
Can mandible morphology help predict feeding habits in Antarctic amphipods?

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Abstract :

In Antarctica, amphipods form a highly diverse group, occupy many different ecological niches and hold an important place in food webs. Here, we aimed to test whether differences in Antarctic amphipod feeding habits were reflected in their mandible morphology, and if mouthpart specialization could be used to describe amphipod trophic ecology. To do so, we compared mandible morphology in nine species spanning seven families and five functional groups (grazers, suspension feeders, generalist predators, specialist predators and scavengers). Mandible morphology adequately depicted some aspects of amphipod trophic ecology, such as the trophic level at which animals feed or their degree of dietary specialization. On the other hand, links between mandible morphology and amphipod diet were seldom unambiguous or straightforward. Similar adaptations were found in distinct functional groups. Conversely, mandible morphology could vary within a single functional group, and phylogenetic effects sometimes complicated the interpretation of form-function relationships. Overall, mandible morphology on its own was generally not sufficient to precisely predict amphipod feeding strategies. However, when combined with other methods (e.g. gut contents, trophic markers), it constitutes a valuable source of information for integrative studies of amphipod ecological diversity in the Southern Ocean.

Keywords : diet analysis, food webs, Peracarida, scanning electron microscopy, Southern Ocean, trophic ecology

Introduction

In Antarctic shelf ecosystems, Amphipoda constitutes, despite its relatively low biomass, a significant group in terms of energy flux (Dauby *et al.* 2001a, Dauby *et al.* 2003). These crustaceans are among the most diversified Antarctic benthic taxa with respect to number of species, lifestyles, habitats and size spectra (De Broyer & Jazdzewska 2014, Momo *et al.* 1998, Watling & Thurston 1989). Since the group is highly diverse, one would expect that amphipods occupy an important number of different ecological niches to exploit the full spectrum of resources. Previous work based on gut content examination and trophic markers has accordingly shown that Antarctic amphipods exhibit a high trophic diversity and cover a wide range of consumed preys, feeding behaviours, and eco-functional roles (Dauby *et al.* 2001a, Dauby *et al.* 2001b, Graeve *et al.* 2001, Nyssen *et al.* 2002, Nyssen *et al.* 2005). Adaptation to a wide variety of ecological niches should be reflected in the diversity of functional morphological traits. Changes in mouthpart morphology, and particularly in mandible anatomy, have notably been interpreted as adaptations to presumed preferred food sources (Momo *et al.* 1998, Watling 1993).

In amphipods, the mandible serves both for cutting and grinding (crushing) food (Manton 1977). It consists of four main structures (Fig. 1), starting distally and going to the mouth opening: the incisor process, a projection of the mandibular body that sometimes bear cusps or teeth; the *lacinia mobilis*, an articulated and sometimes toothed plate, inserted close to and generally in line with the incisor; the seta row, filling the space between incisor and molar, and presumably preventing food particles from falling away from the molar; and the molar process, a columnar structure projecting from the mandibular body and often provided with multiple tritulative surfaces (Watling 1993; Fig. 1). In typical peracarid biting, the left and right mandibles have distinct roles. The right incisor process enters the gap between the left incisor and the left *lacinia mobilis*, which glides into the gap left between the right incisor and the right *lacinia*, when the latter is present. The *lacinia mobilis* contributes to cutting, and helps to guide the incisor processes into the right planes and to lock them into their final closing position. Also, a toothed or spiny *lacinia mobilis* probably helps to hold food particles in place during the bite (Dahl & Hessler 1982, Watling 1993). Due to their different role in feeding, asymmetry between left and right mandibles is commonly observed in amphipods. The left *lacinia mobilis*, notably, is often larger, stronger and/or bears more ornamentation than the right one (Mayer *et al.* 2013, Watling 1993). Besides this, a large panel of modifications to the abovementioned typical mandible morphology pattern can be observed among amphipods. They include reduction or overgrowth of the incisor process, disappearance of the setae row, and reduction or complete loss of the molar process (Watling 1993).

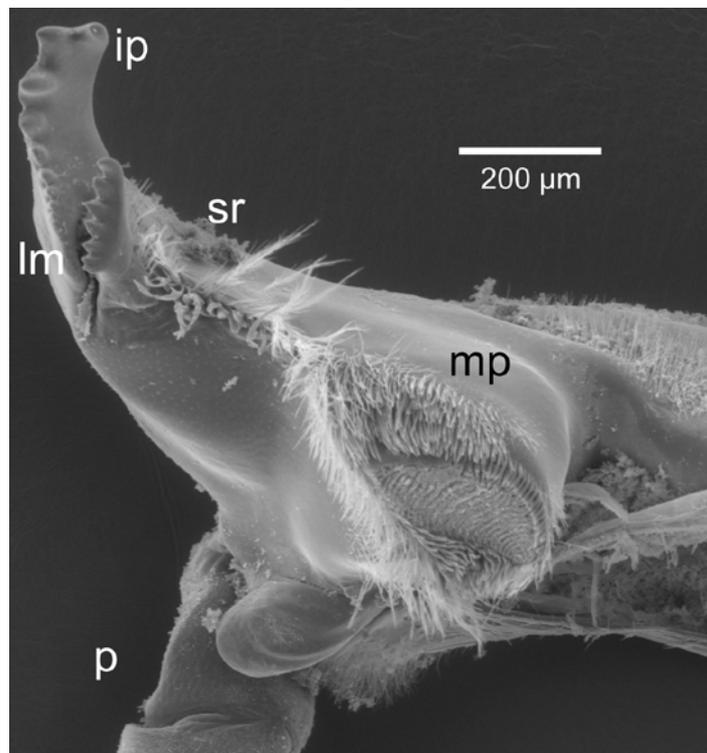


Figure 1: Structure of a typical gammaridean amphipod mandible. ip: incisor process, mp: molar process, lm: *lacinia mobilis*, p: mandibular palp, sr: setae row.

Many studies have suggested associations between specific mouthpart morphologies and feeding strategies (Arndt *et al.* 2005, Coleman 1989a, Coleman 1989b, Coleman 1990, Mayer *et al.* 2009, Mekhanikova 2010, Pavesi & Olesen 2017). The “basic” amphipod morphology, observed in most gammaridean families, is typically associated with detritivory and with suspension and/or deposit feeding. The compact mandible body bears a toothed incisor, a well-developed and toothed *lacinia mobilis* and a columnar molar, designed for crushing (Watling, 1993). Filter-feeders often combine this basic mandible morphology with an increased mouthpart setation (Caine 1974, Cole & Watkins 1977, Mayer *et al.* 2009). Detritus feeders which primarily feed by processing benthic sediments or biofilms have proportionally larger and more strongly dentate mandibles, and increased molar grinding surfaces, suggesting an adaptation to mastication. Moreover, they have teeth and cuspidate setae on maxillae and maxillipeds for scraping off adherent food from substrates (Arndt *et al.* 2005, Caine 1974, Coleman 1991, Mayer *et al.* 2009). Enlarged, strong, toothless and sharp incisors are generally associated with increasingly predatory feeding habits (Coleman 1990, Guerra-García & Tierno de Figueroa 2009, Haro-Garay 2003, Hutchins *et al.* 2014). Predators feeding on relatively soft prey (e.g. some cnidarians, sponges, holothurians or polychaetes) appear to share an absent or greatly reduced molar (Coleman 1989a, Coleman 1989b, Coleman 1990, Guerra-García & Tierno de Figueroa 2009, Watling 1993), whereas a broader and triturative molar is presumably used to crush more rigid food items, such as crustacean exoskeletons (Caine 1974, Haro-Garay 2003, Sainte-Marie 1984). Highly adapted scavenger species generally combine wide, sharp and toothless shearing incisors (to bite off large pieces of carrion) with tall, conical, posteriorly projecting, non-triturative molars, which push pieces of food into the stomodeum without chewing (Arndt *et al.* 2005, Dahl 1979, Sainte-Marie 1984, Seefeldt *et al.* 2017, Steele & Steele 1993, Watling 1993). Facultative scavengers often show some degree of omnivory, and appear to have a somewhat distinct mandible morphology compared to exclusive scavengers, characterized by a slender incisor and a lower triturative molar process (Arndt *et al.* 2005, Dahl 1979, Momo *et al.* 1998, Seefeldt *et al.* 2017). Finally, herbivorous habits are generally associated with sturdy and sharp incisors combined with wide and highly structured (rasp-like) molars, as this mandible morphology is well-suited for cutting and grinding firm plant material (Mayer *et al.* 2009, Pavesi & Olesen 2017, Watling 1993).

Overall, amphipod mouthpart morphology seems to be correlated with their trophic ecology. However, the functional significance of many morphological traits or modified structures in amphipods is still incompletely understood. Here, we aimed to test whether differences in feeding habits were reflected in the morphology of the mandible in multiple Antarctic amphipod groups. To do this, we examined mandible morphology in nine species spanning seven families and five functional groups (grazers, suspension feeders, generalist predators, specialist predators and scavengers; table 1), and related our findings to available information on their feeding ecology (generated by gut content analyses, stable isotopes and fatty acid trophic markers). We hypothesized that mandible morphology and feeding habits would be closely related, and that the mandible should show strong morphological differences between species belonging to different functional groups or having different feeding habits, while mouthparts of species showing similar feeding habits would bear more resemblance.

Material and Methods

Specimens of six species (table 1) were collected with Agassiz, benthopelagic and bottom trawls, and dredges in the eastern Weddell Sea during three Antarctic summer cruises of RV Polarstern: EPOS leg 3 (1989), EASIZ I (1996) and EASIZ II (1998). Individuals of *Oradarea walkeri* and *Djerboa furcipes* were sampled on King George Island (South Shetland Islands, Antarctic Peninsula) in 1993. They were either hand-collected in the upper part of the infralittoral of Admiralty Bay during spring tides, or dredged in shallow water in the vicinity of the Henryk Arctowski Polish Antarctic station during the summer season. Finally, *Oradarea* sp. were also collected on King George Island during the summer of 1993, but at depths of 400 metres, using traps baited with different macroalgae (*Desmarestia menziesii* or *Iridaea* sp.).

For each species, a single adult (see body sizes in table 1) female specimen was selected for mouthpart morphology examination. Animals preserved in 10% formalin were dissected under a binocular microscope (Leica MZ12), using forceps and scissors. After dissection, amphipod mandibles were dehydrated through an alcohol series, critical point-dried and sputter-coated with carbon then gold to be observed by scanning electron microscopy (SEM).

Table 1: Families, functional groups (according to literature), sampling locations and specimen size for the 9 studied amphipod species

Species [Family]	Functional group	Sampling location	Size (mm)
<i>Ampelisca richardsoni</i> Karaman, 1975 [Ampeliscidae]	Suspension feeder (Dauby <i>et al.</i> 2001a, Dauby <i>et al.</i> 2001b, Graeve <i>et al.</i> 2001, Nyssen <i>et al.</i> 2002, Nyssen <i>et al.</i> 2005)	Eastern Weddell Sea	22
<i>Epimeria cf. similis</i> Chevreux, 1912 [Epimeriidae]	Cnidarian predator (Dauby <i>et al.</i> 2001a, Dauby <i>et al.</i> 2001b, Graeve <i>et al.</i> 2001, Nyssen <i>et al.</i> 2002) Omnivore (Nyssen <i>et al.</i> 2005)	Eastern Weddell Sea	36
<i>Iphimediella cyclogena</i> K.H. Barnard, 1930 [Iphimediidae]	Holothurian predator (Nyssen <i>et al.</i> 2002)	Eastern Weddell Sea	35
<i>Eusirus perdentatus</i> Chevreux, 1912 [Eusiridae]	Generalist predator (Dauby <i>et al.</i> 2001a, Dauby <i>et al.</i> 2001b, Graeve <i>et al.</i> 2001, Nyssen <i>et al.</i> 2002, Nyssen <i>et al.</i> 2005)	Eastern Weddell Sea	38
<i>Parschisturella carinata</i> (Schellenberg, 1926) [Uristidae]	Scavenger (Nyssen <i>et al.</i> 2002)	Eastern Weddell Sea	20
<i>Uristes murrayi</i> Walker, 1903 [Uristidae]	Scavenger (Dauby <i>et al.</i> 2001a, Nyssen <i>et al.</i> 2002)	Eastern Weddell Sea	25
<i>Djerboa furcipes</i> Chevreux, 1906 [Pontogeneiidae]	Macroherbivore / grazer (Nyssen <i>et al.</i> 2002)	King George Island	19
<i>Oradarea walkeri</i> Shoemaker, 1930 [Calliopiidae]	Macroherbivore / grazer (Graeve <i>et al.</i> 2001; This study)	King George Island	19
<i>Oradarea</i> sp. [Calliopiidae]	Macroherbivore / grazer (Graeve <i>et al.</i> 2001; This study)	King George Island	25

To assess congruence between mouthpart specialization and feeding habits, mandible morphology was compared to trophic ecology as depicted by feeding behaviour observations, gut content analyses and trophic markers (stable isotope ratios, lipid and/or fatty acid analyses). Gut content data for *Ampelisca richardsoni*, *Djerboa furcipes*, *Epimeria cf. similis*, *Eusirus perdentatus*, *Iphimediella cyclogena*, *Parschisturella carinata* and *Uristes murrayi* were extracted from literature (Aumack *et al.* 2017, Dauby *et al.* 2001a, Dauby *et al.* 2001b, Graeve *et al.* 2001, Nyssen *et al.* 2002). Since, at the time the study was performed, no gut content data were available for *O. walkeri*, *Oradarea* sp., or *D. furcipes*, they were analysed for this study. For 20 specimens of each species, the digestive tract was cut at the oesophagus level and removed together with the midgut glands. It was then separated from the midgut glands, opened, and its content was spread on a microscope slide. Slides were examined under an optical microscope (Leica DMLB equipped with reflection contrast system). Feeding behaviour observations were taken from Klages & Gutt (1990) and Dauby *et al.* (2001b); stable isotope ratios analyses from Aumack *et al.* (2017), Nyssen *et al.* (2002) and Nyssen *et al.* (2005); and lipid and/or fatty acid composition analyses from Aumack *et al.* (2017), Graeve *et al.* (2001) and Nyssen *et al.* (2005). Since no trophic markers measurements were, to the best of our knowledge, available for *Oradarea* sp. or *O. walkeri*, we compared their mandible morphology with results obtained on their congeners *O. edentata* (Graeve *et al.* 2001) and *O. bidentata* (Aumack *et al.* 2017).

Results

Both mandibles of *Ampelisca richardsoni* (Fig. 2a, b) bore well-developed incisor and molar processes. Both incisors presented five rounded cusps, and the left one (Fig. 2a) was ornamented by a strong five-toothed *lacinia mobilis*. The right *lacinia mobilis* was smaller and more spine-like (Fig. 2b). The molars were prominent and some smooth ridges were visible. The most striking feature of those mandibles was the development of the setae rows: on both sides, the row was composed of about 15 serrate setae nearly as long as the *lacinia mobilis*.

Gut composition of *Oradarea* sp. depended drastically on the used macroalgal bait. When they were made of pieces of the brown algae *Desmarestia menziesii*, 95% of the gut volume was filled with fragments of this algae. On the other hand, when baits consisted of pieces of *Iridaea* sp., the gut content was composed of mineral particles, of frustules of different kind of diatoms, of chitinous structures and, in some specimens, of a small amount of pieces of *Iridaea* sp. Mandibles were asymmetrical regarding the morphology of the *lacinia mobilis* (Fig. 2c, d). Incisor bore ten rounded cusps. The left *lacinia* (Fig. 2c) was similar to the incisor but smaller and with only seven more acute cusps. The right *lacinia* (Fig. 2d) was slender. The setae row consisted only of stout serrate setae (Fig. 2d) on the right mandible, while an additional parallel row of plumose slender setae was present on the left one (Fig. 2c). The molar process was massive, sub-columnar, and the triturative area was closely surrounded by short and thick setae and topped by a dense crown of setae. The grinding surface of the molar process was provided with densely set short spinules and ridges with serrate distal margins (Fig. 2e).

Ninety percent of gut contents of *Oradarea walkeri* were composed of pieces of macroalgae. The 10% left consisted of different types of diatoms and of inorganic material. Mandible morphology was nearly identical to the one described for the other *Oradarea* species (Fig. 2f). Both incisors were well-developed and bore ten strong teeth. The rather flat mandibular body was bordered ventrally by a double row of stout serrate setae and of slender plumose setae. The large and tall molars were topped by a dense fringe of setae and bore a large triturative area provided with ridges and other rasp-like structures. Both mandibles were flanked with a *lacinia mobilis*, which was strong and seven-toothed on the left mandible (Fig. 2f) and weaker and spine-like on the right one (not shown).

Examination of gut contents of *Djerboa furcipes* revealed that they were composed of more than 85% of pieces of macroalgae, 10% of pennate diatoms and a small amount of chitinous parts. All dissected specimens also had mineral particles in their guts. As for both species of the genus *Oradarea*, *D. furcipes* displayed basic gammaridean amphipod mandibles (*sensu* Watling 1993; Fig. 2g). They had ten-toothed incisors, a left *lacinia mobilis* with ten teeth, but only three on the right one (Fig. 2g), large (yet smaller than in the genus *Oradarea*) cylindrical and triturative molars crowned by a dense fringe of small setae, and the setae row, like in *O. walkeri*, was composed of two different types of setae (plumose and serrate).

Both mandibles of *Epimeria cf. similis* (Fig. 3a, b) bore strong ten-toothed incisors and tall sub-columnar molars provided with well-developed and smooth ridges. The molars were surrounded by a row of stubby setae and fringed with bundles of hair-like setae at their dorsal margin. The left *lacinia mobilis* (Fig. 3a)

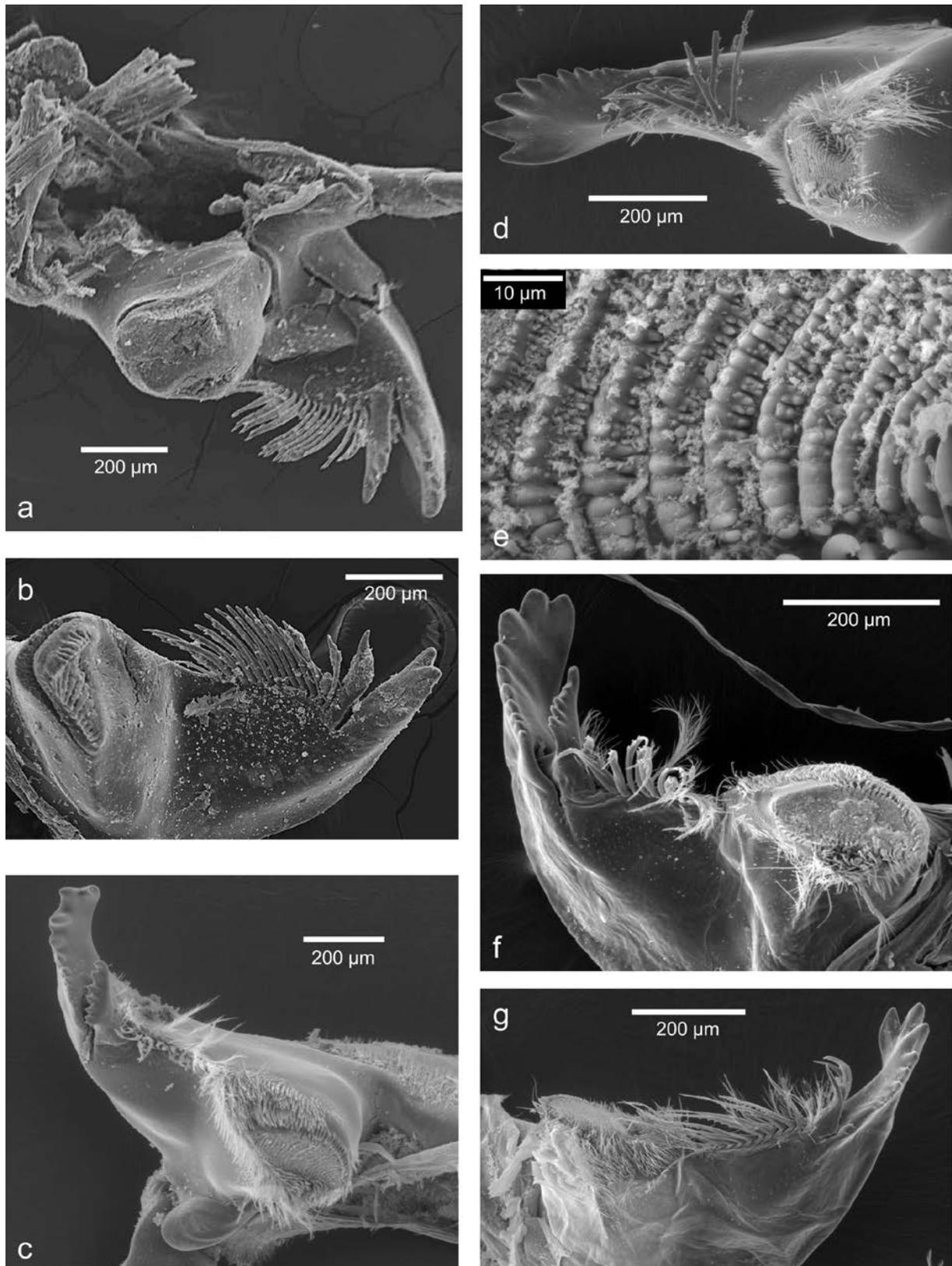


Figure 2: SEM photographs of mandibles of studied amphipods (primary consumers). a: *Ampelisca richardsoni*, left mandible. b: *Ampelisca richardsoni*, right mandible. c: *Oradarea* sp., left mandible. d: *Oradarea* sp., right mandible. e: *Oradarea* sp., details of molar process. f: *Oradarea walkeri*, left mandible. g: *Djerboa furcipes*, left mandible.

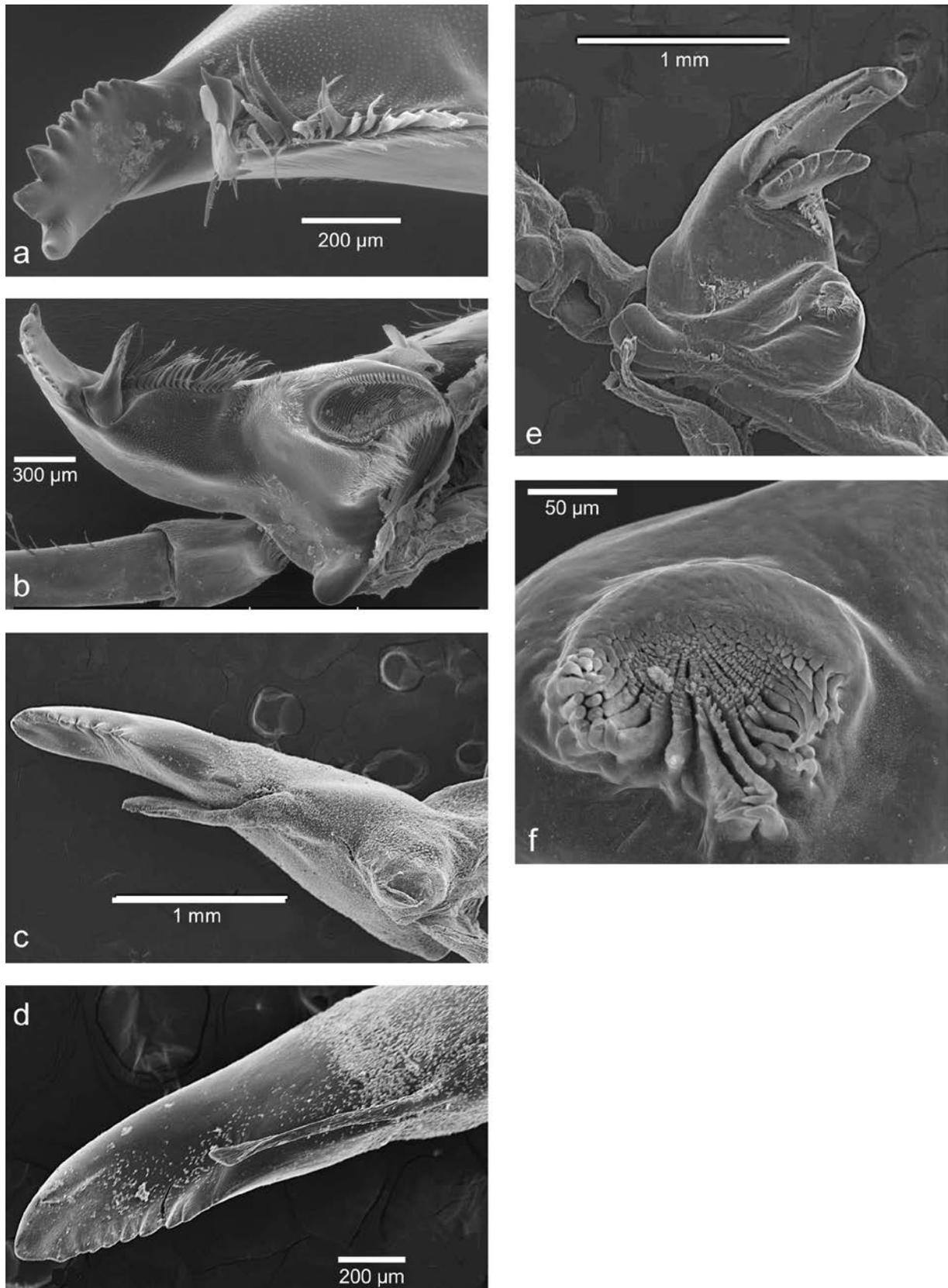


Figure 3: SEM photographs of mandibles of studied amphipods (predators). a: *Epimeria* cf. *similis*, left mandible. b: *Epimeria* cf. *similis*, right mandible. c: *Iphimediella cyclogena*, left mandible. d: *Iphimediella cyclogena*, details of right *lacinia mobilis*. e: *Eusirus perdentatus*, left mandible. f: *Eusirus perdentatus*, details of molar process.

was armed with seven cusps, whereas the right one was smaller, bifurcated and spine-like (Fig. 3b). The setae row was composed by stout denticulate setae, flanked on the ventral side by slender setae. *Iphimediella cyclogena*'s mandibular body was elongate, tapering into the incisor that was much narrower than in all other species considered in this work (Fig. 3c). Both incisor processes were toothed (ten cusps) and bore a long *lacinia mobilis* that was inserted close to the molar process, which was reduced to a small fleshy cone. The left *lacinia mobilis* (Fig. 3c) was thick and strongly chitinised, but the right one (Fig. 3d) was reduced to a thinner twiggy structure. A major modification was the change in the orientation of the incisor, cutting in the vertical front plane.

Eusirus perdentatus' mandibles bore strong incisors and molar processes (Fig. 3e) The incisor was wide and smooth, flanked by a single cusp at each end. The left *lacinia mobilis* was denticulated and seven-toothed (Fig. 3e), whereas the right one was armed with just two teeth. The short setae row was composed of about ten setae. The apex of the tall sub-columnar molar bore a small tritulative area with rasp-like structures (Fig. 3f).

The proximal parts of *Uristes murrayi*'s incisors were narrow, and the structures were broadening distally (Fig. 4a). The cutting edge was smooth and flanked by a single cusp at each end. The *lacinia mobilis* was present on both mandibles. The setae row was composed of three setae, followed by a dense string of hairy setae bordering the mandibular body. The molar was oval-shaped and surrounded by a ventral fringe of setae that partly overlapped the relatively smooth tritulative area (Fig. 4a).

The incisor processes of *Parschisturella carinata* were smooth, and its edge was rather sharp and flanked by a single cusp at each end (Fig. 4b). The slightly concave mandibular body was bordered by a row of seven or eight thick setae. The left mandible bore a weak digitiform, apically bidentated *lacinia mobilis* (Fig. 4b). Molars were tall and ornamented with setae. The oval tritulative area bore several series of deeply toothed ridges and rasp-like structures, and was more developed than in *U. murrayi* (Fig. 4c).

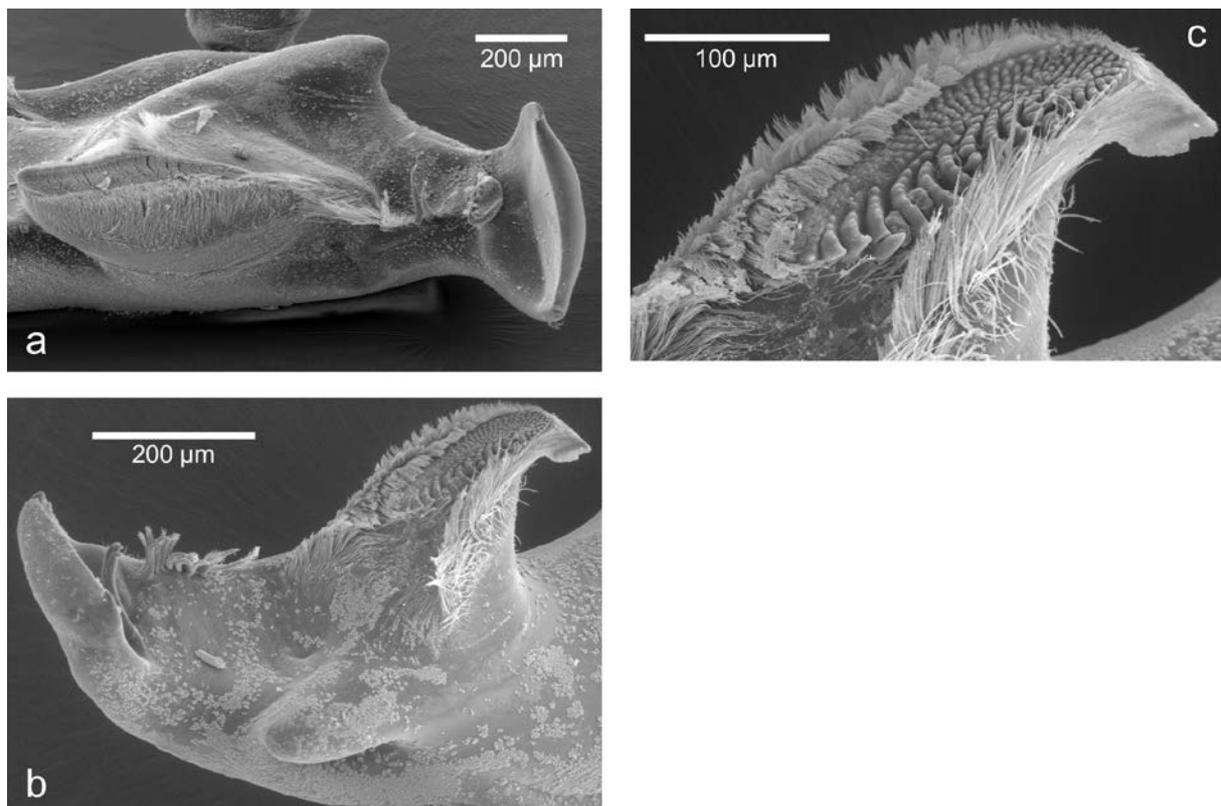


Figure 4: SEM photographs of mandibles of studied amphipods (scavengers). a: *Uristes murrayi*, right mandible. b: *Parschisturella carinata*, left mandible. c: *Parschisturella carinata*, details of molar process.

Discussion

Macroherbivores / grazers

While our study is, to the best of our knowledge, the first one to investigate feeding habits from *Oradarea* sp. and *Oradarea walkeri*, gut contents and lipid analyses suggested that *Oradarea edentata* showed a high, year-round reliance on macroalgal tissues (Graeve *et al.* 2001). Joint use of gut contents, stable isotopes and fatty acid trophic markers pointed out that *Oradarea bidentata* was also a primary consumer, and that it relied not only on macroalgae, but also on their epiphytic diatoms and endo/epiphytic filamentous algae (Aumack *et al.* 2017). The two *Oradarea* species studied here, whose guts mostly contained macroalgal fragments and/or diatom frustules, seem to have feeding habits that are comparable with their congeners. Interestingly, *Oradarea* sp. had specific grazing preferences. It fed avidly on *Desmarestia menziesii*, but did not consume *Iridaea* sp. This preferential consumption could be linked with the ability of red algae from the genus *Iridaea* to develop chemical defences against herbivores (Amsler *et al.* 1998). *O. bidentata* was also found to have distinct dietary preferences when sampled in association with Desmarestiales or red algae (Aumack *et al.* 2017).

Aumack *et al.* (2017) analysed gut contents of *Djerboa furcipes*, and found it ingested items similar to the ones we observed here (macroalgae, diatoms and crustacean parts), albeit in different proportions. This might point out that some dietary plasticity exists in this species. Nevertheless, our gut contents results are consistent with this species mostly consuming living or recently dead macroalgal material (Aumack *et al.* 2017). Combined use of stable isotopes and fatty acid markers confirmed that *D. furcipes* held a low trophic position, and mostly relied on macroalgae for its nutrition (Nyssen *et al.* 2005).

For feeding on live macrophytes and/or fresh macrophyte detritus, it is necessary to possess structures for biting off pieces of plant material and for grinding it prior to ingestion. The mandibles of *Oradarea* sp., *Oradarea walkeri* and *Djerboa furcipes* were very similar to one another and to those of other previously described herbivorous species (e. g. Mayer *et al.* 2009, Pavesi & Olesen 2017), and all appear well-suited for this kind of feeding. All these species have incisors with a sharp dentition, which may facilitate cutting pieces off firm plant material (Mayer *et al.* 2009, Pavesi & Olesen 2017). The left mandible of the three species also had a strong, sharply denticulate *lacinia mobilis* that probably works as an additional cutting edge (Coleman 1989b, Pavesi & Olesen 2017). The setal row was well-developed, constituted of a range of numerous serrate and plumose setae in all three species. The molars of the three grazers, as other herbivorous species, were structured like 'rasps', i.e. ornamented with a dense concentration of strongly denticulate/spinous ridges (see Fig. 2e; *Oradarea* sp. molar details). This has been interpreted as a mechanism to triturate hard particles such as tough macroalgal tissues (Mayer *et al.* 2009, Momo *et al.* 1998, Pavesi & Olesen 2017, Watling 1993).

Suspension feeder

Gut contents of *Ampelisca richardsoni* contained mostly planktonic items, notably diatoms (Dauby *et al.* 2001a, Dauby *et al.* 2001b). Lipid markers suggested that phytoplanktonic organisms (including but not limited to diatoms) made up most of this species' diet (Graeve *et al.* 2001, Nyssen *et al.* 2005). Stable isotopes confirmed that this species occupied a low trophic position (2.1, Nyssen *et al.* 2002). Overall, *A. richardsoni* appeared to be a primary consumer relying mostly on the water column for its nutrition.

Generally speaking, denser setation of the mouthparts and other feeding-related appendages (antennae, gnathopods) has been linked to a greater trend towards filter-feeding (Caine 1974, Caine 1977). Small particles in the water column are indeed collected passively or actively by setiferous antennae, which are cleaned by gnathopods and transferred to the mouthparts (Caine 1974, Caine 1977). The setae row between the molar and the incisor is used to retain suspended organic matter collected from the surrounding water; and is thereby well-developed in filter-feeders (Dixon & Moore 1997, Poltermann 2001). In *A. richardsoni*, the two dense setae rows were composed of long serrate setae which join together when the mandible closes, thereby forming a kind of bridge between the tip of the mouthparts and the mouth itself. This appears particularly well-suited for retaining particles. Well-developed molar processes with blunt lamelliform cusps can be used to masticate hard material, such as diatom shells, that may be present in marine snow (Caine 1974, Haro-Garay 2003). *A. richardsoni* possessed such well-developed and structured molars, although the ridges were fewer and blunter than what is observed in herbivorous species. This is consistent with the fact that smaller planktonic items require less processing prior to ingestion than tough algal tissues. On the other hand, the incisor of filter-

feeders — *A. richardsoni* included — although similarly toothed, was less elongated and not as broad as the grazers' incisors, a trend that may be indicative of a reduced need for cutting.

Generalist predator

Feeding behaviour observations suggested that *Eusirus perdentatus* acted as a "sit-and-wait" passive predator (Dauby *et al.* 2001b, Klages & Gutt 1990). Crustacean hard parts and pieces of polychaetes formed more than half of its gut contents, which also contained unidentifiable organic and inorganic material (Dauby *et al.* 2001a, Dauby *et al.* 2001b). Stable isotopes confirmed the status of *E. perdentatus* as a predator, with a trophic position of 3.0 (Nyssen *et al.* 2005). It showed no particular specialization in terms of lipid or fatty acid compositions, suggesting that the species feeds on a wide range of items (Graeve *et al.* 2001, Nyssen *et al.* 2005). This was corroborated by the important variability of its carbon isotopic ratios (Nyssen *et al.* 2002). Overall, *E. perdentatus* seems to be a generalist predator, able to switch between dietary items (crustaceans, polychaetes), possibly according to food availability.

Wide and sharp, usually toothless ('axe-like') incisors with one broadened *lacinia mobilis*, columnar molars with a generally reduced triturative area, and a lower number of small-sized setae have all been interpreted as adaptations to feeding on animal prey (Haro-Garay 2003). *E. perdentatus*' mandibles showed these typical characteristics of predators. However, apart from these general trends, there seems to be a great degree of variation involved in the form of exclusively predatory mandibles, likely linked to prey nature and size (Watling 1993). Stout molars have also been associated with predatory habits — notably in opportunistic scavengers which also rely on active predation — and could be used to crush masses of soft food, such as decaying tissue, but also to break microzooplankton exoskeletons (Haro-Garay 2003, Sainte-Marie 1984). Predators' mandible morphology also appears to vary with the size of the animal, which reflects the size fraction of preys it can potentially exploit (Arndt *et al.* 2005). Wider incisors and taller molars with a more reduced triturative surface would occur in larger species/individuals (Haro-Garay 2003). *E. perdentatus*, as one of the largest Antarctic amphipod species (up to 75 mm), appeared to follow this trend. Some other opportunistic predators also present a toothed incisor, which may indicate that they feed on softer preys, such as gelatinous plankton, or could be indicative of a lower reliance on predation, i.e. an increased tendency to omnivory (Haro-Garay 2003).

Cnidarian predator / omnivore

Gut content analyses of *Epimeria cf. similis* specimens suggested that this species was a specialist predator of cnidarians (Dauby *et al.* 2001a, Dauby *et al.* 2001b, Nyssen *et al.* 2002, Nyssen *et al.* 2005). Their stomach content was indeed composed of 63% cnidarian remains on average — although other items such as sponge spicules, microalgae and pieces of polychaetes were identified as well (Dauby *et al.* 2001a, Dauby *et al.* 2001b). However, the presence of sediments in its guts also suggested partial reliance on deposit-feeding, and the fatty acid composition suggested assimilation of sediment-associated micro-organisms (Nyssen *et al.* 2005). In addition, stable isotope analyses pointed out that this species relies on a large spectrum of organic matter sources (Nyssen *et al.* 2002, Nyssen *et al.* 2005).

Overall, insights from multiple methods suggest that *E. cf. similis* might be an omnivore rather than a strict cnidarian predator. Examination of its mandibles corroborated this view. Their morphology was indeed basic, and showed no modifications that could be associated with a specialized regime. This is consistent with an omnivore having a wide prey spectrum. Mandibles were very similar to those of other omnivorous species (Arndt *et al.* 2005, Mayer *et al.* 2009, Mekhanikova 2010), which feature roughly toothed incisors and *lacinia mobilis* used to tear apart food items, and a strong and ridged or rasp-like molar, able to reduce food morsels to small pieces and crack and grind even hard plant material and diatom shells (Arndt *et al.* 2005). Conversely, mandibles of specialized cnidarian micropredators such as *Maxilliphimedia longipes* (Coleman 1989b), *Andaniexis* sp. or *Stenothoe brevicornis* (Moore *et al.* 1994) seem adapted to take large bites of soft tissues and ingest food as large whole lumps. As such, the latter species bear an enlarged incisor cutting blade with very small or no teeth, for cutting and slicing soft food, and when a molar is present, it is reduced or soft, without any grinding surface (Moore *et al.* 1994).

Holothurian predator

Stable isotopes suggested that the iphimeriid *Iphimeriella cyclogena* is a predator (trophic position: 3.7; Nyssen *et al.* 2002). Food items observed in its guts were mainly holothurian ossicles (70%), and to a lesser extent, polychaetes remains and plankton (Nyssen *et al.* 2002). This led the authors to conclude that this species is a predator specialized in holothurians (Dauby *et al.* 2001b, De Broyer *et al.* 1999, Nyssen *et al.* 2002). Holothurians are a quite uncommon food source for invertebrates, because their integument is strong and leathery, with embedded ossicles. Few amphipod species are known to feed on holothurians (Dauby *et al.* 2001b) and a single one was recognized to date as a specialized predator of this unusual prey, the Stilipedidae *Alexandrella schellenbergi* (previously *Bathypanoploea schellenbergi*; Coleman 1990).

The mouthparts of *A. schellenbergi* are highly adapted to cope with the tough tissues of these echinoderms (Coleman 1990). Its mandibles are remarkably stout, with strongly serrate incisors and left *lacinia mobilis*, providing three strong edges to cut through the firm body wall of holothurians (Coleman 1990). Although gut contents suggested that *I. cyclogena* mainly relied on the same food source as *A. schellenbergi*, its mandible morphology presents some notable differences (Fig. 3j, k). Its mandibular body was more elongated, with much narrower incisors and a smooth *lacinia mobilis* on both sides. These differences could be explained by a change in the orientation of the cutting plane. Unlike *A. schellenbergi*, which possesses transversely-oriented incisors, the incisor of most iphimeriids — including *I. cyclogena* — cuts in the vertical frontal plane (Watling & Thurston 1989). The longitudinally-oriented toothed cutting edge of *I. cyclogena*'s incisor (Fig. 3j, k) accordingly suggested that it cuts in a scissor-like manner. Because of this change in orientation, to offer a longer cutting edge, the incisor of *I. cyclogena* does not have to be wide, like in *A. schellenbergi*, but rather elongated. In both species, molars were reduced and without any grinding surface, but incisors presented many teeth. This suggests that reduction of food in small pieces relies solely on the action of the incisors and *lacinia mobilis* (Coleman 1990).

Scavengers

Gut contents suggested that *Uristes murrayi*'s diet was mostly made of carrion (Nyssen *et al.* 2002). Nevertheless, their guts also contained crustacean and polychaete remains, and, when kept in tanks, this species could exhibit active predation behaviour (Dauby *et al.* 2001b). Likewise, in aquaria, *Parschisturella carinata* readily fed on any dead material provided, without preference, but crustacean remains could also be found in the guts of this species (Dauby *et al.* 2001b). Stable isotopes ratios confirmed that these two species occupied high trophic positions (3.8 and 3.9, respectively), which is consistent with scavenging behaviour (Nyssen *et al.* 2002). Furthermore, their isotopic ratios were close for both carbon and nitrogen (Nyssen *et al.* 2002), suggesting they might rely on similar resources.

Many Lysianassoidea amphipods are highly mobile scavengers, and have developed morphological, physiological and behavioural traits in relation with this lifestyle. Scavenging amphipods were previously classified in two distinct functional groups. The first is composed of voracious, rapid feeders, processing food in large bites that they swallow directly into the oesophagus. These usually larger species possess large and flexible guts and can survive long starvation periods (Seefeldt *et al.* 2017, Steele & Steele 1993). Their mandibles are therefore adapted to take off large bites of muscular food items. They have a bowl-shaped and enlarged mandibular body, a wide and sharp shearing incisor edge and a molar that is either non-tritulative (like a 'setose tongue'), or only bears a vestigial patch of tritulative area (Dahl 1979, Sainte-Marie 1984). Such species' molars therefore do not have a grinding function, but can serve to push pieces of food into the stomodeum when the mandible is adducted (Steele & Steele 1993). Members of the second functional group have a continuous and less rapid feeding behaviour, and likely do not survive long starvation periods (Seefeldt *et al.* 2017). The mandible of these usually smaller species bears a slender incisor process and a tritulative molar (Arndt *et al.* 2005, Momo *et al.* 1998, Sainte-Marie 1984, Seefeldt *et al.* 2017). The development of the molar suggests that these animals rely on a broader dietary regime (Sainte-Marie 1984). This group would therefore be composed of more facultative scavengers, which could also act as predators and/or deposit-feeders (Seefeldt *et al.* 2017). Here, both *Uristes murrayi* and *Parschisturella carinata* appeared to belong to this second functional group, as previous ecological studies suggested that they are also able to prey on live animals (Dauby *et al.* 2001b). Accordingly, they both displayed the typical mandible morphology of this group: a wide, smooth and sharp incisor combined with a tritulative molar (Fig. 4n, o).

Conclusions

For each of the nine Antarctic amphipod species presented here, mandible morphology seemed to be, to some extent, congruent with what is known of their feeding habits. This tends to confirm the links between mandible morphology and feeding strategies that were previously suggested for amphipods from other regions. These trends seem consistent across a wide variety of taxa and/or at large spatial scales, suggesting that mandible morphology can be a good descriptor of some aspects of trophic ecology. For example, differences in mouthpart morphology are usually seen between primary (herbivores and/or microphages) and higher trophic level consumers (predators and/or scavengers). Similarly, mandible morphology can help to discriminate between more generalist or specialist feeding strategies. The latter is exemplified in this study by the case of *E. cf. similis*, for which the absence of mandible modifications that could be associated with a specialized diet seems consistent with findings from other methods classifying this species as an omnivore with wide prey spectrum.

On the other hand, links between mandible morphology and amphipod diet were seldom unambiguous or straightforward. Similar adaptations were found in distinct functional groups. For instance, grazers, suspension feeders and omnivores all showed very similar mandible morphologies, close to the basic plan. Conversely, a great variability of mandible morphologies can also be found within the same functional group, as observed for generalist predators. Characters states related to the mode of nutrition are plastic and may be independently lost or acquired in the course of evolution (Rüber *et al.* 1999), and phylogeny can have an obfuscating effect on form-function relationships (Hutchins *et al.* 2014). In the case of *I. cyclogena*, a change in the orientation of the cutting plane during the evolution of iphimeriids led to a mandible morphology very different from another unrelated species using the same food source, which can be misleading if phylogenetic effects are not taken into consideration. Finally, in some cases, dietary specialization can be linked with marked changes in the morphology of other mouthparts and/or accessory feeding appendages (e.g. antennae or gnathopods). For instance, the main characteristic of filter-feeders' mandible, differentiating it from grazers or omnivores showing a similar basic morphology, is the increased setation, which is more visible on other feeding-related body parts than on the mandible (Arndt *et al.* 2005, Cole & Watkins 1977, Mayer *et al.* 2009).

Overall, in many cases, mandible morphology on its own could not precisely predict amphipod feeding strategies. Like all trophic ecology methods, such as behavioural observations, gut content analysis or trophic markers, it possesses its own strengths and limitations. Nevertheless, when used in combination with some of the abovementioned methods, morphological observations of mouthparts proved to be able to complement them efficiently, by shedding light on specific issues. As such, it could constitute a valuable source of information for integrative studies of amphipod ecological diversity, in the Southern Ocean and beyond.

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