RESEARCH

Testing dental microwear as a proxy for characterising trophic ecology in fossil elasmobranchs (chondrichthyans)

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Abstract

Dental microwear analysis is a well-established technique that provides valuable information about the diets of extant and extinct taxa. It has been used effectively in most major groups of vertebrates. However, in chondrichthyans, these methods have been implemented only recently in the form of dental microwear texture analysis, with conflicting results. Causes intrinsic to chondrichthyan biology, such as limited food-to-tooth contact, low diversity in terms of trophic categories or fast tooth replacement, have been suggested to reduce diet-related wear on individual teeth, hindering the use of this approach for reliable dietary reconstruction. Here, we explored the relationship between diet and dental microwear in chondrichthyans by using 2D analysis, which can provide finer-scale identification and accurate definition of scratch morphology from tooth surfaces a priori. Scratches were counted and measured on the teeth of 34 extant elasmobranchs grouped into three categories (piscivorous, durophagous and generalist) according to dietary preferences. Our results revealed specific patterns of tooth microwear as a function of dietary abrasiveness, enabling the discrimination of trophic groups and thus establishing a useful comparative framework for inferring aspects of trophic ecology in fossils. We then used this information to study dental microwear in six fossil species from the same locality and stratigraphic levels. First, analyses of the enameloid surfaces of the fossil show that *post-mortem* alterations are distinguishable, allowing reliable quantification of diet-related *ante-mortem* microwear signatures. Discriminant analysis allowed the recognition of microwear patterns comparable to those of living sharks and linked them to specific trophic groups with high probability levels (> 90%). Thus, microwear features developing on chondrichthyan teeth during feeding are intense enough to retain information regarding diet preferences. 2D microwear analysis can track this information, proving to be a useful tool for providing significant information not only about diet but also about oral processing mechanisms in extinct chondrichthyans.

Keywords Dental microwear analysis, Elasmobranchii, 2D-SEM, Fossil tooth, Enameloid, Oral processing

Introduction

Assessing the feeding ecology and diets of extinct vertebrates in general and extinct chondrichthyans in particular is primarily based on indirect evidence. Methods used in living taxa, such as direct observation (e.g., Gordon, 1995; Strong et al., 1990) and stomach content analysis (Baker et al., 2014; Cortés, 2011; Hyslop, 1980), are scarce for the fossil record.

In the absence of other evidence, dental morphology has generally been used as a proxy for inferring dietary

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preferences in fossil chondrichthyans (see Peyer, 1968; Lund, 1990; Gottfried & Fordyce, 2001; Whitenack et al., 2002; Elliott et al., 2004; Botella et al., 2009; Cappetta, 2012; Pla et al., 2013; Manzanares et al., 2018, 2020; and references therein). Nevertheless, a number of recent studies have shown that many living elasmobranchs feed on a different range of prey than expected according to their dentition; therefore, the relationship between tooth morphology and dietary proclivities may not be as accurate as previously assumed (see below but also Scenna et al., 2006; Bergman et al., 2017; Dean et al., 2017; Soekoe et al., 2022). For instance, Collins et al. (2007) revealed that Atlantic cownose rays (*Rhinoptera bonsasus*) can behave as feeder opportunists, depending on the availability of prey, although their crushing dentition suggests that they are hard-prey specialists. Whitenack and Motta (2010) investigated the putative relationship between form and function in shark teeth belonging to three other general categories of selachian dental types: tearing-type, cutting-type, and cutting-clutching type (sensu Cappetta, 1986, 2012). Their results showed that different tooth morphologies are functionally equivalent to each other, drawing and puncturing in the same way. Furthermore, it is now well-known that some extant sharks, such as the white-spotted bamboo shark (*Chiloscyllium plagiosum*), can change their tooth orientation by means of dental ligaments, allowing for a dual role of their single tooth morphology: passing from clutching soft-bodied prey to crushing hard prey (Ramsay & Wilga, 2007).

Overall, these studies demonstrate the need to implement additional analyses beyond tooth morphology to obtain more accurate trophic inferences in fossil chondrichthyans. In recent years, several authors have evaluated the use of dental microwear analysis to assess the diets of extinct chondrichthyans (McLennan & Purnell, 2021; Weber et al., 2021a). Dental microwear refers to micro-metre-scale wear indentations that result from dietary abrasiveness and feeding events (Romero & De Juan, 2012). The proportion and length of microwear signatures in the form of scratches provide information about the mechanical and abrasive effects on tooth surfaces during oral processing (Romero et al., 2012), allowing the inference of oral features such as the type of food processed and aspects of masticatory biomechanics (see Gordon, 1988). Although the earliest works on dental microwear analysis date back to the 1950s and employed a qualitative approach based on optical light microscopy (Baker et al., 1959; Butler, 1952; Mills, 1955), the technique of choice shifted to scanning electron microscopy (SEM) (i.e., 2D-SEM approach) by the late 1970s. This technique allowed for a quantitative analysis of the features present on enamel surfaces and enabled researchers

to explore the relationship between diet and microwear patterns, particularly in early hominins (Grine, 1977, 1986; Rensberger, 1978; Walker et al., 1978). Since the work of Grine et al. (2002) and Semprebon et al. (2004), dental microwear analysis has been developed mainly using texture analysis (i.e., 3D approach) (see Scott et al., 2005, 2006; Ungar et al., 2003). Nevertheless, both 2D-SEM and 3D-surface texture analysis have been demonstrated to be useful tools for inferring the dietary tendencies of extant and extinct vertebrates, including mammals (e.g., Adams et al., 2020; Merceron et al., 2004; Rivals et al., 2022; Teaford & Robinson, 1989), dinosaurs (e.g., Barrett, 2001; Ösi et al., 2022; Williams et al., 2009), reptiles (e.g., Bestwick et al., 2019; Gere et al., 2021; Winkler et al., 2019), bony fishes (e.g., Purnell & Darras, 2015; Purnell et al., 2006, 2007, 2012, 2013), and conodonts (e.g., Purnell, 1995).

Compared with that in other groups, dental microwear analysis in chondrichthyans has been implemented only recently and is still limited. To the best of our knowledge, 2D-SEM analysis has been used only by Itano (2019) to investigate the feeding biomechanics of the enigmatic symphyseal tooth whorls of the Carboniferous "scissor sharks" (*Edestus*). Recently, McLennan and Purnell (2021) and Weber et al. (2021a) explored the use of 3D dental microwear surface texture analysis (DMTA) in chondrichthyans, albeit with contradictory results. McLennan and Purnell (2021) showed that tooth microwear varies with diet in the extant (captive and wild) sand tiger shark (*Carcharias taurus*) and proposed that microwear texture analysis can be a powerful tool for dietary analysis in living and extinct elasmobranchs. In contrast, in a more extensive study including both living and fossil taxa, Weber et al. (2021a) found no clear link between diet and *ante-mortem* dental wear patterns, suggesting that DMTA is difficult to use for reliable dietary inference. According to these authors, this lack of correlation could be explained by the fast tooth replacement rates and the lack of oral food processing, which limited food-to-tooth contact. These authors consider feeding categories in sharks to be less distinct and varied than those in other groups and food-to-tooth contact to be rather limited because only larger prey is manipulated before swallowing. However, this perspective can be reconsidered. Sharks exhibit a remarkable variety of feeding mechanisms and prey upon practically any aquatic animal, ranging from small hard-shelled prey—such as bivalves or gastropods—to giant mammals. Nevertheless, although some shark species are generalists or opportunists, consuming a diversity of prey in various habitats, many other species are certainly ecological specialists, preferring some types of prey over others (see, e.g., Wilga et al., 2007; Motta & Huber, 2012; Bazzi et al., 2021).

This fact is reflected in the great dental morphological disparity in the group, which can be classified into the following dental types corresponding to trophic adaptations (i.e., the method of prey capture/manipulation): clutching, tearing, cutting, cutting-clutching, grinding, crushing, clutching-grinding, cutting-grinding and crushing-grinding (see Cappetta, 1986, 2012). Indeed, many of these trophic adaptations involve complex oral processing, with repeated short compressions and/or lateral movements of the jaws before deglutition. For example, oral processing is used to process/separate hard parts of armoured prey in crushing or grinding types or for reducing prey size in cutting and tearing types (see, e.g., Dean et al., 2005; Huber et al., 2005, 2006; Kolmann et al., 2016; Wilga & Ferry, 2015 and references therein). Furthermore, the reason why continuous tooth replacement has a critical impact on dental microwear analysis in chondrichthyans is also debatable. Dental microwear has been used effectively to infer diet in other groups with rapid and constant tooth replacement, such as fish or reptiles (Baines et al., 2014; Bestwick et al., 2019; Gere et al., 2021; Winkler et al., 2019). In fact, although some neoselachian sharks may have higher replacement rates (the speed at which shark teeth progress forwards from one row to the next (but see Fraser & Thiery, 2019; Botella et al., 2009)) than reptiles, they also present a greater number of functional teeth, which means that the teeth can remain functional for long periods of time (see Fraser & Thiery, 2019).

Evidently, when analysing dental microwear in fossil teeth, it is important to consider the possible *post-mortem* alterations that they may have undergone. These alterations may result from taphonomic processes and from the collection, cleaning, preparation, and moulding of fossil specimens. To determine the impact of *post-mortem* processes on dental microwear analysis, several studies have been conducted (e.g., Böhm et al., 2019; Gordon, 1983, 1984; King et al., 1999; Maas, 1994; Martínez & Pérez-Pérez, 2004; Puech et al., 1985; Romero & De Juan, 2012; Teaford, 1988; Uzunidis et al., 2021; Weber et al., 2021b, 2022). These studies indicate that *post-mortem* alterations are visually distinguishable from *ante-mortem* ingestion-related wear features and, therefore, rarely can cloud dietary inferences with a detailed preliminary examination of fossil material (Böhm et al., 2019; King et al., 1999; Weber et al., 2021b). In this sense, the continuing enlargement of a database of *post-mortem* features is a useful effort for facilitating the identification of this type of alteration in tooth wear analysis (see, e.g., Bohm et al., 2019; Weber et al., 2021b).

Under these considerations, we carried out a study to re-evaluate the correlation between diet and dental microwear in extant chondrichthyans, with the aim of establishing a comparative framework for inferring general aspects of trophic ecology in extinct taxa. In this work, we tested the effectiveness of 2D-SEM-based dental microwear analysis for capturing dietary differences in living species that exhibit different trophic ecologies based on direct studies of stomach contents. For this purpose, we explored microwear variables that have been shown to be useful for correlating dental microwear patterns with dietary preferences in other vertebrate groups (see, e.g., Teaford & Robinson, 1989; Solounias & Moelleken, 1992; Merceron et al., 2004; Nelson et al., 2005; Purnell et al., 2006; Romero & De Juan, 2012; Fahlke et al., 2013). The results obtained from 2D-SEM analysis are compared with those of previous studies using 3D surface texture analysis. Additionally, for the first time, we provide a visual catalogue of *post-mortem* enameloid alterations in fossil chondrichthyans and compare it with similar datasets available for the enamel of other vertebrate groups.

Material

Dental microwear was analysed in 34 extant wild-caught chondrichthyan species. Taxa were selected with an aim of spanning the phylogeny of the group, representing seven of the nine extant orders and covering a wide range of the diet variability present in the group (Table 1; Fig. 1). For each taxon, we analysed in situ functional teeth from complete arcades. For homogenisation, in all cases, we examined one tooth from the upper jaw and one from the lower jaw, present on the anterior third of the jaw from the symphysis. This make a number of more than 14 teeth for each of the tropic groups compared in the analysis (see below). With the exception of *Etmopterus spinax,* the selected species lack dignathic heterodonty. All the extant studied materials are housed at the Museu Cau del Tauró, MCTA (Tarragona, Spain).

In addition, dental microwear was analysed in seven fossil taxa from a Middle Triassic coastal chondrichthyan community of the Iberian Chains, Spain (Fig. 2 and Table 1). To analyse *post-mortem* alterations of fossil teeth, we also considered a different type of mineralised element, termed scales (i.e., dermal denticles). All the selected fossils came from comparable stratigraphic levels of two close sections (Bugarra and Henarejos) of the Iberian Range, Spain (see Pla et al., 2013; Ferron et al., 2014 for further information). We suggest that fossil scales are a great model for evaluating *post-mortem* alterations in teeth because the elements present similar histological

Table 1 Extant and extinct species used for analysing dental microwear

Species	Trophic group	Diet	Registration number
Alopias pelagicus	Piscivorous	Teleost fishes and cephalopods[1]	MCTA00186
Alopias vulpinus	Piscivorous	Teleost fishes and cephalopods[2]	MCTA00201
Carcharias taurus	Piscivorous	Teleost fishes, elasmobranchs and cephalopods[2]	MCTA08133
Isurus oxyrhinchus	Piscivorous	Cephalopods, teleost fishes, elasmobranchs and small cetaceans[2]	MCTA08172
Sphyrna mokarran	Piscivorous	Teleost fishes, elasmobranchs and a few crustaceans[2]	MCTA01274
Squatina africana	Piscivorous	Cephalopods and teleost fishes[2]	MCTA02143
Tetronarce marmorata	Piscivorous	Teleost fishes[3]	MCTA08144
Atlantoraja castelnaui	Generalist	Teleost fishes and crustaceans[4]	MCTA08124
Beringraja binoculata	Generalist	Crustaceans and teleost fishes[5]	MCTA08151
Carcharhinus sealei	Generalist	Teleost fishes, cephalopods and crustaceans[2]	MCTA01221
Chiloscyllium griseum	Generalist	Teleost fishes, crustaceans, gastropods and bivalves[2]	MCTA08134
Chiloscyllium punctatum	Generalist	Teleost fishes and crustaceans[6]	MCTA00492
Dipturus batis	Generalist	Teleost fishes and crustaceans[5]	MCTA08142
Etmopterus spinax	Generalist	Cephalopods, crustaceans and teleost fishes[2]	MCTA08127
Leucoraja naevus	Generalist	Crustaceans and teleost fishes[5]	MCTA08161
Mustelus antarcticus	Generalist	Teleost fishes, cephalopods and crustaceans[7]	MCTA01249
Mustelus henlei	Generalist	Teleost fishes, cephalopods and crustaceans[2]	MCTA08136
Rhizoprionodon terraenovae	Generalist	Teleost fishes, cephalopods and crustaceans[2]	MCTA01216
Scyliorhinus canicula	Generalist	Teleost fishes and crustaceans[8]	MCTA04808
Squalus acanthias	Generalist	Teleost fishes, elasmobranchs and crustaceans[2]	MCTA00010
Stegostoma fasciatum	Generalist	Gastropods, teleost fishes and crustaceans[2]	MCTA01873
Aetobatus narinari	Durophagous	Bivalves, gastropods, crustaceans and a few cephalopods[3]	MCTA08173
Amblyraja radiata	Durophagous	Crustaceans, molluscs and a few teleost fishes and polychaetes[9]	MCTA08062
Atlantoraja cyclophora	Durophagous	Crustaceans and a few teleost fishes[10]	MCTA08132
Heterodontus francisci	Durophagous	Crustaceans and echinoderms, bivalves[2]	MCTA08135
Heterodontus portusjacksoni	Durophagous	Echinoderms, bivalves, crustaceans and a few fishes[11]	MCTA03138
Mustelus asterias	Durophagous	Crustaceans and a few teleost fishes[2]	MCTA08128
Mustelus mustelus	Durophagous	Crustaceans and a few teleost fishes[2]	MCTA01213
Pseudobatos glaucostigma	Durophagous	Crustaceans and crabs[12]	MCTA08153
Raja brachyura	Durophagous	Crustaceans and a few teleost fishes[13]	MCTA08167
Raja clavata	Durophagous	Crustaceans and a few teleost fishes[13]	MCTA08082
Rhina ancylostomus	Durophagous	Crustaceans, bivalves and a few teleost fishes and cephalopods[14]	MCTA08174
Rhynchobatus djiddensis	Durophagous	Crustaceans, bivalves and a few teleost fishes and cephalopods[15]	MCTA01295
Rioraja agassizii	Durophagous	Crustaceans, molluscs and a few teleost fishes[5]	MCTA08148
Hybodus plicatilis †	–	–	MGUV36508
Lissodus sp. †	–	–	MGUV25857
Omanoselache bucheri †	–	–	MGUV25805
Omanoselache contrarius †	–	–	MGUV25824
Paleobates angustissimus †	–	–	MGUV25893
Pseudodalatias henajerensis †	–	–	MGUV25873a
Pseudodalatias henajerensis †	–	–	MGUV25873b

Extant taxa arranged by trophic categories according to diet preferences (see text). Data were compiled from [1] Calle-Morán and Galván-Magaña (2020); [2] Cortés (1999); [3] Jacobsen and Bennett (2013); [4] Soares et al. (1992); [5] Ebert and Bizzarro (2007); [6] Gauthier et al. (2019); [7] Simpfendorfer et al. (2001); [8] Mnasri et al. (2012); [9] McEachran et al. (1976); [10] Viana and Vianna (2014); [11] Powter et al. (2010); [12] de la Rosa-Meza et al. (2013); [13] Mulas et al. (2019); [14] Purushottama et al. (2022); and [15] Abdurahiman et al. (2010). MCTA (Museo Cau del Tauró); MGUV (Museo de la Universitat de València d'Història Natural)
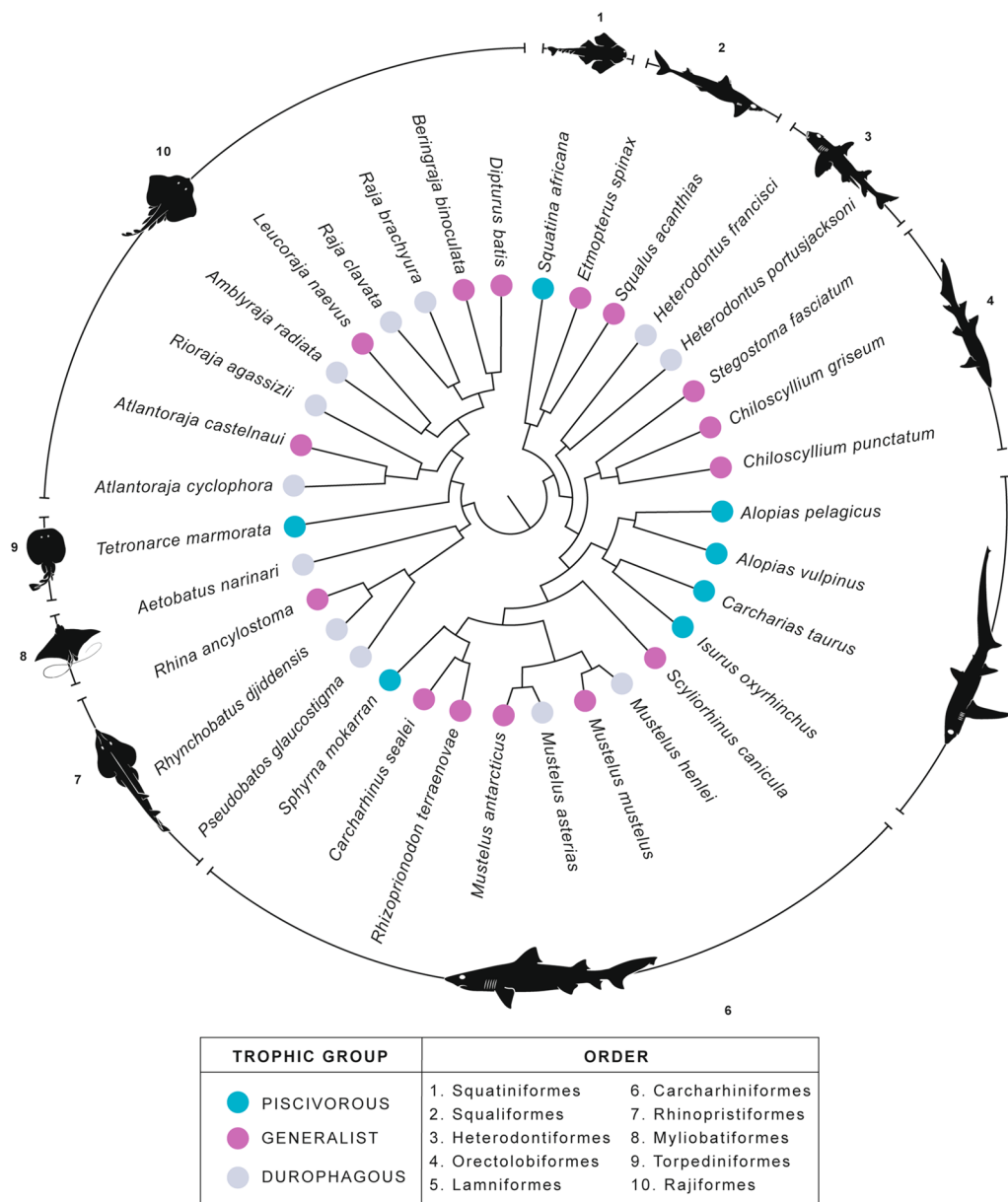
Fig. 1 Phylogenetic position of the 34 extant species studied with indications of major taxonomic lineages and trophic groups. Phylogenetic tree based on Kousteni et al. (2021)

structures, made of dentine with a capping outer layer of enameloid (see Donoghue et al., 2006; Botella et al., 2009; Manzanares et al., 2014). Consequently, the response from *post-mortem* processes will be comparable, showing similar alterations. However, as scales are excluded in oral processing, they are not expected to show diet-related features. All the fossil specimens used in this study are stored at Museu d'Història Natural de la Universitat de Valencia, MHNUV (Spain).

Methods
Analyses of diet-related dental microwear
Establishment of the trophic groups
Diet was determined for all 34 species of living neoselachians based on stomach contents. These data were obtained from previous literature (Table 1). On the basis of their diet preferences, the species were grouped into the following trophic categories (see Table 1 and Fig. 1): (1) piscivorous, species whose diets contain a high percentage (more than 80%) of soft prey, such as
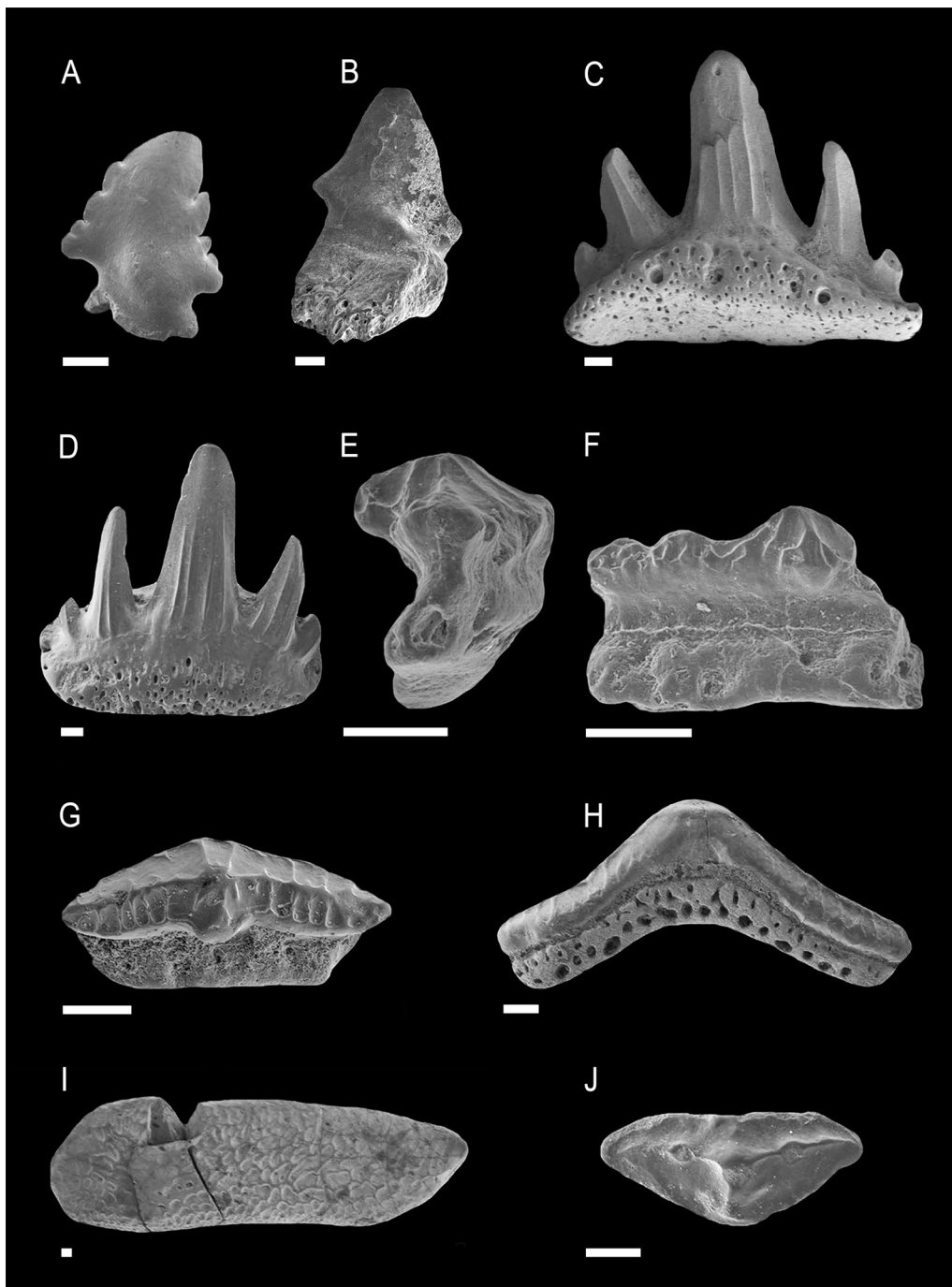
Fig. 2 Scanning electron microscopy images of fossil teeth. **A**, **B** *Pseudodalatias henajerensis*; **A** MGUV2568, lower tooth crown, Henarejos section (He-18), labial view; **B** MGUV25869, upper tooth, Henarejos section (He-14), lingual view; **C**, **D**; *Hybodus plicatilis*; **C** MGUV25837, symphyseal tooth, Bugarra section (Bu1-26), lingual view; **D** MGUV25837, symphyseal tooth, Bugarra section (Bu1-26), labial view; **E**, **F** *Omanoselache contrarius*; I MGUV25821, distal tooth, Henarejos section (He-14), distal view; **F** MGUV25818, distal tooth, Bugarra section (Bu1-26), lingual view; **G**, **H** *Omanoselache bucheri*; **G** MGUV25802, mesial tooth, Bugarra section (Bu1-26), occlusal-lingual view; **H** MGUV25803, mesial tooth, Bugarra section (Bu-pl), labial view; **I** *Paleobates angustissimus*, MGUV25791, tooth crown, Bugarra section, occlusal view; **J** *Lissodus* aff. L. *lepagei*, MGUV25865, lateral tooth crown, Bugarra section (Bu1-26), occlusal view. Scale bar = 200 μm. Modified from Pla et al. (2013)

fishes or cephalopods; (2) generalist, species with a diet composed of both soft and hard prey, with neither type accounting for more than 80% of the diet; and (3) durophagous, species whose diet is composed of more than 80% hard prey, such as bivalves, echinoderms, or decapods. Grouping the species into these categories allowed us to determine whether there were differences in the dental microwear patterns at the trophic group level.

Sample preparation and micrograph acquisition

The original in situ tooth crowns were first cleaned with cotton swabs moistened with ethanol, washed with distilled water and air-dried. High-resolution casts of whole crowns were made using President Microsystem Affinis® Regular Body (Coltène-Whaledent®) polyvinylsiloxane. Replicas were then produced from moulds using EPO 150 two-base component epoxy resin (CTS®, Spain) and a hardener following established procedures (Galbany et al., 2006). The resulting epoxy replicas were subsequently examined using a stereomicroscope to determine their suitability for microwear analysis. For fossil teeth and teeth from some extant taxa of excessively small size (i.e., *Chiloscyllium punctatum*, *Scyliorhinus canicula*, *Etmopterus spinax*, *Amblyraja radiata* and *Tetronarce californica*), microwear was analysed on original tooth surfaces to prevent poor moulding from producing artefacts because of the small size of the teeth. The final sample comprised 68 upper and lower teeth of extant species (58 replicas and 10 original teeth) and 7 fossil teeth (Table 1).

Both tooth replicas and original teeth of extant and extinct species were sputter-coated with gold palladium and examined using a Hitachi S-4800 scanning electron microscope (SEM) at 5 kV in secondary electron emission (SE) mode. All micrographs were taken at a standardised magnification of 1000× following previous protocols (Purnell et al., 2006). SEM micrographs of teeth were taken in the middle of the labial surfaces, and molariform teeth were oriented and imaged in occlusal view (Purnell et al., 2012). All the micrographs were enhanced in contrast using a high-pass (50-pixel) filter and automatic grey level adjustment applied in Photoshop CS6 (Adobe™).

Data acquisition

Dental microwear was analysed following previously outlined methods (Galbany et al., 2005). Individual scratches at least four times longer than wide were counted and measured (in micrometres; μm), and their slope was registered (0°–180°) from SEM micrographs (Fig. 3). A total of ten microwear variables were considered, including
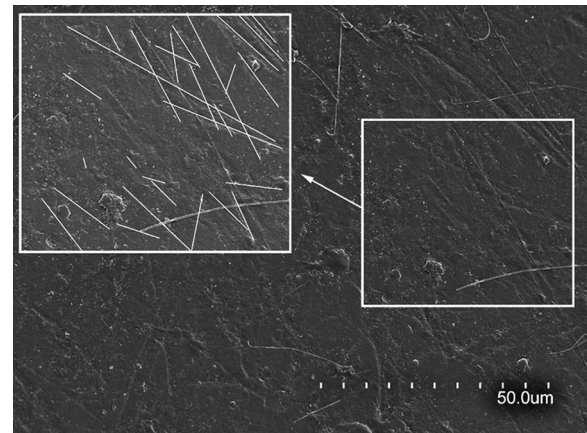

Fig. 3 Scanning electron micrograph of *Scyliorhinus canicula* (MCTA04808) showing the scratch count and measurements processed from well-preserved surfaces. Scratches were classified according to their orientation (0°–180°). See material and methods section for further details

the scratch density (N) and average length (X, in μm) of all observed linear scratches (NT and XT, respectively) and eight independent microwear density and length variables, which were classified by 45° orientation intervals (from 0° to 180°) into vertical (NV and XV), horizontal (NH and XH), left oblique (NL and XL) and right oblique (NR and XR) scratch categories on tooth surfaces to obtain the microwear pattern of each dietary grouping. Microwear metrics were counted, measured and classified using SigmaScan ProV (SPSS™ v.15) by a single observer (MVP-A) to mitigate interobserver effects (Galbany et al., 2005; Purnell et al., 2006).

Statistical analysis

The Kolmogorov–Smirnov test (Z = 1.05 to 0.41; $p > 0.05$) was used for initial data exploration, which showed that tooth microwear variables were normally distributed for each dietary group (piscivorous, generalist and durophagous). Two-way factorial analysis of variance (two-way ANOVA) was performed to assess whether tooth type (upper or lower) and dietary grouping of species had significant effects on the microwear results. In addition, single classification of variance (one-way ANOVA) and Tukey's honestly significant difference (HSD) post hoc test were used as needed to determine the sources of significant microwear variation between the three dietary groups. Canonical variate analysis (CVA) was conducted on the density (NH, NV, NL, and NR) and length (XH, XV, XL, and XR) microwear variables to determine the major trends in the dental microwear patterns among the extant groups based on dietary categories for which the canonical values were computed by using the functions

derived from the CVA. The NT ($r = 0.4$ to 0.8; $p < 0.001$) and XT ($r = 0.3$ to 0.5; $p < 0.001$) variables were not included in the CVA because of their high collinearity levels with other microwear-derived variables according to orientation categories. Fossil specimens were then included in the discriminant analysis performed on extant shark teeth, allowing the evaluation of their assignment to one ecological group. Descriptive and statistical procedures were carried out using IBM® SPSS® Statistics 22.0 (IBM, Armonk, NY, USA) and PAST 4.02 (Hammer et al., 2001). The significance level was set at $\alpha = 0.05$.

Analyses of post-mortem alterations
Micrographs and data acquisition
Fossil elements were examined in detail via SEM (see above) with the aim of identifying *post-mortem* alterations based on comparisons with teeth of current taxa and with available reference datasets of non-ingesta-related dental wear in other vertebrate groups (Böhm et al., 2019; Espurz et al., 2004; King et al., 1999; Martínez & Pérez-Pérez, 2004; Pérez-Pérez et al., 2003; Uzunidis et al., 2021; Weber et al., 2021b, 2022). All identified *post-mortem* surface alterations were photographed and described qualitatively.

In addition, to test the *post-mortem* nature of the alterations that may conflict with ingestion-related dental microwear (i.e., scratches), we developed the statistical analysis described in section "Statistical analysis" in "Analyses of post-mortem alterations". For this purpose, micrographs of the enamel surface were taken from four different categories: (1) fossil scale crowns, (2) occlusal surfaces of fossil teeth considering the labial tooth surface on the middle and upper parts of the crown, (3) non-occlusal surfaces of fossil teeth (i.e., the area closest to the base of the teeth) and (4) occlusal surfaces of current teeth considering the labial tooth surface on the middle and upper parts of the crown (here, ten teeth were randomly selected to avoid differences in sample sizes between different categories). All micrographs were taken at 1000× magnification. A high-pass filter (50 pixels) and automatic grey level adjustments were applied using Photoshop CS6 (Adobe™).

From each SEM micrograph, a 75 μm × 75 μm area was randomly selected. In these areas, we counted the total number of scratches (including both diet-related and *post-mortem* scratches). Thus, the ratio of identified *post-mortem* striae/total striae (PM/T ratio) present in the different categories analysed was used for the statistical analyses (see the following section). All these analyses were carried out using SigmaScan ProV (SPSS™ v.15) and by a single observer (MVP-A).

Statistical analysis
Differences in the PM/T ratios of the four categories considered (fossil scale crowns, occlusal and non-occlusal areas of fossil teeth, and occlusal areas of current teeth) were statistically evaluated using Tukey's honestly significant difference (HSD) post hoc test. Considering that both fossil scales and the non-occlusal area of fossil teeth are not directly related to oral processing, diet-related scratches should be virtually absent, whereas those related to *post-mortem* processes should constitute the total number of scratches. Accordingly, if *post-mortem* marks have been properly identified, the PM/T ratio should be significantly greater for these two groups than for the group corresponding to the occlusal area in fossil teeth and, especially, in teeth from extant species. Statistical analysis was performed as described previously (see section "Statistical analysis" in "Analyses of diet-related dental microwear").

Results
Diet-related dental microwear in extant chondrichthyans
Two-way ANOVA showed that microwear density (NL, NV, NH, NR and NT) and two length (XL and XT) variables were significantly affected by dietary preferences among extant species with different trophic ecologies but not the interaction between tooth type (upper or lower jaw) and diet (Table 2). Therefore, both the upper and lower jaw teeth were considered for further analyses. Descriptive statistics and one-way ANOVAs also revealed that tooth microwear density variables and average scratch length (XT) significantly differed among the dietary groups (Table 3). Overall, durophagous species displayed a significantly greater number of shorter tooth surface scratches than did generalists and piscivorous species.

Pairwise differences (Tukey's HSD; $p < 0.05$) revealed the general pattern of differences between the dietary classes and revealed the strength of the relationship between dietary demands and the functional mechanisms of tooth microwear formation processes (Table 4). The greatest number of pairwise differences was found for tooth microwear density. Accordingly, durophagous individuals tended to exhibit more scratches according to orientation category, followed by generalist and piscivorous individuals. No scratch length by orientation categories could separate the species according to diet.

CVA produced an ordination of two canonical axes with significant discriminatory power (Wilks' Lambda: 0.123; $p < 0.001$) and revealed that tooth microwear patterns differ according to dietary ecology. CV1 (99.4%) was strongly correlated (Pearson's $r > 0.5$; $p < 0.001$) with microwear density variables (NL, NV, NH and

Table 2 Two-way ANOVA of tooth microwear variables for tooth position and dietary groups

Effects	Tooth microwear density variables									
	NL		NV		NH		NR		NT	
	F	p	F	p	F	p	F	p	F	p
Model	20.739	**0.000**	7.687	**0.000**	3.759	**0.005**	19.271	**0.000**	48.055	**0.000**
Tooth	0.165	0.686	0.098	0.755	0.755	0.388	0.001	0.976	0.070	0.792
Diet	51.484	**0.000**	18.927	**0.000**	8.608	**0.001**	46.724	**0.000**	120.008	**0.000**
Tooth × Diet	0.216	0.806	0.197	0.822	0.405	0.669	1.453	0.242	0.067	0.935

Effects	Tooth microwear length variables									
	XL		XV		XH		XR		XT	
	F	p	F	p	F	p	F	p	F	p
Model	1.840	0.118	1.149	0.345	1.296	0.277	1.013	0.418	8.878	**0.000**
Tooth	0.749	0.390	0.921	0.341	2.298	0.135	2.890	0.094	0.283	0.596
Diet	3.177	**0.049**	2.349	0.104	0.038	0.962	0.843	0.435	21.805	**0.000**
Tooth × Diet	1.345	0.268	0.128	0.880	2.521	0.089	0.649	0.526	0.148	0.863

Effects: Tooth includes upper and lower teeth. Diet: piscivorous, generalist and durophagous species

Scratch density (N) and average scratch length (X; in µm) by orientation category [left oblique (L); vertical (V); horizontal (H) and right oblique (R)] and total (T) scratches

Significant differences at $p < 0.05$ (in bold)

Table 3 Summary statistics and differences (one-factor ANOVA) for tooth microwear variables and dietary groups

Variable	Piscivorous		Generalist		Durophagous		ANOVA	
	Mean	SD	Mean	SD	Mean	SD	F	p
NL	2.571	1.828	7.844	5.144	17.864	6.190	53.352	**0.000**
XL	44.174	32.428	39.356	16.570	29.700	10.020	3.185	**0.048**
NV	2.357	1.499	5.719	3.050	9.955	5.867	19.660	**0.000**
XV	43.857	25.936	39.454	19.340	31.975	15.000	2.422	0.097
NH	1.571	1.651	3.063	3.435	6.682	5.437	8.800	**0.000**
XH	57.804	60.307	46.739	38.245	39.743	19.513	0.036	0.964
NR	2.357	1.737	8.219	3.765	15.500	6.022	46.790	**0.000**
XR	47.381	39.471	39.536	18.439	32.010	13.555	0.838	0.437
NT	8.714	2.585	24.844	10.315	50.000	7.329	125.294	**0.000**
XT	66.651	15.093	42.710	13.090	33.201	9.534	22.577	**0.000**

Significant differences at $p < 0.05$ (in bold)

NR), whereas it displayed significant negative loadings ($r = -0.2$; $p < 0.05$) for scratch length (Fig. 4). Therefore, species with greater levels of durophagy exhibit denser scratches that are shorter in length as a consequence of an increase in abrasive items in the diet. In contrast, CV2 (0.6%) captured mainly intragroup diet-related tooth microwear variability and was found to be positively correlated ($r > 0.2$, $p < 0.05$) with microwear density (NL and NH) and length (XL, XV, XH and XR) variables and significantly negatively correlated

($r = -0.2$; $p < 0.01$) with the NV ($r = -0.2$; $p < 0.001$) and NR ($r = -0.4$; $p < 0.001$) metrics. Therefore, overlapping specimens suggest that microwear patterns are also capable of reflecting specific variability in food resource exploitation and biomechanical demands. In turn, the ANOVA results for CV1 ($F_{2,65} = 137.5$; $p < 0.001$) and CV2 ($F_{2,65} = 0.759$; $p = 0.472$) further support that increased dietary abrasiveness is significantly tracked by increasing CV1 scores. The average probabilities of group assignment for correctly classified specimens were 89.7.2% and 86.8% after jackknife cross-validation,

Table 4 Tukey matrices of pairwise mean differences for evaluating tooth microwear variables among dietary groups

Tooth microwear density variables		P	G	Tooth microwear length variables		P	G
NL				**XL**			
Piscivorous	P			Piscivorous	P		
Generalist	G	**− 19.207**		Generalist	G	1.169	
Durophagous	D	**− 42.490**	**− 23.282**	Durophagous	D	13.311	12.142
NV		**P**	**G**	**XV**		**P**	**G**
Piscivorous	P			Piscivorous	P		
Generalist	G	**− 20.828**		Generalist	G	2.772	
Durophagous	D	**− 33.841**	**− 13.012**	Durophagous	D	12.746	9.974
NH		**P**	**G**	**XH**		**P**	**G**
Piscivorous	P			Piscivorous	P		
Generalist	G	− 9.473		Generalist	G	0.875	
Durophagous	D	**− 24.194**	**− 14.721**	Durophagous	D	1.818	0.943
NR		**P**	**G**	**XR**		**P**	**G**
Piscivorous	P			Piscivorous	P		
Generalist	G	**− 22.330**		Generalist	G	1.419	
Durophagous	D	**− 42.142**	**− 19.812**	Durophagous	D	7.538	6.119
NT		**P**	**G**	**XT**		**P**	**G**
Piscivorous	P			Piscivorous	P		
Generalist	G	**− 22.964**		Generalist	G	**23.200**	
Durophagous	D	**− 48.396**	**− 25.431**	Durophagous	D	**35.357**	**12.156**

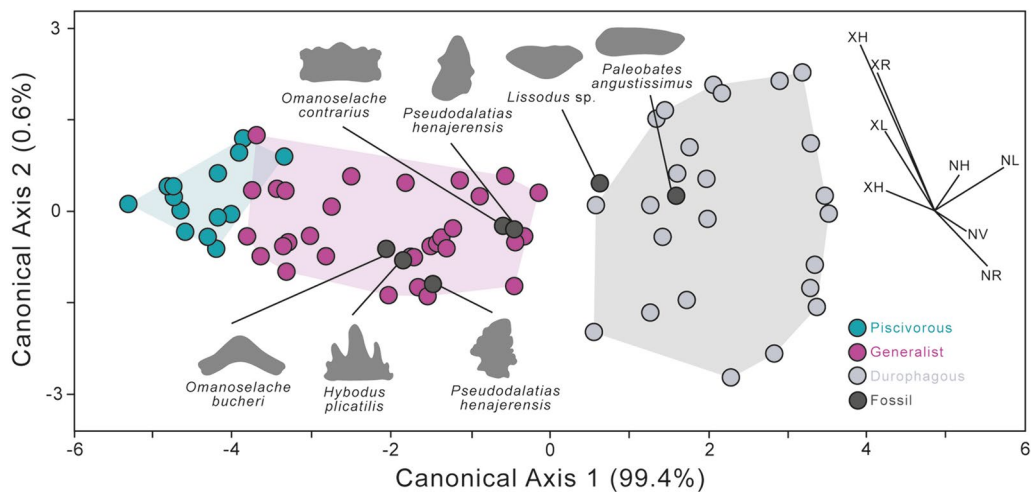Significant differences at p < 0.05 (in bold)

Fig. 4 Scatter plot of the first two canonical variates of dental microwear patterns showing diet-related variability between extant species and the trophic position of fossil Chondrichthyans. The labelled rays show the loading scores for each dental microwear variable on the canonical axes. Fossil specimens were classified post hoc with the derived canonical variates

with 92.87% for piscivorous individuals, 70% for generalists and 100% for durophagous individuals.

Analyses of post-mortem alterations

SEM observations allowed us to identify a variety of abrasive patterns in both fossil chondrichthyan teeth and scales that can be attributed to *post-mortem* processes.
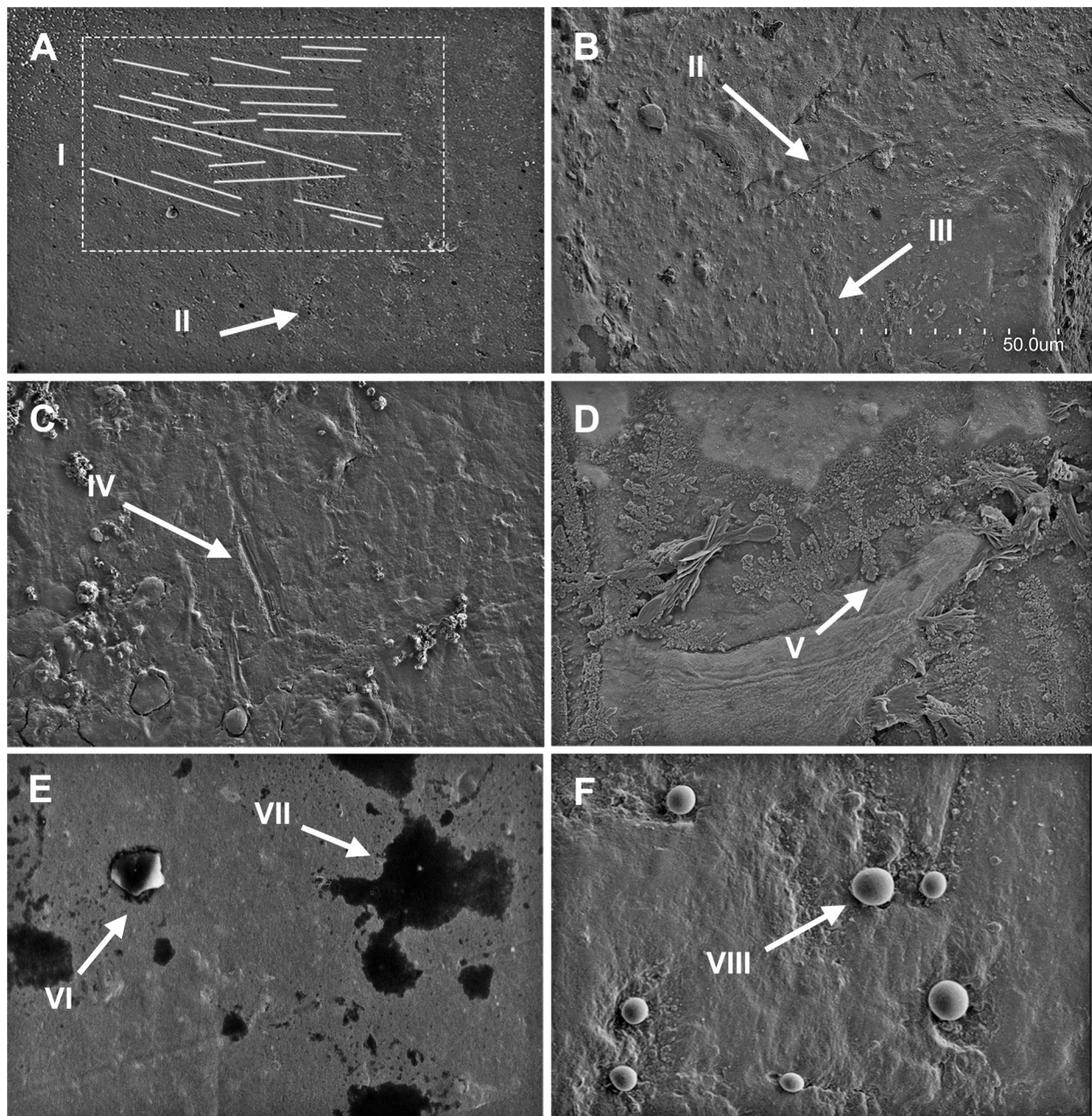
Fig. 5 Scanning electron images of chondrichthyan tooth surfaces showing *post-mortem* alterations. **A** Shark fossil scale; **B** non-occlusal area of fossil tooth; **C** tooth of *Raja clavata* (MCTA08082); **D** fossil teeth; **E** tooth of *Mustelus henlei* (MCTA01213); **F** tooth of *Aetobatus narinari* (MCTA08173). Roman numerals specify the different *post-mortem* alterations: I. Parallel and superficial scratches with similar directions. II. Fracture on the enameloid. III. Sinuous scratch. IV. Wide scratch with multiple indentations. V. Pyrolusite overlapping the fossil tooth surface. VI. Remaining varnish. VII. Adherent dirt. VIII. Varnish bubbles

These alterations included (1) parallel and superficial scratches with similar orientations found in both fossil scales and teeth (Fig. 5A); (2) cracks in the enameloid of fossil teeth producing fractures that are not straight but are frequently deep and wide (Fig. 5A and B); (3) scratches with sharper margins and a sinuous track (Fig. 5B) found in both scales and in the non-occlusal and occlusal areas of fossil specimens; (4) scratches with multiple deep furrows (Fig. 5C) identified in fossil teeth and in some extant specimens; (5) pyrolusite growth overlapping the occlusal surface found only in fossil teeth (Fig. 5D); (6) remaining varnish (Fig. 5E); and (7)

Table 5 Tukey matrices of pairwise mean differences for the ratio of *post-mortem* processes

	Scale	Non-occlusal area of fossil tooth	Occlusal area of fossil tooth	Occlusal area of extant tooth
Scale	–	− 2.057	**− 8.300**	**− 16.800**
Non-occlusal area of fossil tooth	2.057	–	**− 10.357**	**− 18.857**
Occlusal area of fossil tooth	**8.300**	**10.357**	–	**− 8.500**
Occlusal area of extant tooth	**16.800**	**18.857**	**8.500**	–

Significant differences at *p* < 0.05 (in bold)

adherent dirt (Fig. 5E) and the presence of varnish bubbles on both extant and fossil teeth (Fig. 5D).

Overall, the identified *post-mortem* alterations are easily distinguishable from ingestion-related *ante-mortem* microwear, even considering the *post-mortem* scratches. Thus, *post-mortem* parallel scratches (Fig. 5A) are denser and tend to be less pronounced than diet-related scratches. The sinuous scratches (Fig. 5B) displayed more irregular, wider and sharper edges than the dental microwear. In addition, while dental microwear scratches usually display a single dale, those identified as *post-mortem* in teeth from fossil and extant specimens were generally wider with multiple furrows (Fig. 5C).

Significant pairwise differences (Tukey's HSD; *p* < 0.05) were found when *post-mortem* scratch patterns were compared (Table 5), with the exception of fossil scales compared with the non-occlusal area of fossil teeth (i.e., the categories indirectly involved in oral processing; mean difference 2.057; *p* > 0.05). As expected, greater differences were detected when these categories were compared with the area of extant-taxon teeth [mean differences (16.800; *p* < 0.05) and (18.857; *p* < 0.05)], although significant differences were also found upon comparison with the occlusal area of fossil teeth. The differences between the occlusal areas of fossil teeth and extant taxon teeth were also statistically significant (8.500 *p* < 0.05).

Dental microwear in fossil chondrichthyans
Fossil teeth were assigned to trophic categories after their inclusion in the CVA. *Hybodus plicatilis*, *Omanoselache bucheri*, *Omanoselache contrarius* and *Pseudodalatias henajerensis* were classified as generalists. *Lissodus* sp. and *Paleobates angustissimus* were assigned to the durophagous group. Posterior probabilities were high, close to 1 (*p* > 0.9; df = 2), indicating that the assignment of fossil shark teeth to trophic categories was performed reliably. The conditional probabilities of shark fossil teeth were high (*p* > 0.816; df = 2) for *Hybodus plicatilis*, *O. bucheri* and *Paleobates angustissimus*, whereas the teeth of *Lissodus* sp. and *O. bucheri* and the two teeth of *Pseudodalatias henajerensis* displayed lower values (*p* < 0.550; df = 2).

For probabilities of classifications for all specimens, both extant and extinct, see Supplementary Table S1.

Discussion
Dental microwear and dietary signals in extant elasmobranchs.
The dental microwear patterns of 34 species of living neoselachians whose trophic ecology and dietary habits are well known were analysed with the aim of testing the relationship between dietary group and dental microwear and therefore creating a comparative framework useful for inferring general aspects of trophic ecology in extinct taxa. The studied species were grouped into three trophic categories (piscivorous, generalist and durophagous) based on their dietary preferences. Canonical variable analysis (CVA), including these trophic categories as defined groups, revealed a clear connection between dental microwear and dietary proclivities in elasmobranchs. The discriminant punctuation of the original cases (Fig. 4), as well as two-way ANOVA and pairwise differences (Tukey's HSD) (Table 4), indicated great separation among all three trophic groups.

Evidently, the wide ecological trophic diversity of chondrichthyans could lead to more detailed classifications being established, exemplified by the arrangement of chondrichthyans into eight groups related to trophic adaptations proposed by Cappetta (1986, 2002). However, the three groups proposed here are simple enough to evaluate the correlation between diet and dental microwear. In fact, the CVA correctly classified 86.8% of cases by cross-validation, demonstrating high predictive power and therefore providing a useful approximation for inferring general trophic aspects of extinct chondrichthyans according to the trophic categories established in the present study.

Our findings indicate that the dental microwear pattern correlates with dietary abrasiveness. When hard items are included in the diet, the density of scratches increases while the scratch length decreases, possibly because of prey brittleness, which increases the speed of breakage. As a result, durophagous species exhibit a dental microwear pattern characterised by a greater density

of shorter scratches on the tooth surface (NT = 50.000; XT = 33.201 μm) than observed in generalist species (NT = 24.844; XT = 42.710 μm), which consume both hard and soft items, particularly piscivorous species (NT = 8.714; XT = 66.651 μm). Furthermore, elasmobranchs that feed on hard prey exhibit greater anisotropy in scratch orientation than piscivorous elasmobranchs, suggesting that dental microwear analysis not only tracks diet but also provides information about jaw movements (compare Charles et al., 2007; Gordon, 1984; Williams et al., 2009). This indicates that the processing of hard items involves more horizontal and oblique jaw movements than does the processing of softer prey, aimed at reducing prey and separating inedible material from edible tissue. In contrast, the processing of soft items involves more vertical movements, as biting by grasping may be sufficient to handle these types of items. Thus, our analyses show that dental microwear reflects the ability of elasmobranchs to process food (see Dean & Motta, 2004; Kolmann et al., 2016; Wilga & Motta, 1998) and to adapt their feeding mechanism based on the physical and mechanical properties of the prey (see Gerry et al., 2010; Wilga et al., 2012; Kolmann et al., 2016).

Overall, the dental microwear patterns found in chondrichthyans are comparable to those reported from other groups of aquatic vertebrates, where variables such as the density of scratches and scratch length have been revealed to be useful for correlating dental microwear patterns with dietary preferences. For example, marine mammals such as *Odobenus rosmarus* and *Eumetopias juratos*, which consume organisms with mineralised exoskeletons, exhibit a greater density of scratches than other marine mammals whose diets are primarily composed of fish and squids (Fahlke et al., 2013). Similarly, the quantitative analysis of dental microwear in *Gasterosteus aculeatus* (Purnell et al., 2006) revealed that wild fishes feeding on hard prey requiring manipulation exhibit a dental microwear pattern characterised by a greater number of shorter scratches than in fishes feeding on items requiring minimal handling.

In summary, our study revealed that analysis of dental microwear through two-dimensional scanning electron microscopy can reveal dietary differences in elasmobranchs and reveal a significant relationship between dietary preferences and dental wear features. This finding agrees with the results obtained by McLennan and Purnell (2021) through 3D surface texture analysis but contrasts with the conclusion reached by Weber et al. (2021a) using the same approach (see Introduction). Evaluating the effectiveness of 3D surface texture analysis in chondrichthyans is beyond the scope of this work. However, the results obtained through a 2D-SEM approach reveal that (1) food-to-tooth (or tooth-to-tooth) contact during food intake (i.e., prey capture and oral processing) in elasmobranchs is intense enough to produce quantifiable wear on individual teeth; (2) the diversity of prey preferences and oral processing mechanisms between species is large enough to produce significant differences in their tooth wear patterns; and (3) despite the high tooth replacement rates, teeth remain functional long enough to produce diet-related dental wear. Therefore, further research is needed before suggesting the use of 3D approaches in chondrichthyans. A comparison between 2D tooth microwear and 3D sub-micro-metre-scale tooth surface textures revealed the effectiveness of both methods for dietary discrimination in other groups of aquatic vertebrates (Purnell et al., 2012). Furthermore, the positive correlation found between microwear density and microtextural data (surface roughness) was strongly indicative of similar levels of information regarding the effects of feeding on teeth (Purnell et al., 2012).

Post-mortem alterations on enameloid surfaces

Previous studies assessing *post-mortem* alterations on fossil teeth focused on the enamel surfaces of tetrapod teeth (e.g., Böhm et al., 2019; King et al., 1999; Maas, 1994; Martínez & Pérez-Pérez, 2004; Puech et al., 1985; Romero & De Juan, 2012; Teaford, 1988; Uzunidis et al., 2021; Weber et al., 2021b, 2022). However, specific studies on chondrichthyan tooth enameloid surfaces are lacking. Enamel (present in tetrapod and sarcopterygian teeth) and enameloid (covering the teeth of Chondrichthyes, Actinopterygii and larval stages of some caudate amphibians) are morphologically and functionally similar, but the two tissues have different origins as well as different chemical compositions and internal microstructural organisations (see Enax et al., 2012; Manzanares et al., 2016 and references therein). Specifically, enamel is secreted solely by cells from the inner dental epithelium (ameloblasts), whereas enameloid has epithelial and mesenchymal origins (ameloblasts plus odontoblasts) (Sasagawa et al., 2009; Sire et al., 2009). The mineral phase of enamel typically consists of hydroxyapatite $(Ca_5(PO_4)_3OH)$ crystallites, whereas the mineral phase of chondrichthyan tooth enameloid consists of fluoro-apatite $(Ca_5(PO_4)_3F)$ (see Enax et al., 2012, 2014; de Renzi et al., 2015 and references therein). Finally, the internal microstructure of chondrichthyan tooth enameloid is also substantially different from that of tetrapod tooth enamel. The microstructure of chondrichthyan tooth enameloid varies from a simple single-crystallite layer in extinct lineages and living batoids (Gillis & Donoghue, 2007; Botella et al., 2009; Manzanares et al., 2014, 2016) to a triple-layered bundled enameloid in modern sharks (i.e., non-batoid neoselachians) (Reif, 1974, 1977; see also Cuny & Risnes, 2005). However, despite the chemical and

microstructural differences of these tissues, some studies have indicated that the mechanical properties (i.e., hardness and Young's modulus) of "true" enamel and chondrichthyan enameloid (i.e., hardness and Young's modulus) are comparable (Enax et al., 2012; Whitenack et al., 2010).

Here, for the first time, we have assessed *post-mortem* alterations on enameloid surfaces in detail. *Post-mortem* features are described and illustrated (Fig. 5). We provide an increasing dataset to help researchers discern between *post-mortem* (mainly taphonomic) altered surfaces and well-preserved surfaces (see Böhm et al., 2019; Weber et al., 2021b). As in tetrapod enamels (Böhm et al., 2019; Gordon, 1983, 1984; King et al., 1999; Maas, 1994; Martínez & Pérez-Pérez, 2004; Puech et al., 1985; Romero & De Juan, 2012; Teaford, 1988; Uzunidis et al., 2021; Weber et al., 2021a, 2021b), *post-mortem* alterations of chondrichthyan enameloid can be easily visually differentiated from *ante-mortem* ingesta-related wear. Therefore, a preliminary examination of fossil elements allows *post-mortem* altered surfaces to be excluded from tooth microwear analysis, as is logically recommended (see King et al., 1999; Martínez & Pérez-Pérez, 2004; Weber et al., 2021a, 2021b).

In general, the *post-mortem* alterations identified in the enameloid of fossil chondrichthyans are comparable to those described in the dental enamel of other vertebrates (Böhm et al., 2019; Gordon, 1983, 1984; King et al., 1999; Maas, 1994; Martínez & Pérez-Pérez, 2004; Puech et al., 1985; Romero & De Juan, 2012; Teaford, 1988; Uzunidis et al., 2021; Weber et al., 2021b). For example, surfaces with a high density of superficial scratches that run parallel to each other recognised on fossil scales and fossil teeth of elasmobranchs (Fig. 4A) are similar to those identified on human and other mammal teeth associated with sedimentary transport during taphonomic processes (see Böhm et al., 2019, Fig. 9; King et al., 1999, Fig. 1b; Martínez & Pérez-Pérez, 2004, Fig. 3; Pérez-Pérez et al., 2003, Fig. 1f). Similarly, deep scratches with sharper edges and sinuous tracks (Fig. 5B) have been reported on enamel surfaces (Espurz et al., 2004, Fig. 2b; King et al., 1999, Fig. 2e, f; Martínez & Pérez-Pérez, 2004; Romero & Juan de Dios, 2012, Fig. 14.5; Uzunidis et al., 2021, Fig. 3). Although the exact cause of these marks is unclear (Weber et al., 2021b), their presence only in fossils and their association with other *post-mortem* alterations, such as fractures in the enameloid, suggest a taphonomic origin. In other cases, scratches with multiple indentations identified on extant chondrichthyan teeth (Fig. 5C) are similar to those found on the teeth of reptiles (Weber et al., 2021b, Fig. 1) and hominids (Teaford, 1988, Fig. 17), as well as on other bones (Marin-Monfort et al., 2018,

Fig. 9a, b). These scratches are likely the result of the use of drills or sharp objects during sample preparation, cleaning, and manipulation (Weber et al., 2021b; Marin-Monfort et al., 2018). Finally, alterations derived from errors in moulding processes (Fig. 5E, F) (see Weber et al., 2021b, Fig. 1) or the growth of pyrolusite in fossil samples could overlap with the dental microwear pattern.

Therefore, our survey suggests that despite differences in chemical composition and internal microstructure, *post-mortem* processes (i.e., the taphonomic process and extraction/preparation of fossil specimens) produce comparable alterations on both enamel and enameloid surfaces, probably because of their similar mechanical properties.

Dental microwear as a proxy for inferring diet in extinct chondrichthyans

Chondrichthyans represent one of the oldest and longest-living lineages of vertebrates. Their fossil record begins at least with the Early Devonian (Botella, 2006; Ginter et al., 2010), but it extends to the early Silurian if we include the stem-chondrichthyan "acanthodians" (Andreev et al., 2022; Zhu et al., 2022) or even to the Ordovician if we consider some isolated putative chondrichthyan scales (see Andreev et al., 2015 and references therein). Certainly, teeth are the most abundant elements in the fossil record of chondrichthyans, and indeed, most of the extinct taxa are known only from them (Ginter et al., 2010). Moreover, their stratigraphic record has been remarkably continuous since their first occurrence in the Lower Devonian (Botella, 2006; Botella et al., 2009). Consequently, chondrichthyans represent an exceptional study model for understanding the ecological context of vertebrate tooth evolution (Bazzi et al., 2021; Fraser et al., 2020).

As noted above, until recently, trophic inference in extinct chondrichthyans has been largely based on tooth morphology. However, although dental morphology evidently constitutes an important source of information about the diet of extinct taxa, several studies on current elasmobranchs have shown that the relationship between tooth morphology and diet is not always exact (Bergman et al., 2017; Collins et al., 2007; Dean et al., 2017; Ramsay & Wilga, 2007; Scenna et al., 2006; Soekoe et al., 2022; Whitenack & Motta, 2010). Therefore, additional sources of information to help detect possible disconnections between tooth shape and diet in fossils and to carry out more precise studies on the dietary preferences of extinct chondrichthyans are desirable. In this sense, the good results obtained in our analysis of extant chondrichthyans suggest that dental microwear analysis will become a

useful tool for providing additional information to reconstruct the diet of extinct taxa.

When fossil teeth were included in the CVA (Fig. 4; Supplementary Table S1), the diversity of fossil tooth wear patterns fit well within the wear patterns and trophic groups recognised/established in living sharks. All the fossil teeth were assigned to two of the trophic groups defined in the current taxa with high posterior probabilities (i.e., the probabiily of belonging to the group), near 1 in most cases (Supplement Table S1), which indicates that the assignment of fossil teeth to trophic groups was performed reliably. The Mahalanobis distance to the centroid for fossil taxa is similar to that for current elasmobranchs in all cases.

Indeed, for most of the cases, the trophic category assigned in dental microwear analysis corresponds with that expected from tooth morphology (see Pla et al., 2013), which provides additional support for the good functioning of the methodology. Thus, two fossil taxa (*Lissodus* sp. and *Paleobates angustissimus*) are assigned to the durophagous species. Both of these taxa present teeth of the grinding type with low and flat crowns (Fig. 2J and I) and highly specialised for feeding on hard prey with resistant shells (see Cappetta, 1986, 2012). The high density of dental microwear scratches supports a diet based mainly on these types of prey (i.e., bivalves, gastropods, crustaceans and/or echinoderms). All the other fossil teeth included in the analysis were assigned to generalist species (i.e., a mixed diet composed of soft and hard prey). Among these, *Omanoselache bucheri* and *O. contrarius* possess teeth of the grasping-crushing dentition type (Manzanares et al., 2018; Pla et al., 2013), which are morphologically similar to those present in some neoselachians, such as heterodontid sharks or several Rajiformes (see Cappetta, 2012). Similarly, the lateral (mesial) teeth of *O. bucheri* present a flatter and lower crown than those of *O. contrarius* and lack lateral cusplets, similar to a grinding-type morphology (Fig. 2E–F). This finding is in significant agreement with the information obtained from dental microwear analysis, which identifies *O. bucheri* as a generalist feeder but very similar to a durophagous specialist (Fig. 4), suggesting an important component of hard-shelled prey in its diet. Particularly interesting is the case of *Pseudodalatias henarejensis*. Pseudodalatiids are a family of Triassic non-neoselachian sharks with uncertain phylogenetic affinities (Botella et al., 2009 and references therein). They present strong, dignathic heterodonty, reflecting a cutting-clutching dentition. The upper jaw teeth present crowns with a conical main cusp flanked by small lateral cusplets, whereas the lower jaw teeth have a triangular crown with coarsely serrated edges. The lower jaw teeth form a continuous, serrated blade along the occlusal

jaw margin and are replaced as a single unit (i.e., shedding all the teeth of the same dental row at the same time; see Tintori, 1980; Pla et al., 2013). Interestingly, this particular dentition pattern is currently present in some squaliform sharks. Species with this type of dentition are usually very voracious generalist feeders (feeding on fishes, cephalopods, crustaceans, molluscs, gastropods or bivalves), although several taxa of the Dalatiidae family have developed a specialised feeding strategy, cutting sections of large prey, culminating in some ectoparasitic species (Underwood et al., 2016). We included two current squaliform sharks in our analysis: *Squalus acanthias* (without dignathic heterodonty) and *Etmopterus spinax* with extremely marked ("Pseudodalatias-like") heterodonty (i.e., blade-like teeth in the lower jaw and pointed teeth in the upper). Both species are assigned to the group of generalist feeders according to the dental microwear analysis. *S. acanthias* feeds on a great variety of prey (Table 1) but consume a high proportion of bony fishes, being capable of tearing apart relatively large prey with its blade-like teeth (Wilga & Motta, 1998). In the dental microwear analysis, it was positioned very close to the piscivorous species in the representation of discriminant scores for the original cases (Fig. 4) and was assigned to the piscivores as the second most likely group. *E. spinax* presents a clear mixed diet, feeding on cephalopods, crustaceans and teleost fishes [Table 1; Jacobsen & Bennett (2013)]. Dental microwear analysis assigned both the upper and lower jaw teeth of this taxon to the generalist category with high posterior probabilities (0.776–0.984) and placed them in the central part of the generalist species area. The lower tooth of *P. henajerensis* was positioned close to that of *E. spinax.* In contrast, the upper tooth of *P. henajerensis* was closer to the durophagous feeders (Fig. 4). Based on the dental similarities with recent dalatiids, some authors (Manzanares et al., 2018; Pla et al., 2013) have suggested that pseudodalatiids fed by 'parasitic' bites, excising portions from larger oceanic animals using their narrow upper teeth to anchor to the prey while the teeth of the lower jaws sliced into them. Further specific dental microwear analyses based on a larger number of squaliform species (including Dalatiids) could provide definitive evidence to test this (possible) amazing case of evolutionary convergence. Finally, *Hybodus plicatilis* possesses multicuspidate teeth, which have large cusps with sharp edges. This morphology corresponds with clutching or tearing types (present in sharks that consume principally soft-bodied prey such as fishes, mammals and soft-bodied invertebrates) (Cappetta, 1986, 2012). Dental microwear analysis, however, assigned *H. plicatilis* teeth to the category of generalist taxa with a relatively high posterior probability (0.971), indicating that this hybodont shark could include a certain portion

of hard prey in its diet. In this sense, the robust ridges that strongly ornament the tooth cusps of *H. plicatilis* have a mechanical reinforcement function for the tooth, which could indicate an adaptation to a more generalist diet (Pla et al., 2013).

In summary, dental microwear patterns correlate with the percentage of hard prey included in the diet. Therefore, dental microwear analysis combined with information obtained from other sources (e.g., tooth morphology and stable isotope analysis) can provide more reliable information on the trophic ecology of fossil taxa. This could allow for the design of further specific analysis for particular cases (by example, for the study of basal groups of chondrichthyans (including the earliest toothed vertebrates), the split of certain lineages, etc.). Dental microwear analysis can reveal cases where the diets of fossil species deviate from those predicted by their dental morphology, depending on the availability of prey. This approach may also be very useful for palaeoecological studies if diets inferred by dental microwear analysis are compared for the same taxa in different palaeogeographic localities and at different geological ages. Moreover, as Itano (2019) proposed, dental microwear patterns could also provide information about the feeding biomechanics of extinct taxa whose cranial anatomy differs from that of the living lineages of sharks.

Conclusions

Our study reveals that analysing dental microwear using the 2D-SEM approach is a valuable method for tracking diets in elasmobranchs. Despite continuous tooth replacement, the teeth in this group remain functional long enough to record measurable dental microwear produced by tooth–food interactions during feeding. Species-level differences in prey preferences and/or oral processing mechanisms lead to significant differences in the wear patterns of their teeth. Thus, microwear variables such as scratch density and length correlate with the abrasiveness of the diet, with durophagous species displaying dental microwear patterns characterised by a greater density of shorter scratches on their tooth surface. Moreover, our study suggests that anisotropy in scratch orientation can provide information about feeding biomechanics.

Canonical variable analysis can distinguish between piscivorous, generalist and durophagous taxa with great predictive power, thus establishing a useful comparative framework for the study of fossil elasmobranchs. Therefore, fossil teeth from a Middle Triassic chondrichthyan community were included in the CVA and assigned to trophic groups with high statistical probabilities. Our

results demonstrate the potential of dental microwear analyses not only as a palaeodietary indicator but also for the assessment of the evolution of oral processing mechanisms in extinct species of chondrichthyans, the oldest lineage of living toothed vertebrates.

Additionally, following the initiatives of previous authors, we described and illustrated *post-mortem* alterations on the enameloid surfaces of chondrichthyans and compared them with previous compiled datasets of non-ingesta-related dental wear on tetrapod tooth enamels, which could be useful for future dental microwear investigations.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13358-024-00322-9.

> Supplementary Material 1.

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