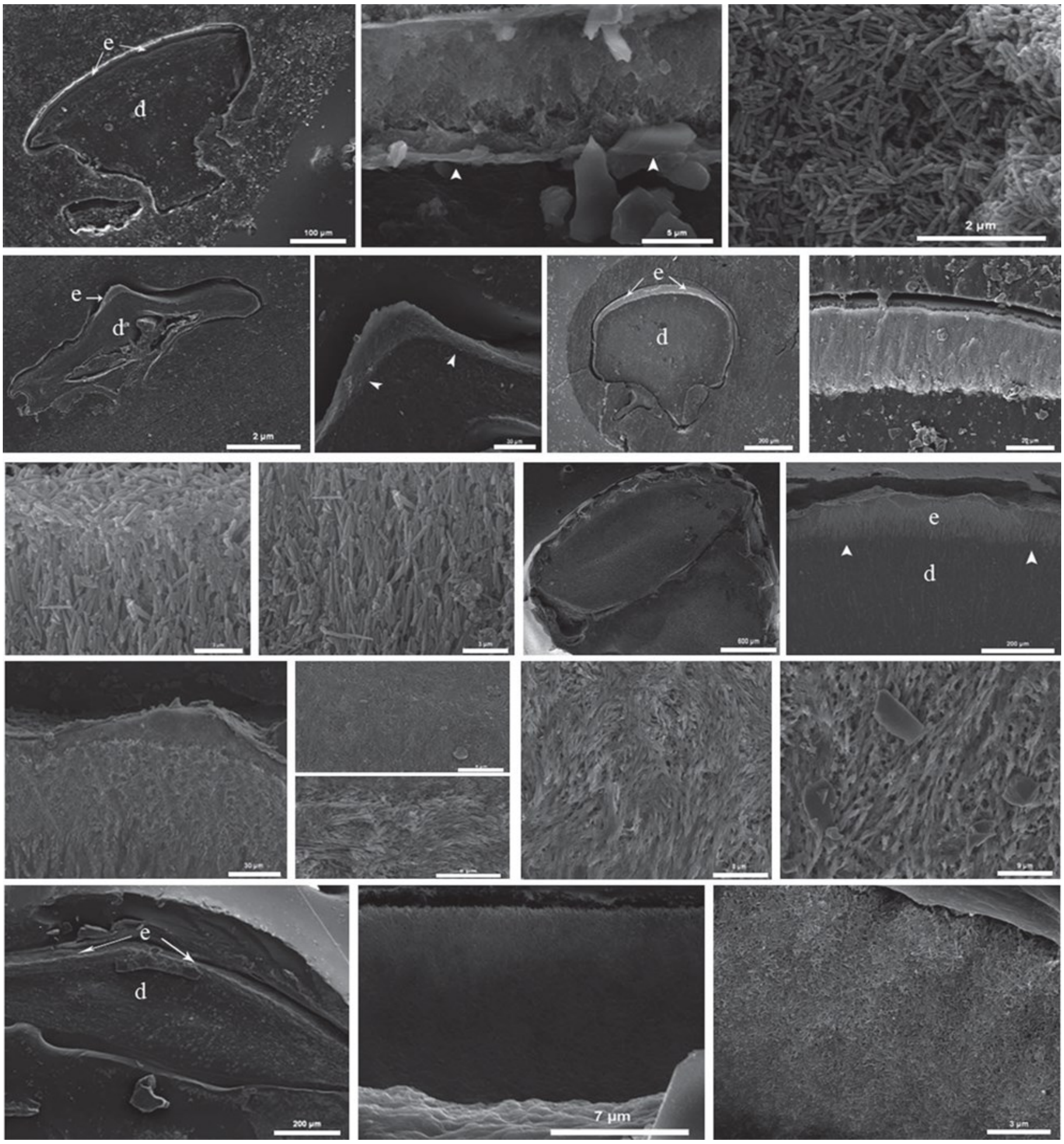
laterally, being widest at the cusp apex and becoming thinner towards the base (Fig. 1Q, T). The orientation of enameloid crystallites also varies depending on the area, thus near the top of the cusp hydroxiapatite crystallites are highly compacted and are predominantly oriented perpendicular to the teeth surface (Fig. 1Q–S), whereas in the lateral side of the cusp and in the base of the crown crystallites are less compacted and randomly oriented (Fig. 1T). Noticeably, the size and shape of the crystallites also vary depending on their position: those in the tip of the crown are elongated and 2 μm in length (Fig. 1R, S), whereas crystallites near the base are more round in shape and measure less than 1 μm in length (Fig. 1T)

3.2. Thornbacks

Platyrhinoidis triseriata Jordan & Gilbert, 1880 (Fig. 2A–C)

Sexual heterodonty is apparent, with a relatively high, elongated principal cusp on the teeth of males, versus lower cusps in females. Only female teeth were analysed here. Their crushing-type teeth show a layer of SCE lacking any type of microstructural differentiation. The thickness of the layer is homogeneous through the flattened crown of the teeth (Fig. 2A). Individual crystallites appear to be randomly distributed and compacted, although towards the EDJ the crystallites are less compacted (Fig. 2B). The hydroxipatite crystallites are

Figure 2. Scanning electron micrograph of the enameloid in batoids: Platyrhinoidei (A–C); Torpedinoidei (D–E); Rhinobatoidei (F–I); Rhinoidei (J–P); and Dasyatidae (Q–S). A–C, tooth of Platyrhinoidis triseriata embedded and etched for 1 day in H2O2, MGUV 27755; A, overview of the whole tooth; B, enameloid layer and the enameloid–dentine junction (EDJ); C, detail of the randomly oriented hydroxiapatite crystallites. D–E, embedded tooth of Torpedo marmonata, etched for 2 s in 10% HCl, MGUV 27756; D, general view of the polished surface of the tooth; E; detail of (D), individual crystallites are not discernable in MGUV 27756 but a monolayer enameloid is clearly distinguishable from the dentine below. F–I, tooth of the guitarfish Rhinobatos productus, etched for 5 s in 10% HCl, MGUV 27757; F, embedded tooth showing the enameloid layer and the dentine core; G, detail of the whole enameloid layer; H, crystallites randomly distributed in a plane parallel with the surface of the tooth, whereas in the inner part the crystallites are more compacted and are distributed in a preferred orientation, perpendicular to the dentine; I, crystallites near the EDJ; J–P, teeth of Rhyna ancylostoma, etched for 5 s in 10% HCl,



MGUV 27758; J, embedded tooth in longitudinal section; K, general view of the EDJ; L, aspect of the enameloid in a crest of the crown, organized in two different layers, with the limit between the layers clearly defined; M, detail of (L) showing the outer layer composed of randomly oriented crystallites; N, detail of (L) showing the limit between the outer single-crystallite enameloid and the inner bundled-crystallite enameloid layers; O, inner layer showing bundles in an interwoven texture; P, bundles near the EDJ. Q–S, teeth of a female individual of Dasyatys americana etched for 3 h in H2O2, MGUV 27759; Q, embedded tooth in longitudinal section; R, detail of the whole enameloid layer showing no microstructural differentiation; S, enameloid crystallites randomly oriented. Arrowheads point to the EDJ; d, dentine; e, enameloid. Scale bars: A, 100 lm; B, 5 lm; C, D, M, N, 2 lm; E, G, L, 30 lm; F, K, Q, 200 lm; H, I, S, 3 lm; J, 600 lm; o, 8 lm; P, 9 lm; r, 7 lm.

elongate and measure 1 μm in length (Fig. 2C).

3.3. Electric rays

Torpedo marmonata Risso, 1810 (Fig. 2D, E)

No dental sexual dimorphism has been described in this species. The small clutching teeth of this species present a single-pointed central cusp. To observe the enameloid layer, the minute size of the available teeth made the etching process difficult. Nevertheless, although individual hydroxiapatite crystallites are not discernible, a thin enameloid layer is histologically distinguishable from the underlying dentine core. It clearly lacks the tubule-rich texture of dentine matrix. The enameloid layer is homogenous and reveals no indication of any degree of microstructural differentiation within it, and thus the presence of a monolayer of SCE, similar to that present in Rajiformes, can be inferred.

3.4. Guitarfishes

Rhinobatos productus Ayres, 1854 (Fig. 2F, I)

Sexual heterodonty is shown by low cusps on the anterior and lateral side of male teeth, versus a slightly arched transverse keel in females (Herman *et al.,* 1997). Only female jaws were available for the study. Their crushing-type teeth possess a thin layer (i.e. 34 μm thick in a tooth of 572 μm in depth) of SCE capping their crown surface (Fig. 2F, G). In the outermost part of the enameloid layer, crystallites are randomly distributed in a plane parallel with the surface of the tooth, whereas in the inner part crystallites are more compacted and are in a preferred orientation perpendicular to the dentine core (Fig. 2G, H). Towards EDJ, the crystallites are more randomly oriented and less compacted than in the rest of the layer (Fig. 2I) – a pattern that is also found in the tooth edges. The enameloid crystallites are elongate and reach up to 3 μm in length; they are easily differentiated individually.

Rhina ancylostoma Bloch & Schneider, 1801 (Fig. 2J–P)

Sexual dimorphism has not been reported in the teeth of this species. The crushing teeth of *Rhina* are covered with a thick layer of enameloid that becomes progressively thinner towards the edges (Fig. 2J). The enameloid is organized into two different layers (Fig. 2L–P). The limit between the layers is very well defined (Fig. 2L, N). The outer layer is composed of densely packed crystallites that are randomly oriented (Fig. 2K–M). The inner layer exhibits complex organization. In the outer part of this layer, adjacent to the SCE layer, crystallites appear to be arranged in short bundles of interwoven texture (Fig. 2L, N, O). In the innermost part, reaching the EDJ, bundles are longer and generally oriented perpendicular to the EDJ, although they are crossed by bundles oriented in other planes (Fig. 2L, P). The border with the underlying dentine is uneven, with dentine tubules penetrating into the enameloid layer. Crystallites within a bundle show similar orientation. The relative thickness of the enameloid layers varies along the teeth: the outer SCE layer is thicker in the ridges that ornament the crown, but becomes thinnerin the spaces between ridges, whereas the inner layer has a more continuous thickness (~50–60 μm in the teeth studied). Therefore, in some parts of the crown (e.g. the spaces between ridges and the labial and lingual shoulder of the crown; Fig. 2N) the outer layer is reduced or absent and most of the enameloid layer is formed by short bundles of woven texture (Fig. 2O). The hydroxiapatite crystallites are differentiated individually and are elongate, with a total length of 1 μm

3.5. Stingrays

Dasyatis americana Hildebrand & Schroeder, 1928 (Fig. 2Q–S)

Sexual dimorphism is present in this species, but only the teeth of female were available. A thick layer of SCE caps the surface of their crushing teeth (Fig. 2Q). The hydroxiapatite crystallites are randomly organized along the entire layer and are highly compacted (Fig. 2R); however, each crystallite can be easily distinguished (Fig. 2S). The enameloid crystallites are elongate with pointed ends, and are of no more than 1 μm in length.

4. DISCUSSION

4.1. Batoid enameloid structure

Our SEM analysis of the enameloid microstructure in the teeth of a selection of taxa covering the phyLogeny of batoid fishes provides evidence of the presence of a superficial layer of SCE capping the teeth of most of the species studied, i.e. in the Rajoidei (*Raja clavata*, *Atlantoraja platama*, *Sympterygia acuta*, and *Rioraja agassizii*), Platyrhinoidei (*Platyrhina triseriata*), Rhinobatoidei (*Rhinobatos productus*), Torpedinoidei (*Torpedo marmonata*), and Myliobatoidei (*Dasyatis americana*). In all of these species, the enameloid consists of a homogenous monolayer made of individualized crystallites with no apparent superior microstructural differentiation (i.e. bundles or fibres; Figs 1 and 2A–I, Q–S). The oly exception to this general pattern was found in the enameloid of the Rhinoidei *Rhina ancylostoma*, where crystallites of the outermost part of the layer are individualized and highly compacted (SCE), whereas in the innermost part single crystallites are organized into bundles with variable orientation (Fig. 2J–P) As noted above, in some parts of the teeth the SCE component is reduced or is not present, and the enameloid layer is mostly formed by short entangled bundles of woven texture (Fig. 2O). It is probably this fact that induced Reif (1977) to consider that only a TFE (TBE) is present in *Rhina ancylostoma*. The organization of the enameloid of *Rhina ancylostoma* is in fact very similar to that found recently in the Late Cretaceous batoids *Parapalaeobates* cf. *atlanticus*

Arambourg, 1952 and *Ptychotrygon* sp. (Enault *et al*., 2015). Recent studies (Cappetta, 2012; Enault *et al*., 2013) have noted that the crystallites of enameloid in batoid fishes are poorly individualized and that, moreover, the morphology of the bundles of 'fibres' of batoids differs from the bundles of sharks, which are composed of microcrystallites. Our analysis, however, evidenced that well-individualized crystallites are clearly discernable in all batoid taxa studied. Even in the case of the double-layered enameloid found in *Rhina ancylostoma*, the bundles of the inner layer are composed of single crystallites that remain individually discernable (Fig. 2O, P).The cases of *Raja* and *Dasyatis* deserve special consideration, as the structure of their enameloid found in our analysis differs from other previous interpretations. Thus, according to Enault *et al*. (2013), the enameloid of *Raja clavata* and most myliobatiforms, including *Dasyatis*, consists of at least two layers: an outer, very compact layer of poorly individualized crystallites, without clear microstructure; and an inner layer formed by parallel 'bundles of fibres' that are perpendicular to the crown (complex B, sensu Enault *et al*., 2013). Their figures do not allow for the clear identification of any kind of bundles (or fibres), however, and the existence of the two layers is doubtful (see Enault *et al*., 2013: ftgs 2A, C–K). In fact, Enault *et al*. (2015) have later stated that only a SCE layer is present in Myliobatiformes, noting that their previous descrip- tion of two distinct layers in the enameloid of several Myliobatiforms was a misinterpretation resulting from the poor resolution of some scanning electron microscopy (SEM) micrographs. In our opinion, the identiftcation of a double-layered enameloid in other taxa studied by Enault *et al*. (2013) is also dubious, and needs further discussion Thus, the differences observed in the innermost part of the enameloid layer in the specimen of *Raja clavata* described in Enault *et al*. (2013: fig. 2A) probably result from the presence of abundant dentine tubules extending up to the enameloid, across the EDJ (an usual character of chondrichthyan enameloid; see Gillis & Donoghue, 2007), or are signs of the reticular structure of the EDJ. In order to better support our results, we analyzed several teeth of both females and males and both upper and lower jaws of *Raja clavata*. In all of the specimens studied the enameloid consists of a SCE monolayer lacking any higher microstructural differentiation. Single crystallites are clearly individualized and arranged perpendicular to the EDJ, except in the very outermost part of the layer where they become oriented parallel with the tooth

A double-layered enameloid was also identifted by Enault *et al.* (2013) in extant *Rhynchobatus* sp. The picture depicting this case (Enault *et al.*, 2013: fig. 2B) shows an enameloid layer with two different zones: a compact outer thick zone and an inner zone with thick enameloid 'pillars' intermingled with dentine, but the magnification of the figure prevents the unambiguous identification of crystallite bundles in the inner layer. However, Cappetta (2012) provide more detailed SEM images of *Rhynchobatus* [*R. pristinus* (Probst, 1877), middle Miocene, France] where the presence of a bundled enameloid, with parallel bundles normal to the EDJ in the inner part, and with interwoven bundles towards the crown surface, is more clear. Thus, although more precise illustrations of the enameloid of this genus are required to support their observations conclusively, the double-layered enameloid identifted in *Rhynchobatus* by Cappetta (2012) and Enault *et* al. (2013) clearly differ from the SCE present in the other 'rhinobatoid' taxa studied here, *Rhinobatos productus*, and seems somewhat similar to the 'double-layered' enameloid found in *Rhina ancylostoma*. Remarkably, the genera *Rhina* and *Rhynchobatus* have long been included in a single family, Rhinidae (=Rhynchobatidae) by several authors (e.g. Müller & Henle, 1841; or more recently Compagno & Last, 1999).

Moreover, most recent molecular phylogenies (Aschliman *et al.*, 2012; Naylor *et al.*, 2012) placed them as sister groups nested deeply within ‘guitarftshes’, but closely related to sawfishes. Accordingly, *Rhina* (Rhinidae) and *Rhynchobatus* (Rhynchobatidae) are included in a monophyletic order, Rhinopristiformes, together with Rhinobatidae and Pristidae (Naylor *et al.*, 2012).

4.2. Phylogeny of batoid tooth enameloid

When the enameloid microstructure data for the taxa studied here were mapped on a recent mo-

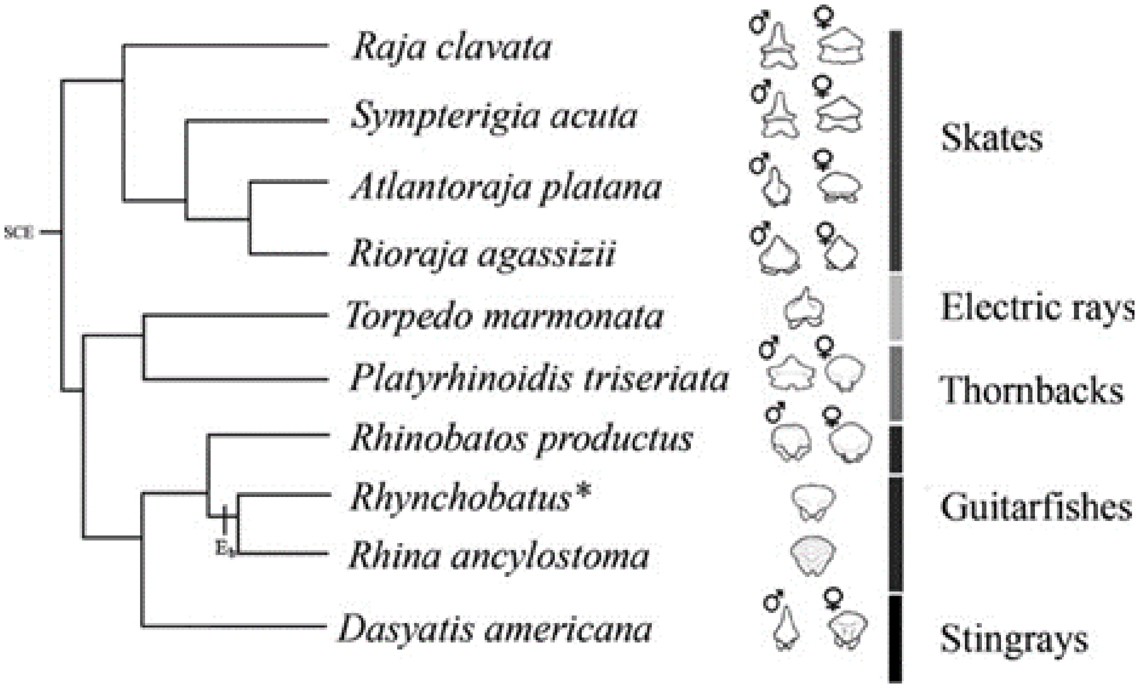


Figure 3. Phylogeny of the extant batoid fishes considered in this study, with the distribution of tooth enameloid microstructure. *Rhynchobatus has not been sampled here, and the presence of a bundled enameloid in this species is based on Cappetta (2012). Only Dasyatys americana has been sampled here as a representative of the stingrays, but the work of Enault et al. (2015) indicated that a classical single-crystallite enameloid (SCE) is present in other Myliobatiformes. Phylogenetic interrelationships are based on Aschliman *et al.* (2012). Skates are recovered as sister group to all other extant batoids and ‘guitarfishes’ are recovered as a polyphyletic group, with sawfishes (not represented here) nested within the clade considered here. Eb mark the occurrence of bundled-crystallite enameloid. Illustrations of teeth are not drawn to scale.

lecular phylogeny of extant batoid fishes (Fig. 3), it provided phylogenetic support to consider the monolayer SCE lacking microstructural differentiation as the primitive condition for batoids. The bundled enameloid in *Rhina ancylostoma* – and presumably in *Rhynchobatus* (see remarks above) – must be considered as a derived character that appeared during the diversifycation of Rhinopristiformes (sensu Naylor *et al.*, 2012). Thus, as recent phylogenetic analysis placed batoids as the sister group of all other neoselachians (Douday *et al.*, 2003; Maisey, Naylor & Ward, 2004; McEachran & Aschliman, 2004; Winchell, Martin & Mallatt, 2004; Aschliman *et al.*, 2012; Naylor *et al.*, 2012), and a SCE is the primitive state for all non-neoselachian groups (Reif, 1973; Gillis & Donoghue, 2007; Botella *et al.*, 2009a), the idea that Batoidea has retained the plesiomorphic condition of enameloid microstructure seems the most parsimonious option, as was suggested by Cuny *et al*. (2009) based on the presence of a SCE in some basal batoids.

Our phylogenetic interpretation contrasts with recent hypotheses that consider double-layered bundled enameloid as the plesiomorphic condition for batoids (Enault *et al.*, 2013, 2015); however, the proposal of Enault *et* al. (2013) was established on a misinterpretation of the enameloid microstructure of several taxa. The enameloid that was interpreted as a bundled two-layered enameloid in some myliobatiforms and in *Raja clavata* is in fact a SCE (see Enault *et al.*, 2015 and below). On the other hand, the suggestion of Enault *et al*. (2015) is based on the finding of a complex bundled enameloid in several fossil batoids. These authors clearly showed the presence of bundled enameloid in two different units of the teeth of *Pytchotrygon* sp. (Turonian, Upper Cretaceous, Morocco), in 'rhinobatoids' *Belemnobatis* sp. (Aptian Lower Cretaceus, France), and in *Parapalaeobates* cf. *atlanticus*

(Campanian, Upper Cretaceous, Morocco); in contrast, a SCE was found in other Rhinobatoid (*Hypsobatis weileri* Cappetta, 1992, Maastrichtian, Late Cretaceous, Morocco) and in some Myliobati-formes. Based on the oldest specimen that they investigated (*Belemnobatis* sp.), they suggest that the ancestral batoid enameloid exhibited some degree of complexity; however, Enault *et al*. (2015) overlooked the fact that a SCE had been recognized previously in older taxa, i.e. the Early Jurassic *Spathobatis moorbergensis* Thies, 1983 and *Spathobatis* sp. (Thies, 1983), the Middle Jurasic *Belemnobatis aominensis* Cuny, Srisuk, Khamha, Suteethorn & Tong, 02009, and probably, according to Cuny *et al*. (2009), in the oldest known batoid, the Early Jurassic *Doliobatis weisi* Delsate & Candoni, 2001 In any case, identifying plesiomorphic conditions based on the stratigraphic position of taxa is problematic, and the phylogenetic relationships of guitarftsh-like batoids from the Jurassic and the Lower Cretaceous are still unclear (Underwood, Mitchell & Veltkamp, 1999; Underwood, 2006; Cuny, 2009). Only a few phyloge-netic studies of batoids have incorporated these extinct taxa (essentially *Spathobatis* and *Belemnobatis*, known from articulated specimens). Furthermore, although it is well established that they present a number of signiftcant differences that separate them from extant rhinobatoids (Maisey, 1984; Aschliman, 2011; and refereneces therein), their position within batoid phylogenies vary among different authors. Thus, Maisey (1984) tentatively placed Torpediformes at the basal level of the batoid phylogeny and a trichotomy containing (*Spathobatis* + *Belemnobatis* + *Pristis*) as the sister group of the remaining batoids. Brito & Seret (1996) considered (*Rhynchobatus* + *Rhina*) as the most basal batoid representatives, and placed the group (*Spathobatis* + *Belemnobatis*) as sister to all other batoids. In Underwood *et al*. (1999), sclerorhynchoidei and (*Spathobatis* + *Belemnobatis*) are recovered as successively distant rootward sister groups to all other batoids, except torpediniforms. This aspect of the topology resembles that of Maisey (1984), except for the placement of *Pristis*. Finally, more recently, Claeson, Underwood & Ward (2013) considered *Sclerorhynchus* and *Spathobatis* as two consecutive stem-batoid groups, with *Spathobatis* as sister to crown batoids. Similarly, the systematic position of *Ptychotrygon* remains controversial, even at a subordinal (or upper) level (Cappetta, 2012; see also Underwood, 2006; Kriwet, Nunn & Klug, 2009). In consequence, the plesiomorphic condition of batoid enameloid remains currently unresolved, as (1) both a SCE and a complex layered bundled enameloid have been found in early batoids, and (2) the exact phylogenetic place- ment of these taxa within batoids is uncertain.

5. CONCLUSION

We have studied the enameloid microstructure in a selection of taxa, trying to cover the phylogeny of living batoids. Our SEM analysis demonstrated that a SCE lacking microstructural differentiation is the most widespread condition among extant batoids, and is probably plesiomorphic at least for crown batoidea, and is not a derived character of the Myliobatiformes, as suggested by Enault *et al*. (2015). In fact, a homogenous SCE monolayer with very conspicuous individualized crystallites is present, capping the teeth of all species studied, with the Rhinoidei *Rhina ancylostoma* being the only exception. Notably, our studies evidenced that SCE enameloid is present in the teeth of both females and males of the species *Raja* cl*a*vata, in contrast with previous interpretations asserting the existence of bundled double-layered enameloid in this taxa.

The double-layered enameloid of *Rhina ancylostoma* has an outermost part in which crystallites are individualized and highly compacted and an innermost part where crystallites are organized into bundles of variable orientation. This microstructure is similar to that described in extant *Rhynchobatus* sp. (Cappetta, 2012), which is placed as sister group of *Rhina* according to most recent molecular phylogenies (Aschliman *et al.*, 2012; Naylor *et al.*, 2012). Moreover, the organization of the enameloid of *Rhina ancylostoma* is also very similar to that found in several distantly related taxa, such as the fossil batoids *Parapalaeobates* cf. *atlanticus* and *Ptychotrygon* sp. (Enault *et al.*, 2015). This suggests that the complex bundled enameloid present in some batoids is a convergent character that appeared more than once during the evolution of batoids, probably as a mechanical adaptation towards moderate durophagous diets.

The plesiomorphic condition of enameloid for the whole group of batoids, including stem lineages, remains unclear, because the phylogenetic position of most guitarftsh-like batoids from the Jurassic and the Lower Cretaceous remains unresolved (Underwood *et al.*, 1999; Underwood, 2006; Cuny, 2009), and both a SCE and a complex layered bundled enameloid are present in these early batoids. Therefore, further studies on both the diversity of the enameloid microstructure in early batoids and on their phylogenetic status are needed to clarify this question.

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CHAPTER 8

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RESEARCH PAPER

On the enameloid microstructure of Archaeobatidae (Neoselachii, Chondrichthyes)

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Purpose In this study, we present new data regarding the enameloid microstructure of the oldest batoid family, Archaeobatidae.

Methods First, all the teeth were etched superficially with HCl 10% for 5 s and photographed in the SEM. Afterwards, the same teeth were embedded in Canadian Balsam, polished and etched again with HCl 10% in order to reveal the enameloid microstructure before being photographed a second time.

Results The enameloid layer of Archaeobatidae consists on a superficial single crystallite enameloid (SCE) with a parallel bundled enameloid (PBE) in all the taxa studied, but only in *Toarcibatis* and *Cristabatis* there exists a tangled bundled enameloid (TBE) under it.

Conclusions The structural complexity and diversity found in Archaeobatidae are comparable to that recently described in others fossil batoids. Our data suggest a general trend to "simplification" in batoid enameloid up till the homogeneous single crystallite enameloid that is present in the majority of current batoids; which contrasts with the increasing structural complexity present in selachimorphs

1. INTRODUCTION

Since pioneer studies of Reif (1973a, b, c) the analyses of chondrichthyan tooth enameloid have been of particular interest to zoologists and paleontologists, proving to be a helpful tool in taxonomic and phylogenetical studies, and providing important information for the understanding of the evolutionary history of the group (e.g. Thies 1993; Cuny *et al*. 2001; Cuny and Risnes 2005; Gillis and Donoghue 2007; Botella *et al.* 2009, b; Guinot and Cappetta 2011; Andreev and Cuny 2012; Enault *et al.* 2013, 2015; Manzanares *et al.* 2016, 2017). During last years, these studies have spread to other fields of research, such physics (Enax *et al.* 2012), geochemistry (Fischer *et al*. 2013), chemistry or archaeology (Drew, Philipp and Westneat 2013) in part due to their potential in the development of novel biomimetic materials. A comprehensive historical background on the evolution of studies on shark enameloid microstructure has been recently published (Enault *et al.* 2015). They also modified the terminology used in the study of chondrichthyan enameloid, unifying and creating new terms to describe its microstructural features, arrangement and diversity. So far, the distinguished two units in the enameloid layer of chondrichthyans: a “Single Crystallite Enameloid” (SCE) unit made of crystallites randomly oriented and the “Bundled Crystallite Enameloid” (BCE) where the crystallites are arranged into bundles with different size and orientation. The orientation of the bundles in this BCE can be parallel to each other [“Parallel Bundle Enameloid” (PBE)] or they can be less organized or “tangled” [“Tangled Bundled Enameloid” (TBE)]. A third possible component of the BCE is the radial bundled enameloid (RBE), where the enameloid crystallites form bundles that originated in the SCE, go perpendicularly through the other BCE components until they reach the enameloid-dentine junction (EDJ). Several of the most recent studies focused on batoid fishes, filling to some degree a previous lack of information regarding the enameloid microstructure in this group (Enault *et al.* 2013; Manzanares *et al.* 2016). These studies demonstrate a great microstructural diversity in the group dismissing earlier assumptions considering batoid enameloid exclusively composed of a single layer of TBE (Gillis and Donoghue 2007) or of SCE (Cuny *et al.* 2009). thus, Enault *et al.* (2015) demonstrate the presence of a complex bundled enameloid organized in different units in several fossil batoids (i.e. *Pytchotrygon* sp., in the ‘rhinobatoid’ *Belemnobatis* sp. or in P*arapalaeobates* cf. *atlanticus*) whereas a SCE is present in other taxa (i.e. the ‘rhinobatoid’ *Hypsobatis weileri* Cappetta, 1992 and in some Myliobatiforms). More recently Manzanares *et al.* (2016) studied the enameloid microstructure through extant batoids phylogeny and found that a homogenous SCE monolayer lacking microstructural differentiation is the most widespread condition, present in all taxa they studied—i.e. in the Rajoidei (Raja *clavata, Atlantoraja platama, Sympterygia acuta* and *Rioraja agassizi*), Platyrhinoidei (*Platyrhina triseriata*), Rhinobatoidei (*Rhinobatos productu*s), Torpedinoidei (*Torpedo marmonata*) and Myliobatoidei—with the unique exception the Rhinoidei *Rhyna ancylostoma* where a double-layered enameloid consisting in an outer layer of SCE and an inner layer of bundles with variable orientation was found.

The high variability and complexity found within batoid dental enameloid, especially among fossil taxa unclear the plesiomorphic condition for the whole group, including stem lineages, and the understanding of the evolution of enameloid microstructure in batoids. Clarifying this question consequently requires further investigation with special focus on the enameloid microstructure in earliest batoids taxa (Enault *et al.* 2015; Manzanares *et al.* 2016).

In the present work, we study the enameloid microstructure in Archaeobatidae, widely accepted as the oldest batomorph family (Underwood 2006; Cuny *et al.* 2009; Aschilman 2011; Aschliman *et al.* 2012; Cappetta 2012; Enault *et al.* 2013, 2015). The family Archaeobatidae was erected by Delsate and Candoni (2001) to include the genera *Doliobatis, Cristabatis* and T*oarcibatis*, all of them represented by isolated teeth found in Toarcian sediments from French, Belgian and Luxembourg localities in the Paris Basin. They were assigned to batoids, based on morphological traits: crushing type crowns, presence of a prominent uvula, hemiaulacorhize roots. The assignment of Archaeobatidae to batoids has been largely followed by posterior authors (e.g. Underwood 2006; Cuny *et al.* 2009; Aschilman 2011; Aschliman *et al.* 2012; Cappetta 2012; Enault *et al.* 2013, 2015; although Cappetta 2012 removed *Doliabatis* from Archaeobatidae and placed it within the family Rhinobatidae).

2. MATERIAL

We studied 5 isolated teeth (Table 1): 2 *Toarcibatis elongata* and 2 *Cristabatis crescentiformis* from Halanzy (HLZ) Localty, 1 *Doliobatis*

Table 1. List of specimens investigated, with information on their locality, age and details of the SEM analysis

Taxon	Family	Age	Locality	Specimen number	SEM	Voltage (kV)
Toarcibatis	Archaeobatidae	Toarcian	Halanzy		S-4100 Hitachii	10
Cristabatis	Archaeobatidae	Toarcian	Halanzy		S-4100 Hitachii	10
Doliobatis	Archaeobatidae	Toarcian	Ginzebierg		S-4100 Hitachii	10

weisi from Ginzebierg (GZB) locality, both locality dated as Toarcian (Fig. 1). The village of Halanzy (Aubange) is situated in the South-East of Belgium, near the French border (Coordinates: East 5º45′10″ North 49º38′23″) (Delsate 1990). The material was collected from a 15–30 cm thick, brown, marly horizon rich in macroinvertebrates (belemnites, ammonites, gasteropods) and phosphatic centimetric "pebbles". The 'Crassum layer' is characterized as a thin conglomeratic level with changes in lateral facies, containIng reworked ammonite faunas of the Bifrons and Variabilis zones. A preliminary analysis of cephalopod (ammonites and belemnites) and shark (micro teeth) faunas provided new data on the biostratigraphic and paleogeographic distribution of these groups in the framework of the Paris basin.

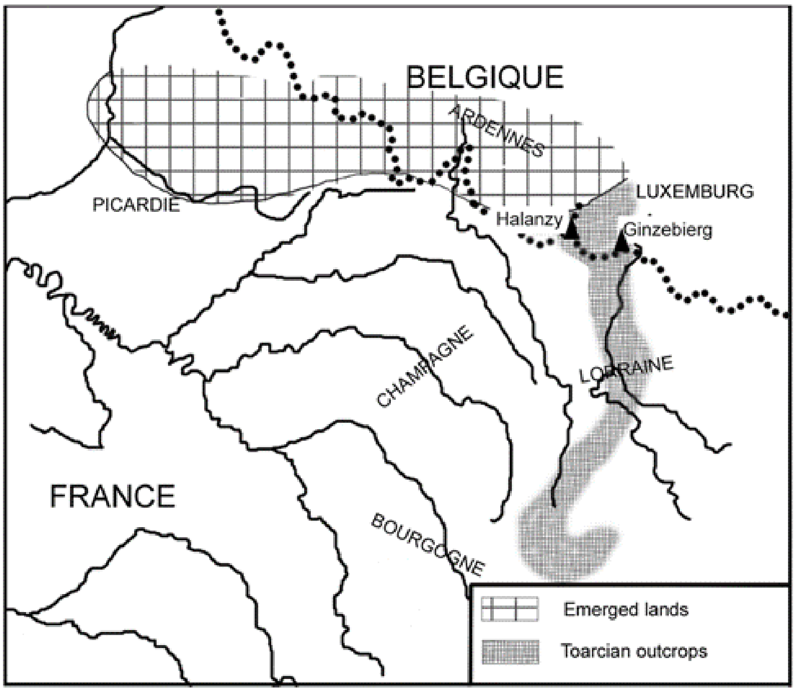

Fig. 1 Situation of the localities of Halanzy (HLZ) and Ginzebierg (GZB) in the Paris Basin. Both localities have been dated as Toarcian. Modified from Delsate and Candoni (2001)

The locality of Ginzebierg (GZB) is situated in the South Grand Duchy of Luxembourg, near Dudelange, at the border between Luxembourg and France (Toarcian Levesquei Zone). The material was collected from a laminated coquina marl on top of the “Grès supraliasique” (top of Toarcian). The impressive abundance of bivalves and their fragments determined the choice of this level in the search of vertebrate predators. Cephalopods are *Mesotheuthis rhenana* Oppel 1856 and *Brevibelus breviformis* Voltz 1830, and the ammonite *Pleydellia subcompta* (Branco 1879), which dates the sediments of the Aalensis subZone (top of the Levesquei Zone).

All the teeth and preparations are deposited in the Museum of Geology of the University of València with catalogue numbers MGUV-36104 to MGUV-36108.

3. METHODS

3.1. Surface study

Due to the scarcity of the material for study, the manipulation and

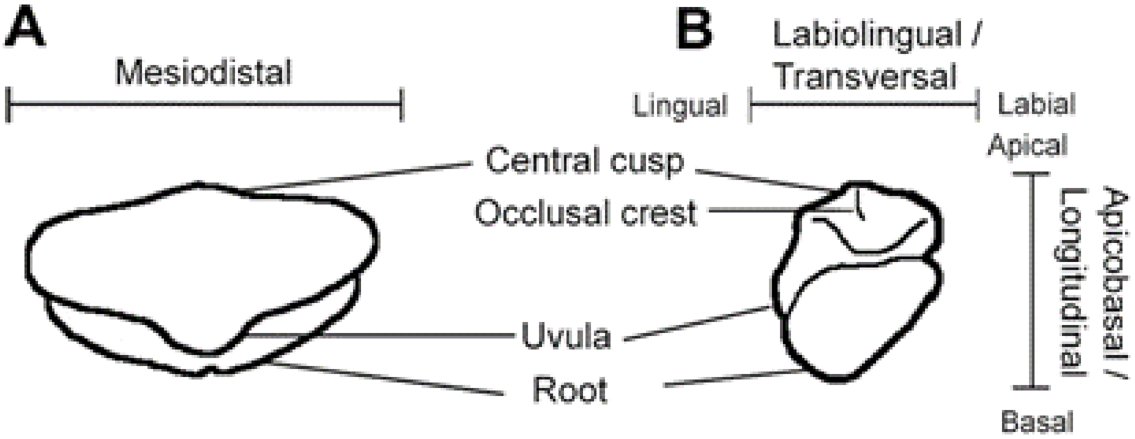

Fig. 2 Sketched tooth of *Toarcibatis* with the descriptive terminology used in this work. **a** Lingual and **b** lateral views

preparation of the teeth were challenging Due to the scarcity of the material for study, the manipu- lation and preparation of the teeth were challenging. To optimize the information that could be obtained, studies of the surface and the internal enameloid structure were car-ried out on each tooth. After each treatment, the teeth were coated in a gold –palladium alloy and photographed on a Hitachi S-4800 scanning electron microscope at the Microscope Service of the Universitat de València. The terminology used in this work is modified from Fischer *et al.* (2011) (Fig. 2a, b).

This method consists of etching the exterior of the tooth with HCl diluted at 10% with a duration of 5–10 s (Reif 1973a, b, c1977, 1978, 1979; Cuny 1998; Cuny and Benton 1999; Cuny *et al.* 2001; Duffin 1980; Guinot and Cappetta 2011; Andreev and Cuny 2012). This allows the acid to penetrate the superficial layers of enameloid and expose its structure, especially the SCE and, depending on how much the acid has penetrated, the PBE that lies beneath it when this layer is present (Andreev and Cuny 2012).

3.2. Section study

This method shows the complete structure of the enameloid layer (Gillis and Donoghue 2007; Guinot and Cappetta 2011; Andreev and Cuny 2012), while the study of the surface does not allow to observe the TBE and the enameloid/dentine junction. Once the superficial analysis was carried out, the same teeth were embedded in Canadian Balsam at 120 °C for two hours before being grounded until the desired plane of section (longitudinal in this work) was reached, then etched with HCl 10% from 5 to 10 s.

4. RESULTS

4.1. *Toarcibatis elongata* (Fig. 3a-l)

The small tooth of Toarcibatis presents a well-defined occlusal crest with a small cusp that shows clear

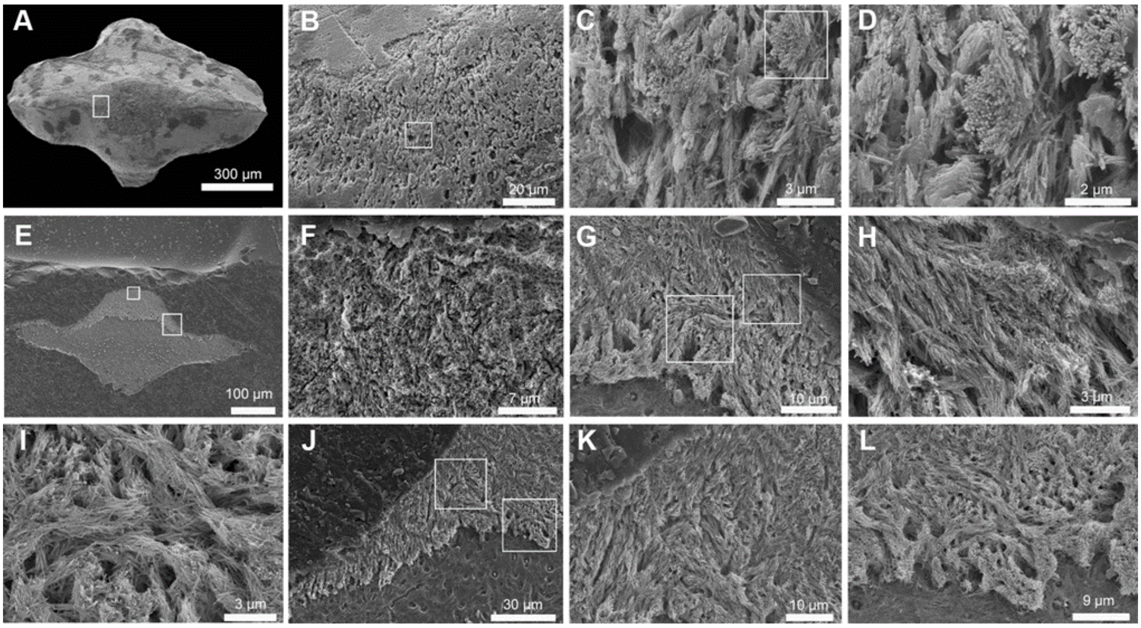

Fig. 3 Tooth of *Toarcibatis* MGUV-36104, **a–d** surface study, **e–l** sectioned study. **a** General view of *Toarcibatis* tooth. **b** Superficial etched surface of the tooth showing the SCE with randomly hydroxyapatite crystallites and the PBE under it, etched for 5 s in HCl 10%. **c** Detail of the PBE with bundles parallel to the crown surface and with some perpendicular to it. **d** Detail of one of the bundles of the PBE normal to the crown surface. **e** Embedded and grounded longitudinal section of the same tooth showing the totality of the enameloid layer, etched in 10% HCl for 10 s. **f** Central part showing bundles of enameloid with no organization. **g** Detail of the enameloid layer showing the PBE and the transition to the TBE that lays underneath it. **h** Close up of the loose bundles of the PBE, with almost of the hydroxyapatite crystallites of each bundles parallel to each other. **i** Bundles of the TBE showing no organization. **j** Detail of the lateral zone of the enameloid layer, showing how the bundles lose their organization in the central part of the tooth. **k** Close up of **j**. **l** Detail of the TBE and the irregular EDJ. The rectangles show the zones where more detailed pictures were taken

signs of wear (Fig. 3a). The longitudinal section reveals a continuous enameloid cover, with a maximum thickness of 78 µm in its central part that becomes thinner distally (Fig. 3e). This tooth shows the most complex microstructure of all the three taxa. The surface study (Fig. 3a–d) shows the presence of a SCE (Fig. 3b) covering the surface of the crown, with a PBE layer beneath it (Fig. 3b–d). In the SCE, the crystallites are randomly oriented whereas in the PBE the crystallites are arranged into well-defined bundles. These bundles are parallel to the crown surface (Fig.

3b–d, h, i). Additionally, some wider bundles (cross-sectioned in surface study) are perpendicular to the occlusal surface, cross the PBE, then reach the SCE (Fig. 3c, d), which will suggest the presence of a RBE. Section studies evidence a TBE (Fig. 3g, i, l) overlying a very irregular but well defined enameloid/den- tine junction (EDJ) (Fig. 3e, g, j, l). In the TBE, the bundles are in general wider and less compacted than the bundles of the PBE (Fig. 3g–l). However, the boundary between these components is diffuse and the PBE component is absent in the lateral-most parts of the teeth (Fig. 3g, j, j).

The central part of the tooth, where the enameloid layer reaches its maximum thickness, displays the most complex microstructure. The bundles of the PBE lose their orienta-tion and cross each other, defining a zone with a compacted interwoven structure that is different from the TBE, which is still clearly distinguishable beneath (Fig. 3f, j–k). The hydroxyapatite crystallites are elongated and measure 2 µm length in the entire enameloid layer.

4.2. *Cristabatis crescentiformes* (Fig. 4a–h)

As in *Toarcibatis*, the tooth of *Cristabatis* presents an occlusal crest with a small central cusp (Fig. 4a). Its enameloid microstructure is organized into a SCE with randomly oriented crystallites covering the surface of the crown (Fig. 4b–c), a PBE and a TBE beneath it (Fig. 3f–g). Surface study shows that bundles of the PBE are oriented parallel to the surface and change their direction near the occlusal crest (Fig. 4c–d) and the section shows that these bundles continue to be parallel to the crown surface (Fig. 4f). Beneath it, the bundles lose this arrangement near the EDJ, where they are less compacted and show the typical woven texture of the TBE (Fig. 4g). In some part of the crown, the enameloid layer shows radial bundles (Fig. 4h) that origi-

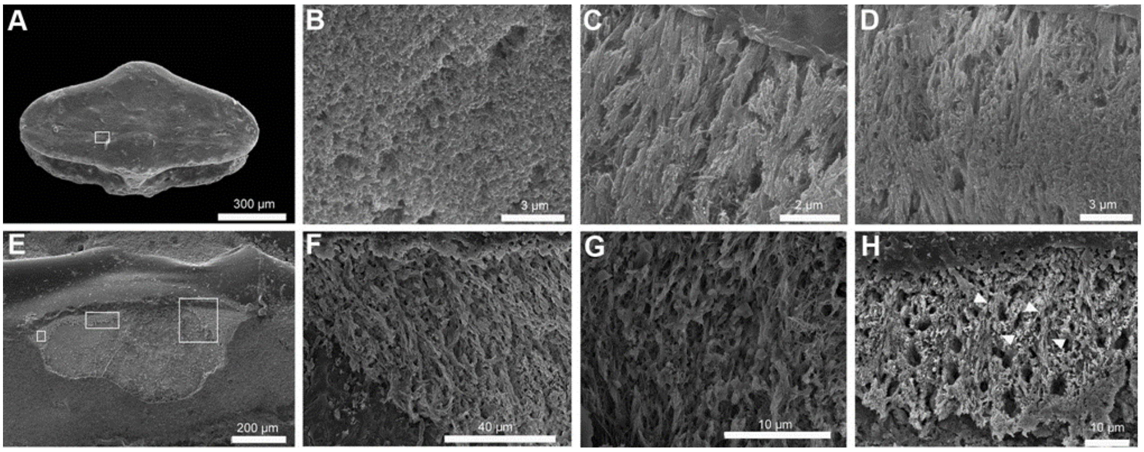

Fig. 4 Tooth of *Cristabatis*, MGUV-36106, **a**–**d** surface study, **e**–h section study. **a** General view of *Cristabatis* tooth, etched for 5 s in HCl 10%. **b** Detail of the etched surface of the crown with the enam- eloid crystallites of the SCE randomly arranged. **c** Detail of the SCE covering the bundles of the PBE in the occlusal crest, note how the bundles are parallel to the surface and change their orientation to fol- low the crown surface. **d** Detail of the bundles. **e** Same tooth embedded, sectioned, polished and etched in 10% HCL for 10 s. **f** Parallel bundles of enameloid crystallites. **g** Interwoven bundles of the TBE, showing less degree of organization than in the PBE. **h** Detail of the enameloid layer near the center of the tooth, note how some radial bundles (arrows) that originated in the SCE run perpendicular to the surface until the reach the EDJ. The rectangles show the zones where more detailed pictures were taken

nated in the SCE and go to the EDJ. The EDJ is irregular but well defined and the enameloid crystallites are elongate and measure ~ 2 µm in length.

4.3. *Doliobais weisi*(Fig. 5a-h)

This tooth is poorly preserved, showing evidences of boring organisms, especially the inner part of the dentine. The surface of the tooth is worn at the level of the central cusp, where the enameloid layer has disappeared completely and exposes the dentine below (Fig. 5a). The interpretation of its microstructure relies mainly on the superficial study which allows for the identification of a SCE with crystallites randomly oriented at the crown surface (Fig. 5b), and a PBE beneath it The bundles in the PBE are well defined and parallel to the crown surface in the superficial study (Fig. 5c-d), but the embedded and polished section shows that the structure of the bundles is less compacted and defined than the bundles

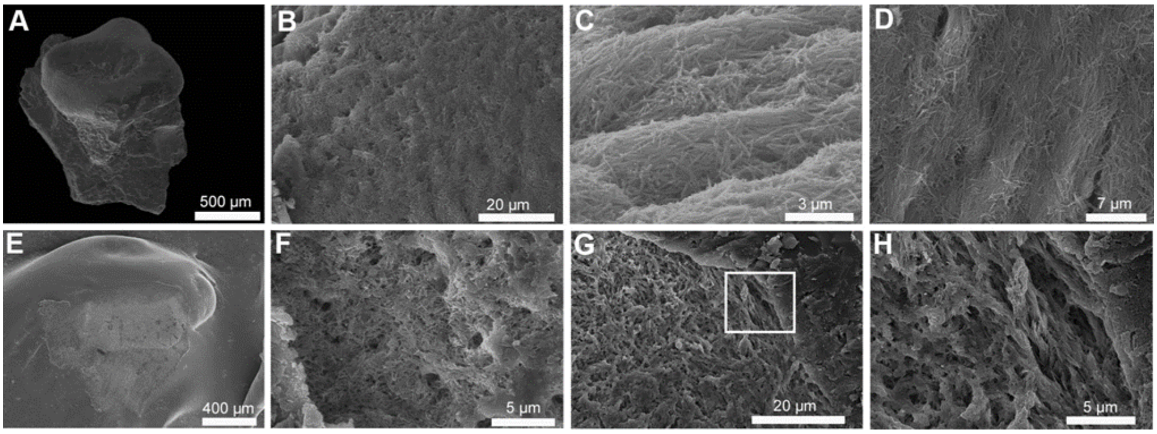

Fig. 5 Tooth of *Doliobatis,* MGUV- 36108. a General view of the tooth. b Etched surface showing the bundles parallel to the crown surface. c–d Close up of some bundles showing the crystallites arranged parallel to each other in each bundles. e Embedded and sections tooth. f Detail of the enameloid layer showing the loss of the organi zation in the bundles, etched 10 s in HCL 10%. g Detail showing the chaotic organization of the bundles. h Detail of the highlighted area of g, showing some bundles parallel to the crown surface. The rectangles show the zones where more detailed pictures were taken

of the other two taxa (Fig. 5e-h). The presence of a TBE layer (present in the other two taxa) can neither be confirmed—nor rejected—in *Doliobatis*, due to the poor results obtained in section studies (Fig. 5g, h). The hydroxyapatite crystallites are elongated and measure around 2 µm in length.

5. DISCUSSION AND CONCLUSIONS

Previous analysis of the enameloid microstructure of Archaeobatidae are limited to the species Doliabatis weisi, where Delsate (2003) described a double layered enameloid with outer SCE and inner TBE. This interpretation was posteriorly questioned by Cuny *et al.* (2009) who consider that the tissue interpreted as a TBE is the underlying dentine, and that only a SCE is present in the species Doliabatis weisi. In fact, the poor quality and resolution of SEM images provided by Delsate (2003; pl

3, Figs. 3, 4, 5) do not allow for a good characterization of the enameloid microstructure. More recently, Enault *et al.* (2015) noted (as Enault, pers. com.) that Archaeobatidae possess a complex dental histology, but neither SEM images nor descriptions are provided to demonstrate it.

Our SEM analysis demonstrates the presence of an enam- eloid layer with a high complex microstructural differentiation in the teeth of all three genus *Doliabatis, Toarcibatis* and *Cristabatis* . In general, the enameloid layer is composed of two clear distinct units; (1) a SCE outermost unit consisting of well individualized randomly oriented crystallites. It appears to cap the complete surface of the tooth crown although the dental wear of the studied specimens does not allow us to assert it with total certainty and (2) an inner bundled layer BCE, where TBE, PBE and RBE components can be identified clearly at least in *Cristabatis* and *Toarcibatis* The poor preservation of the studied tooth of *Doliobatis* only allows for a definitive identification of a SCE outer layer covering a PBE component. However, as said above, a TBE layer has previously been claimed to be present in teeth of *Doliobatis weisi* (Delsate 2003).

The structural complexity and diversity found in Archaeobatidae are comparable to that recently described in other fossil batoids (Enault *et al.* 2015). Thus, a highly complex enameloid with two well-defined units (SCE + BCE) is present in *Ptychotrygon* sp (Sclerorhynchoidei), *Belemnobati*s and P*arapalaeobates* cf. *atlanticus*. *Sclerorhynchoidei* and (*Spathobatis* + *Belemnobatis*) are recovered as successively sister groups to all other batoids (see Claeson *et al.* 2013; Underwood *et al.* 1999) and *Parapalaeobates* is still in unclear phylogenetic position. The bundles oriented parallel to the apical surface of the crown and crossed by wider radial bundles found in *Crista-*

batis and *Toarcibatis* appears very similar to the enameloid microstructure observed in *Ptychotrygon* sp. and *Parapalaeobates* cf. *atlanticus* (Enault *et al*. 2015; Fig. 5h–l). In addition, the complex microstructure described at the level of the cusp and occlusal crest in *Belemnobatis* by Enault *et al*. (2015; Fig. 5f, g), exhibits close similarities with the microstructure found here in the central part of *Toarcibatis* tooth (and probably in *Cristabatis*, but not sectioned at that level) with an outer SCE, a PBE in both lingual and labial sides teeth and a central zone with a compacted interwoven structure and an inner TBE.

The SCE + BCE units identified in Archaeobatidae differ from the triple layered enameloid of modern sharks in both the compaction of the bundles (being more compacted and well-defined in neoselachian sharks) and in the arrangement of the PBE, RBE and TBE components of the BCE (Fig. 6). However, the presence of a complex bundled enameloid in Archaeobatidae, the oldest known batomorph family, and many other ancient batomorphs (Cappetta 2012; Enault *et al.* 2015) suggests the evolution of a complex layered bundled tooth enameloid prior to the dichotomy between Batomorphii and Selachimorpha (considering a sister-group relationship between them; e.g. Douady *et al.* 2003; McEachran and Ashliman 2004; Maisey *et al.* 2004; Aschliman *et al.* 2012). Thus, the new findings indicate that the amalgamation of individual crystals into bundles forming a layered enameloid would not mark the appearance of Selachimorphii as suggested in Andreev and Cuny (2012), and particularly the PBE, long time considered typical of non-batoid neoselachians (e.g. Reif 1977; Delsate 2003; Andreev and Cuny 2012) is more widespread among chondrichthyans than previously considered, including even ctenacanth and Synechodontiformes teeth (i.e. *Neosaivodus flagstaffensis*; Guinot *et al.* 2013; see also Thies *et al.* 2014).

Therefore, the placement within selachians of tooth-based species, some of them pre- viously assigned to batoids (e.g. Hoffman *et al.* 2016) or even to hybodonts (e.g. Reif 1977; Cuny and Risnes 2005; Andreev and Cuny 2012); based only on of the presence of PBE, must be reconsidered.

However, it is important to note that most of the findings of complex bundled enameloid in non-selachians taxa came from the analysis of fossil isolated teeth. Paleontologists know the limitations of these remains for phylogenetical interpretation. Therefore, studies on the enameloid microstructure of teeth taken from articulated specimens, when available, together with phylogenic analysis on the affinities of Jurassic and Cretaceous batoids will provide more definitive information for the understanding of the diversity and evolution of the enameloid in batoid fishes. Anyway, available data suggest a general trend to "simplification" in batoid enameloid from the high complex bundled enameloid present in Archaeobatidae and several other ancient batomorphs (Enault *et al.* 2015 and here) to the homogenous SCE layer present in many other fossil taxa as well as in most of recent

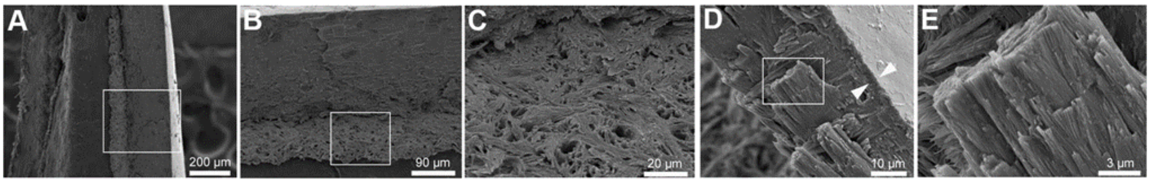

Fig. 6 Sectioned tooth of *Carcharhinus brachyurus* etched in HCl 10% for 5–10 s. **a** Longitudinal section of the tooth showing the com- plete enameloid layer and the dentine below. **b** Close up of the enam- eloid layer, where it is possible to differenciate between the PBE and the TBE. **c** Detail of the interwoven bundles of the TBE. **d** Detail of the bundles of the PBE, the arrows indicate the SCE. **e** Close up of the enameloid crystallites inside bundles of the PBE, showing how close and parallel they are organized

lineages (Manzanares *et al.* 2016). This contrasts with the increasing structural complexity present in selachimorphs (Andreev and Cuny 2012; Enault *et al.* 2015).

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CONCLUSIONS

The main objectives of the present Ph Dissertation can be summarized in: (1) the study of the chondrichthyans of the Middle Triassic of the Iberian Peninsula and (2) to assess on the actual phylogenetical distribution of an homogeneous SCE (non bundled) layer in chondrichthyans. Through the study of isolated remains of chondrichthyans, their taxonomical classification, stratigraphic distribution and/or the study of the ultrastructure of the enameloid layer, I conclude that:

1. The chondrichthyan faunas found in the Betic and the Catalan Coastal Ranges are taxonomical similar to that described from the Ladinian of the Iberain Range by Plá *et al.* (2013), although the remains are considerably less abundant in both basins than in the Iberian Range. The assemblages are comprised of six genera (*Hybodus*, *Omanoselache*, *Lissodus*, *Lonchidion*, *Pseudodalatias* and *Rhomaleodus*) and seven species (*H. plicatilis*, *O. bucheri*, *O. contrarius*, *L.* aff. *L. lepagei*, *L. derenzii*, *P. henarejensis* and cf. *R. budurovi*) belonging to five families, all of them belonging to non-nesoselachian, with the only probable exception of cf. *Rhomaleodus budurovi.* Of those taxa, *P. henarejensis* is endemic of the Iberian Peninsula, and *Lonchidion derenzii* and cf. *Rhomaleodus budurovi* are endemic of the Betic Range. Furthermore, both taxa are restricted to levels of the Boyar section, dated as Carnian by Martín-Algarra *et al.* (1995). The chondrichthyan communities described in this thesis were largely dominated by small durophagous coastal sharks with crushing dentitions well-adapted for preying on crustaceans and mollusks.

2. *Lonchidion derenzii*, sp. nov., represents the earliest record of the genus in Spain, considering its Carnian age (Upper Triassic) according to the bivalve, conodont, and pollen assemblages. Moreover, it can also be considered as the oldest unequivocal record of the genus in Europe. The record of this taxa is limited to a level that represents a very shallow marine platform with close continental influence.

3. The arrival of the chondrichthyans during the Triassic were timing with two transgressive pulses of the Tethys sea. Shark that reach NE Iberia during the first transgressive pulse (middle–late Amirian) of the Tethys Sea show clear affinities with taxa from the Sephardic bioprovince, related to the Neotethys Ocean (i.e. *Omanoselache*). The notable increase in chondrichthyan diversity that occurs in E Iberia during the second transgressive pulse (Ladinian) is due to the arrival of new taxa with clear affinities with European faunas from the Palaeotethys Ocean (i.e. *Paleobates angustissimus*, *Lissodus*, *Hybodus*, *Rhomaleodus*, or *Pseudodalatias*).

4. The colonization patterns reported for chondrichthyans are somewhat inverse to those recently evaluated for other fossil groups. Thus, Anisian bivalves, foraminifers, brachiopods and conodont faunas from E Iberia are almost exclusively compose of taxa from the Alpine/Germanic bioprovince. In contrast invertebrate and conodonts from the Sephardic bioprovince reach the shallow waters of the Iberian Peninsula for first time during the second (Ladinian) transgressive pulse. The existing differences between the colonization patterns are explained on the basis of their dissimilar dispersion strategies: dispersion of bivalves, ammonites and foraminifers, is largely determined by oceanic currents whereas dispersion of coastal sharks depends of adult vagility (very low) and requires continuous appropriate habitats. We propose the existence of narrow, probably short-term, shallow-

water corridors between Palaeo- and Neo-Tethys during the Anisian; the Cimmerian microcontinent would have acted as a barrier for the oceanic currents in the incipient Neotethys; and (3) during the next sea-level rise (Ladinian), the Tethys sea onlapped vast areas of the westernmost Cimmerian microcontinent allowing marine currents from the Neotethys to extend toward the Palaeotethys reaching E Iberian epicontinental seas.

5. Our analysis of *Lonchidion derenzii*, sp. nov., enameloid demonstrates the presence of SCE. Previous studies on the enameloid microstructure of other lonchidiid taxa also have reported the presence of a homogeneous SCE layer in *Lissodus angulatus* (Blazejowski, 2004), *Lissodus minimus* (Cuny and Risnes, 2005), and *Lissodus* aff. *L. lepagei* (Pla *et al.*, 2013; Fig. 3D). Therefore, a single crystallite enameloid without any kind of arrangement into fibers (or bundles) is the widespread condition among Lonchidiidae. This kind of ultrastucture is the most widespread among the teeth of hibodontiformes, although there exist a few exceptions were "loose bundles" of cristallites can be found.

6. The enameloid found in some scales of hybodontiforms (*Lobaticorona*, *Glabrisubcorona* and *Coniunctio*) and neoselachians demonstrates the presence of an outer part of compact SCE and the inner part of SCE, with some densely arranged crystallites parallel to the scale surface. This arrangement with parallel crystallites aligned perpendicular to the enameloid–dentine junction is comparable to that described in various non-neoselachian shark teeth. Taking into account that scales lack feeding functions of teeth the occurrence of incipient microstructural organization/differentiation in scale enameloid (i.e., preferred orientation of crystallites, or "sublayers") cannot be linked with any obvious adaptive function and it could be understood as a con-

sequence of self-organizing processes

7. Our SEM analysis demonstrated that a SCE lacking microstructural differentiation is the most widespread condition among extant batoids and is probably plesiomorphic at least for crown batoidea. The Rhinoidei *Rhina ancylostoma* is the only known exception. The double-layered enameloid of this taxon has an outermost part in which crystallites are individualized and highly compacted and an innermost part where crystallites are organized into bundles of variable orientation. This suggests that the complex bundled enameloid present in some batoids is a convergent character that appeared more than once during the evolution of batoids

8. The study of the enameloid layer in the family Arcaheobatidae shows an enameloid layer with a high complex microstructural diferentiation in the teeth of all three genus *Doliabatis*, *Toarcibatis* and *Cristabatis*. The enameloid layer is composed of two clear distinct units; (1) a SCE and (2) an inner bundled layer BCE, where TBE and PBE and RBE components can be identifed clearly at least in *Cristabatis* and *Toarcibatis.* The poor preservation of the studied tooth of *Doliobatis* only allows for the definitive identification of a SCE outer layer covering a PBE component. However, a TBE has previously been claimed to be present in teeth of *Doliobatis weisi*.

9. The SCE + BCE units identified in Archaeobatidae differ from the triple layered enameloid of modern sharks in both the compaction of the bundles (being more compacted and well-defined in neoselachian sharks) and in the arrangement of the PBE, RBE and TBE components of the BCE. The presence of a complex bundled enameloid in Archaeobatidae, the oldest known batomorph family, and many other ancient batomorphs suggests the evolution of a complex layered bundled tooth enameloid prior to the dichotomy between Batomor-

phii and Selachimorpha. Available data suggest a general trend to “simplifcation” in batoid enameloid from the high complex bundled enameloid present in Archaeobatidae and several other ancient batomorphs to the homogenous SCE layer present in many other fossil taxa as well as in most of recent lineages. This contrasts with the increasing structural complexity present in selachimorphs.

10. The apparition of complex ultrastucutre enmeloid suggests that the complex bundled enameloid present in some batoids (Archaeobatidae, *Rhina ancylostoma*, *Rhynchobatus* sp. and *Parapalaeobates* cf. *atlanticus*) is a convergent character that appeared more than once during the evolution of batoids, probably as a mechanical adaptation towards moderate durophagous diets.

OTHER PROJECTS AND FUTURE PROSPECTS

During the development of my PhD I have had the opportunity of been part of the EVER research group led by Dr. Héctor Botella. This allowed me to be part of different projects in parallel to the study of the fossil record of Spain in which the grant for my PhD was included. Thus, I have participated in the study of the Miocene shark fauna of the province of Alicante, the study of taphonomic processes in the composition of the enameloid layer in chondrichthyan teeth and the study of the diversification patterns that was part of the PhD of Dr. Humberto Ferrón. Thanks to these collaborations, I have also participated in the following papers:

de Renzi, M., **Manzanares, E.**, Marin-Monfort, M. D., & Botella, H. (2016). Comments on “Dental lessons from past to present: ultrastructure and composition of teeth from plesiosaurs, dinosaurs, extinct and recent sharks” by A. Lübke, J. Enax, K. Loza, O. Prymak, P. Gaengler, H.-O. Fabritius, D. Raabe and M. Epple, RSC Adv., 2015, 5, 61612. *RSC Advances*, 6,74384

Martínez-Pérez, C., Esparza, C., Carrillo-Briceño, J. D., **Manzanares, E.**, Hammann, C., Ferrón, H. G, & Botella, H. (2018). A Serravallian (Middle Miocene) shark fauna from Southeastern Spain and its palaeoenvironment significance. *Historical Biology*,. 30(3),422-432.

Ferrón, H. G., Martínez-Pérez, C., Turner, S., **Manzanares, E.**, & Botella, H. 2018. Patterns of ecological diversification in thelodonts. *Palaeontology*. 61(2),303-315. doi: 10.1111/pala.12347.

Botella, H., Olive, S., Pradel, A., Rodriguezcharry, G., Colmenares, F., García-Román, L., **Manzanares, E.**, Navas-Parejo, P., & Martinez-Pérez, C. (In press). First occurrence of fossil vertebrates from the carboniferous of Colombia. *Journal of Vertebrate Paleontology.*

I have also developed two research stays in the Natural History Museum of London under the supervision of doctor Zerina Johanson to conduct the study of the dentition in Holocephali. The results of both stays are published in:

Smith, M., **Manzanares, E.**, Underwood, C., Healy, C., Clark, B., & Johanson, Z. (2020) Holocephalan (Chondrichthyes) dental plates with hypermineralized dentine as a substitute for missing teeth through developmental plasticity..*Journal of Fish Biology*. DOI: 10.1111/jfb.14302

Johanson, Z., **Manzanares, E.**, Underwood, C., Clark, B., Fernandez, V., & Smith, M. (In press) Evolution of the dentition in holocephalans (Chondrichthyes). *Integrative and Comparative Biology*.

Apart from those papers and collaborations, I have also been part of the Organising Committee of three different paleontological conferences:

XIV EJIP/1st IMERP, held in the locality of Alpuente during April of 2016

63rd Meeting of the Palaeontological Association, held in Valencia in December 2019

1st and 2nd PVC, that took place during December 2018 and May 2020 respectively. These two conferences were held completely in a virtual platform, been the first initiative of this nature that has been made in the paleontological field. The first edition of this conference resulted in the edition of a special volume with some of the works of the conference (Vicente D. Crespo and Esther Manzanares. (2020). Palaeontological Virtual Congress: A new way to make sciences. *Palaeontologia Electronica*. DOI:10.26879/1037). The second one drew the attention of the journal Nature ecology and evolution, which held an interview about this congress (Barral, A. (2020) Virtual conferences are the future. *Nature Ecology and Evolution* 4,666–667 https://doi.org/10.1038/s41559-020-1172-z)

Currently, I'm following two lines of research that started as side projects during my PhD.

The first one, is the study of the mineralogical changes in chondrichthyan teeth from the Spanish fossil record, and continues the line started with de Renzi *et al*. (2016). One manuscript is been writing in this moment, trying to understand the changes that the dentine and the enameloid go through the fossilization process. The second one, is the systematic study the enameloid ultrastucture in extant sharks in a complete phylogenetical context. I have directed one final degree thesis for the study of the enameloid layer in the order Galeomorfii, trying to encompass as much as the diversity of feeding strategies and diets of this group of chondrichthyans. These results, along with the study of the Squalomorfii enameloid, the microwear of their teeth and their phylogenetical signals will allow us to understand the complexity of this tissue in modern sharks.

PhD Thesis . 06/2020

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