SYMPOSIUM

Soft Bodies, Hard Jaws: An Introduction to the Symposium, with Rotifers as Models of Jaw Diversity

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From the symposium "Soft Bodies, Hard Jaws: Phylogenetic Diversity of Prey Capture and Processing in Jawed, Soft-bodied Invertebrates" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2015 at West Palm Beach, Florida.

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Synopsis Jaws have evolved numerous times in the animal kingdom and they display a wide variety of structural, compositional, and functional characteristics that reflect their polyphyletic origins. Among soft-bodied invertebrates, jaws are known from annelids, chaetognaths, flatworms, gnathostomulids, micrognathozoans, mollusks, rotifers, and several ecdysozoans. Depending on the taxon, jaws may function in the capture of prey (e.g., chaetognaths and flatworms), processing of prey (e.g., gnathostomulids and onychophorans), or both (e.g., rotifers). Although structural diversity among invertebrates' jaws is becoming better characterized with the use of electron microscopy, many details remain poorly described, including neuromuscular control, elemental composition, and physical characteristics, such as hardness and resistance to wear. Unfortunately, absence of relevant data has impeded understanding of their functional diversity and evolutionary origins. With this symposium, we bring together researchers of disparately jawed taxa to draw structural and mechanistic comparisons among species to determine their commonalities. Additionally, we show that rotifers' jaws, which are perhaps the best-characterized jaws among invertebrates, are still enigmatic with regard to their origins and mechanics. Nevertheless, technologies such as energy dispersive X-ray spectroscopy (EDX) and 3D modeling are being used to characterize their chemical composition and to develop physical models that allow exploration of their mechanical properties, respectively. We predict that these methods can also be used to develop biomimetic and bioinspired constructs based on the full range of the complexity of jaws, and that such constructs also can be developed from other invertebrate taxa. These approaches may also shed light on common developmental and physiological processes that facilitate the evolution of invertebrates' jaws.

Introduction

What *are* jaws? Such a simple question does not always have a clear answer. A basic search of the Internet reveals an extensive and varied fascination with jaws, including sharks, maxillofacial pathologies, and the mechanics and evolution of mammalian jaws and dentition. Naturally, human tendency is to study what we see most often, thus teeth, bones, joints, and muscles are what most of us picture when hearing the word jaw. Consequently, the vertebrate jaw has received a great deal of scientific attention. Its importance in craniate evolution and ecology cannot be overestimated, and it remains a relevant research avenue that has provided valuable insights into

topics such as the mechanics of soft and hard tissues (Sanchez et al. 2013; Trinajstic et al. 2013), developmental patterning (Cerny et al. 2010; Fish et al. 2011; Medeiros and Gage Crump 2012; Kuratani et al. 2013), the evolution of redundancy in physiological systems (Alfaro et al. 2005; Parnell et al. 2008; Holzman et al. 2011), and the correlations between functional complexity and ecology (Parnell et al. 2012). Comparable studies on invertebrates' jaws are lacking despite observations that morphological and functional complexity of such jaws can be extremely intricate (Kristensen and Funch 2000; Sørensen 2002a, 2002b; Paxton 2009), and are therefore predicted to provide a great deal of explanatory

power into the relationships among structure, function, and the use of resources in a wide diversity of animal taxa.

Thus, the original question stands—what are jaws, and can invertebrates be said to possess jaws, as they have no bones? In this symposium, we consider jaws more generally: as a series of rigid, articulated structures that function to grasp, manipulate, and process food at the entrance of an animal's alimentary canal. Based on this broad definition, the term jaw can be applied to any hardened cuticular, sclerotic, or mineralized secretions that either surround the mouth externally or are directly inside the oral cavity. Familiar examples of jaws from the invertebrate world include the Aristotle's lantern of sea urchins (Echinodermata), the bloodletting jaws of leeches (Annelida), and the beaks of cephalopods (Mollusca). Examples from less familiar animals include the gasping spines of arrow worms (Chaetognatha), the trophi of rotifers (Rotifera), the pharyngeal teeth of priapulids (Priapulida), the oral stylets of mud dragons (Kinorhyncha), and the complex jaw-like sclerites of species of Gnathostomulida and Micrognathozoa. In fact, the diversity of jaw types among invertebrates is truly remarkable (one might say "jaw-dropping"). Moreover, as evidenced in the literature, an even looser definition of the term might apply when (1) the jaw-like structures are far removed from the oral cavity but still function in the manipulation of food, as occurs with the anterior proboscis hooks of some kalyptorhynch (Platyhelminthes) flatworms (Uyeno and Kier 2010) or (2) when the jaws are derived from internalized appendages, as occurs in species of Onychophora (Oliveira and Mayer 2013), and possibly species of Tardigrada (Guidetti et al. 2012). One might argue that this overwhelming structural diversity clouds the meaning of the term "jaws", but our purpose is not to study homology; it is well known that most animals' jaws evolved independently over the course of >500 million years (Fig. 1), and yet all have converged on two important functions—to obtain and process food. Altogether, invertebrates as different as annelids, chaetognaths, flatworms, gnathostomulids, micrognathozoans, mollusks, rotifers, and several ecdysozoans may be said to possess jaws. However, unlike the case with vertebrates, their origins, compositions, and functions still remain a mystery.

While almost any invertebrate with oral or extra-oral structures dedicated to the manipulation and/or mastication of food may be said to possess jaws, few invertebrates other than the arthropods have received much attention. Snodgrass' (1950) seminal work on mandibulate arthropods began a long tradition of research on arthropods' jaws, which was followed by Manton and Harding's (1964) comparative functional and evolutionary study of mandibles, maxillae, chelicerae, and other jaw-like elements. Since then scientists have used structure and function of arthropods' jaws as a means of gaining insight into trophic and evolutionary ecology (Krenn 2010; Krenn and Aspöck 2012; Perdomo et al. 2012), as well to help answer important questions about the origin of the head, segmentation, and patterning of the body (Simonnet and Moczek 2011; Angelini et al. 2012; Sharma et al. 2012). We contend that the jaws of other invertebrates provide similar opportunities for their respective lineages even though their jaws differ in most respects from those of arthropods. For example, the jaws of most (but not all) soft-bodied invertebrates appear to be derivatives of the oral cavity, are compositionally different than the surrounding tissues (i.e., they are not derived from a similarly hardened body wall), and are controlled solely by extrinsic muscles (as opposed to both extrinsic and intrinsic muscles as in arthropods). Other differences, for example, in the structure of the jaw joints and their movements, are also present, but the degree of variation among invertebrates has yet to be fully investigated. The soft-body of most jawed invertebrates is also an important consideration because the hydrostatic skeleton is likely to play a significant role in movement of the jaw, and so one might expect a correlation among the jaw's mechanics, the presence of a body cavity (coelom or blastocoel), and characteristics of the body wall (e.g., orientation, thickness, and rigidity of collagen fibers).

In this symposium, our aim is to achieve a greater understanding of the similarities among soft-bodied, jawed invertebrates by exploring the development and structure of their jaws and the mechanistic bases of the capture and processing of prey. A combination of approaches is used to study the structure and evolution of jaws, as well as feeding behavior, including high-resolution light microscopy, immunohistochemistry, confocal laser scanning microscopy (CLSM), electron microscopy, energy dispersive X-ray spectroscopy, and 3D modeling. We further seek to advance our understanding of the importance of jaws in the evolution of invertebrates by combining evidence from studies of (1) behavior, (2) biomechanics, (3) ecology, (4) neuromuscular integration, (5) molecular phylogenetics, and (6) the ultrastructure of animals that span the (a) phylogenetic spectrum (Lophotrochozoa and Ecdysozoa), (b) ecological spectrum (freshwater, marine, terrestrial), and (c) size-spectrum (<1 mm to several meters long). We also
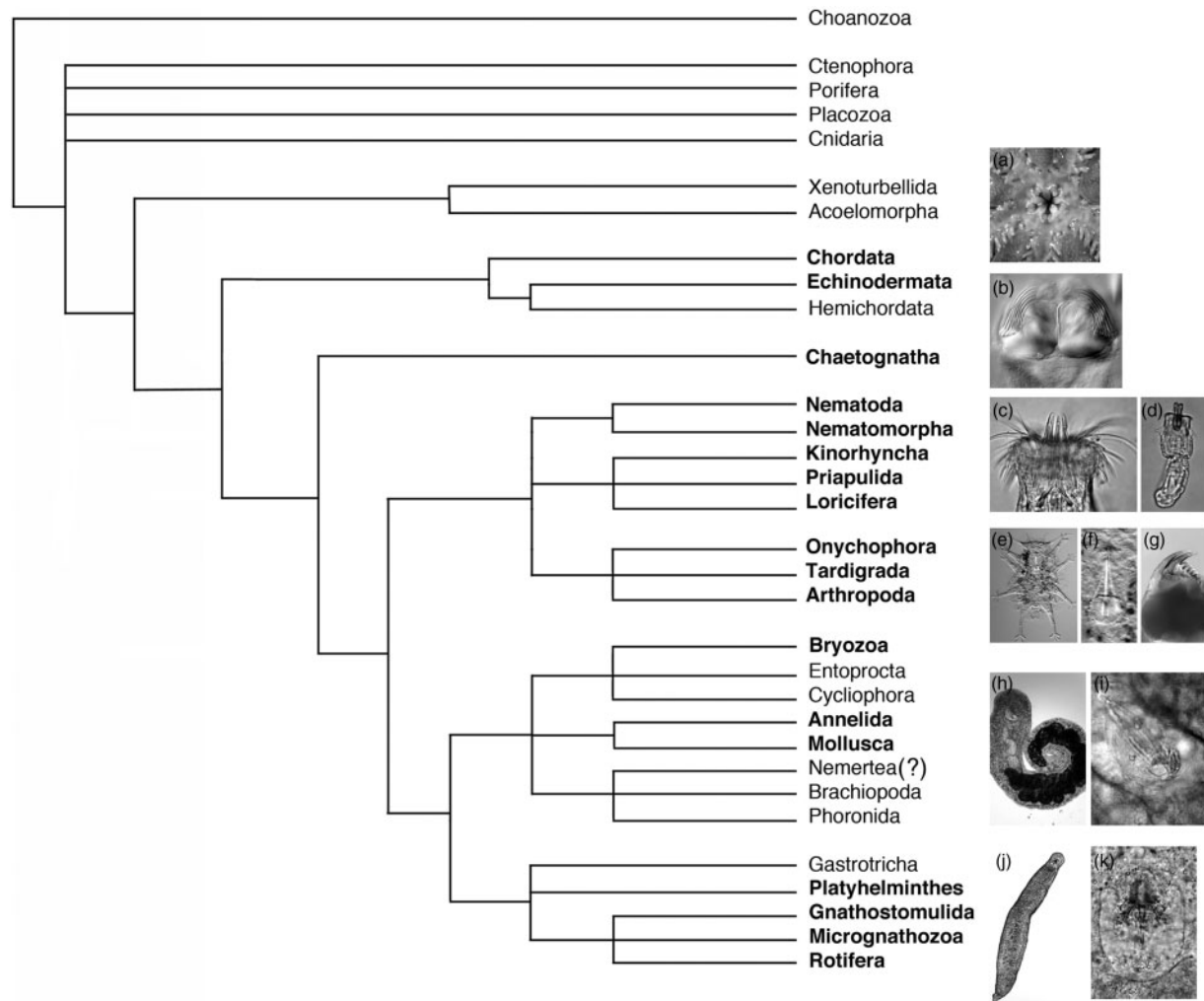
Fig. 1 Animal phylogeny showing the distribution of "jawed" taxa (bold). The phylogeny is based on the results of Edgecombe et al. (2011). (a) Oral view of the brittle star mouth and jaws; (b) chaetognath spines; (c) kinorhynch mouth-cone; (d) nematomorph larva with protruding stylet; (e) marine tardigrade; (f) buccopharyngeal apparatus of tardigrade from (e); (g) jaw of an onychophoran; (h) interstitial species of *Platyhedyle* (Mollusca: Gastropoda); (i) jaws of *Platyhedyle*; (j) gnathostomulid; and (k) jaws of gnathostomulid.

explore how jaws can provide useful systematic information for interpreting phylogenetic trends in trophic ecology and for revealing cryptic species. This symposium provided a framework upon which to build collaborative and integrative research ideas that can enhance cooperation among scientists and guide their common efforts to understand the evolution and function of jaws in soft-bodied invertebrates. Presently, there is no common set of approaches to understand how jaws develop, how they evolved, or how they are used by animals with vastly different body plans and that live in entirely different environments. Ultimately, we expect to combine quantitative functional and ecological data to model the evolution of jaws in diverse invertebrates and search for a common set of principles underling mechanics and adaptability.

Rotifers as models of the complexity of jaws

We open this conversation with a review of phylum Rotifera, an important freshwater taxon comprising >2500 species of unsegmented, bilaterally symmetrical micrometazoans (Wallace et al. 2006; Segers 2007; Segers et al. 2012). To avoid unnecessary complications of phylogeny we ignore Acanthocephala, a small taxon of jawless, obligatory endoparasites formerly given status as a separate phylum (Wey-Fabrizius et al. 2014).

Rotifers hold a peculiar position in science. In fact there is much to promote rotifers as research tools (Wallace 2002; Fussmann 2011). Ecologically, rotifers are basal consumers and thus important components both in the microbial loop (Hart et al. 2000; Fermani
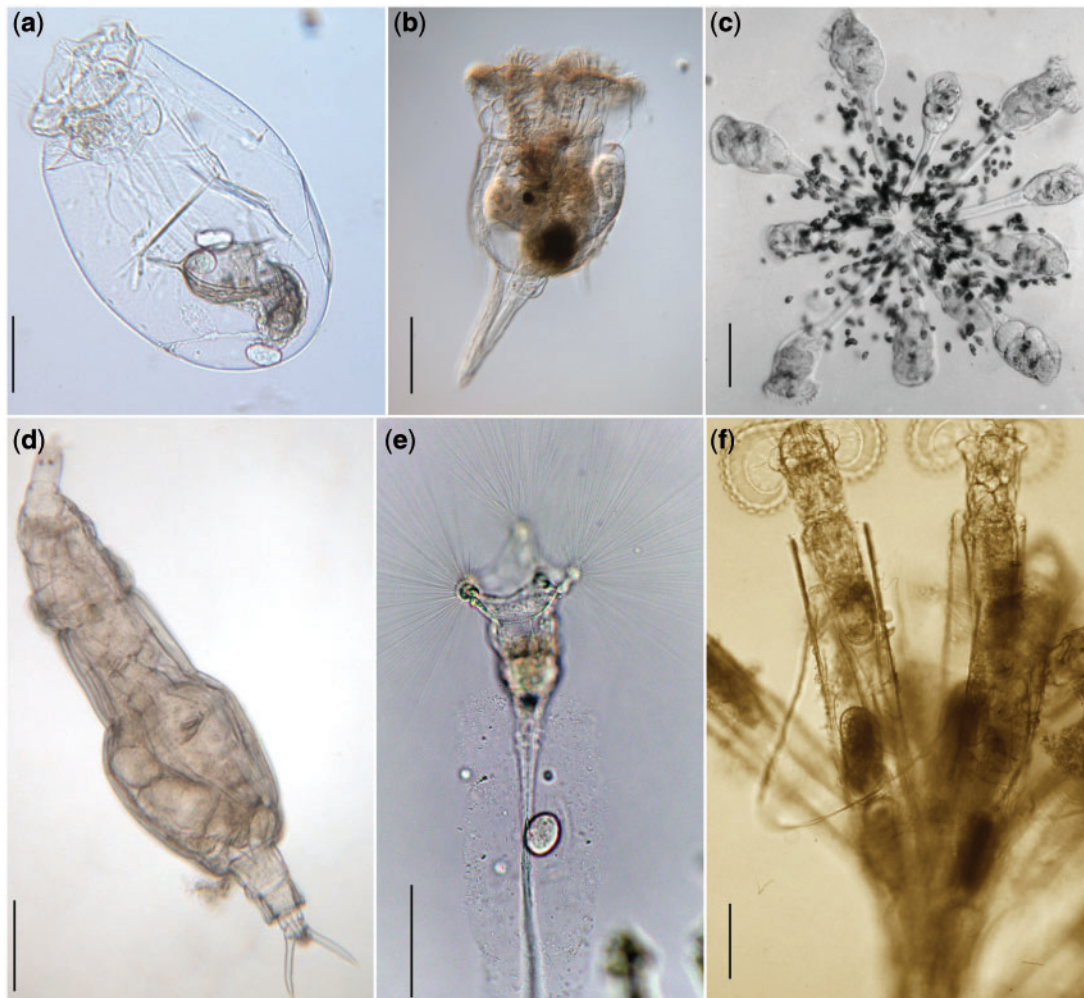
Fig. 2 Diversity in form and function within Rotifera. (**a**) *Asplanchna*—planktonic; raptorial (prey, *Keratella*, in gut); (**b**) *Epiphanes*—planktonic; microphagous; (**c**) *Conochilus*—planktonic, colonial within a common gelatinous matrix; microphagous; (**d**) *Rotaria* (bdelloid)—creeping; microphagous. (**e**) *Collotheca*—sessile, in a solitary gelatinous tube; predator; (**f**) *Limnias*—sessile, in a hardened tube; microphagous—*Collotheca*—sessile, in a solitary gelatinous tube; raptorial. Scale bars = 100 µm.

et al. 2013) and in higher trophic levels. As a taxon, they are recognized as being cosmopolitan in distribution, yet upon closer examination many species are seen as having a biogeography (Segers 1996, 2003). Moreover, molecular studies have revealed a remarkable level of cryptic speciation: 2–10× the diversity than previously realized (Gómez et al. 2002; Fontaneto et al. 2007, 2011). Rotifers are relatively easy to culture (Wallace et al. 2015) and, as a result, are used extensively as feed for commercially important species in aquaculture (Hagiwara et al. 1997) and are used in studies of phylogeny (Sørensen and Giribet 2006; Fussmann 2011), population dynamics (Yoshida et al. 2003; Hampton 2005), ecotoxicology (Dahms et al. 2011), and aging (Snell et al. 2012; Gribble and Mark Welch 2013).

Rotifers are bound to an aquatic existence, being present both in marine and freshwater habitats, as well as in a variety of temporary habitats such as mosses, wet soils, and ephemeral rock pools (Walsh et al. 2014). While diverse in form and function (Fig. 2), rotifers are united by three characteristic features that separate them from other Gnathifera (Giribet et al. 2004; Wallace et al. 2015). (1) All rotifers possess a syncytial body wall embedded with a layer of filamentous proteins called the intracytoplasmic lamina (ICL). The ICL provided the first evidence of the connection between rotifers and acanthocephalans. (2) To varying degrees the anterior end of the adult is elaborated into a ciliated field called the corona. In some species the action of the cilia gives the illusion of a rotating wheel, from which the group derives its name: the wheel bearers. In a few

Table 1 Characterization of the components and functions of rotifers' trophi

Type	Taxonomic distribution	Function	Selected references
Ramate	Bdelloidea	Grinds food particles	Melone et al. (1998), Melone and Fontaneto (2005)
Fulcrate	Seisonidea	Pumping action is achieved by the hypopharynx muscle, which is attached to the strong fulcrum	Segers and Melone (1998)
Malleoramate	Flosculariaceae	Grasps and grinds food before pumping crushed material into the esophagus	De Smet (2005), Wulfken and Ahlrichs (2012)
Uncinate	Collothecidae	Grasps single prey items from a funnel-shaped mouth (infundibulum) pulling them into the stomach	Wright (1958), Bevington et al. (1995), Meksuwan et al. (2013)
Forcipate	Dicranophoridae	Acts like a forceps with the trophi being projected from the mouth to grasp or tear the prey, which is then transferred to the mouth and swallowed	De Smet and Pourriot (1997), Riemann and Ahlrichs (2008)
Incudate	Asplanchnidae	Mastax initiates the capture of prey by creating a suction that draws prey into the mouth, which is then stuffed into the stomach with the aid of the trophi	Salt et al. (1978), Jose de Paggi (2002)
Malleate	*Brachionus, Keratella, Kellicottia*	Grasps and grinds food before pumping crushed material into the esophagus	Kleinow (1998)
Virgate	*Notommata, Polyarthra, Synchaeta*, Ituridae (modified)	Produces a piercing and pumping action; facilitates sucking fluids from plant cells or animal prey	Nogrady et al. (1995), De Smet and Pourriot (1997)
Cardate	Lindiidae	Produces a pumping action	Nogrady and Segers (2002)

sessile species, ciliation is lost or reduced and instead long setae ring the margin of the head; in some bdelloids the anterior end is reduced to a ciliated plane. While highly varied, the corona always functions in the acquisition of food and often in locomotion (3). The jaws of rotifers, called trophi, are located in a muscular pharynx, which is termed the mastax. Nine different kinds of trophi have been recognized.

Diversity and ultrastructure of trophi (rotifers as models of jaw-diversity)

We posit that rotifers are ideal models for the study of jaw-diversity. Within this relatively small phylum, nine distinct forms of jaws (Table 1 and Fig. 3) have evolved, but modifications and transition forms have been described. These forms are classified on the basis of size and shape of their seven major sclerotic elements (Wallace et al. 2015). While all types of trophi share these elements, each is modified slightly. In addition there may be accessory parts (e.g., hypopharynx, epipharynx, and alulae) and some transitional and anomalous variants do exist (Koste 1978; Segers 2004). This level of diversity contrasts with the very complex jaws of micrognathozoans, which possess 2 unpaired and 18 paired elements (De Smet 2002), and the less diverse jaws of gnathostomulids (Sørensen 2000, 2002b; Sørensen and Sterrer

2002). In addition, much more work has characterized the composition and function of rotifers' trophi (see below), thus making them amenable as a template for the diversification of jaws in other taxa.

In rotifers, details of the structure of trophi are often essential in the identification of species. In fact, the features of rotiferan trophi can be unique to families, genera, and even species (Edmondson 1959; Wallace et al. 2015). For example, ramate and fulcrate trophi are found only in Bdelloidea and Seisonidea, respectively, while malleoramate trophi are restricted to the Order Flosculariaceae. At the familial level, uncinate, forcipate, and incudate trophi are associated with Collothecidae, Dicranophoridae, and Asplanchnidae, respectively. Three species can be distinguished by the size of an opening (i.e., the basal window) on the ramus within the *Epiphanes senta* species-complex (Schröder and Walsh 2007).

The morphology of trophi can vary with diet within a given taxon. For example, the only carnivorous bdelloid found to date, *Abrochtha carnivora*, has one of the largest trophi of all bdelloids (Melone and Fontaneto 2005). Furthermore, in a study of eight species of rotifer (4 monogononts and 4 bdelloids), it was found that the trophi grew after hatching in only *Asplanchna priodonta,* a predatory species (Fontaneto and Melone 2005).
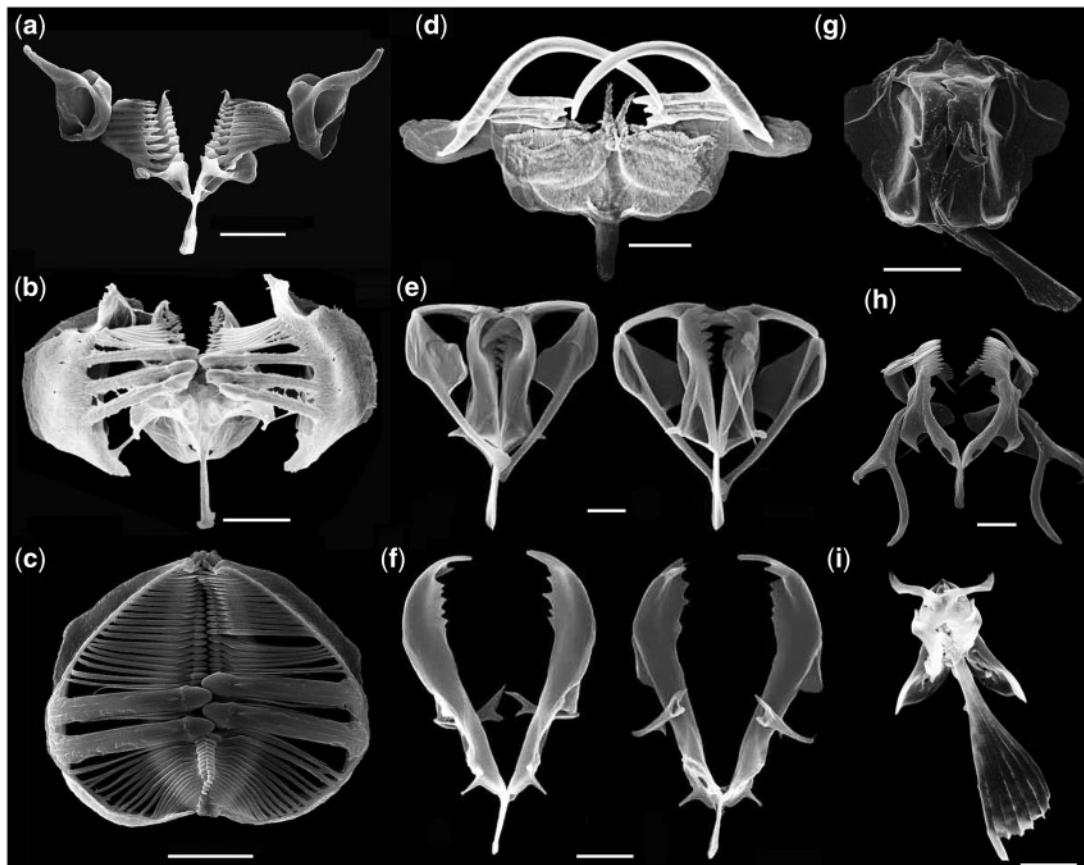
Fig. 3 Examples of the nine types of rotiferan trophi. Grasping and grinding trophi (**a–c**). (a) Malleate: *Cyrtonia tuba* (Ehrenberg, 1834); (b) Malleoramate: *Sinantherina semibullata* (Thorpe, 1889); (c) Ramate: *Rotaria tardigrada* (Ehrenberg, 1830). Grasping trophi (**d–f**). (d) Uncinate: *Cupelopagis vorax* (Leidy, 1857); (e) Forcipate: *Dicranophorus forcipatus* (O.F. Müller, 1786) dorsal (left) and ventral (right); (**f**) Incudate: *Asplanchna priodonta* (Gosse, 1850) ventral (left) and dorsal (right). Pumping trophi (**g–i**). (g) Virgate: *Polyarthra dolichoptera* Idelson, 1925; (h) Cardate: *Lindia deridderae* Koste, 1979; and (i) Fulcrate: *Seison annulatus* (Claus, 1876). Bars = 10 μm. SEMs courtesy of D. Fontaneto.

Muscle supply, neural integration, and mechanics

The rotiferan jaw is part of a larger organ system (the mastax) that includes epithelia, glands, muscles, and nerves that interact to generate complex movements of individual sclerites for processing food (Fig. 4). Many rotifers suck or pump prey items into their mastax using a combination of cilia and muscles, while others actively grab the prey by extruding the free ends of the sclerites out of their mouth. Regardless of the method of ingestion, it is the sclerites (and probably the sclerotic wall of the mastax's lumen) that functions to prepare prey for primary digestion. At first sight, the multi-articulate nature of rotifers' trophi seems to be an evolutionary case of over-engineering, with seven or more elements performing apparently simple tasks such as piercing, grasping, or mastication. Why so many hard parts are required for capturing and processing

prey remains a mystery. Unfortunately, there has yet to be any studies that correlate the movements and fine structure of the sclerites with the ultrastructure of the prey. For example, some algivorous rotifers have trophi with a piercing sclerite that impales cells, but it is unknown whether the physical structure of the sclerite (e.g., composition and hardness) is adapted to the physical attributes (e.g., biomineralized cell walls) of specific types of algae. Alternatively, adapting to softer and more mobile prey such as ciliates might require sclerites of a different composition and with different physical attributes.

Studies on the function of the mastax date back to de Beauchamp (1909), Martini (1912), and Remane (1929–1933), when these early investigators made significant observations on innervation and on the arrangement of muscles that control the jaws. Their observations also laid the foundations for a complex terminology that describes mastax muscles based
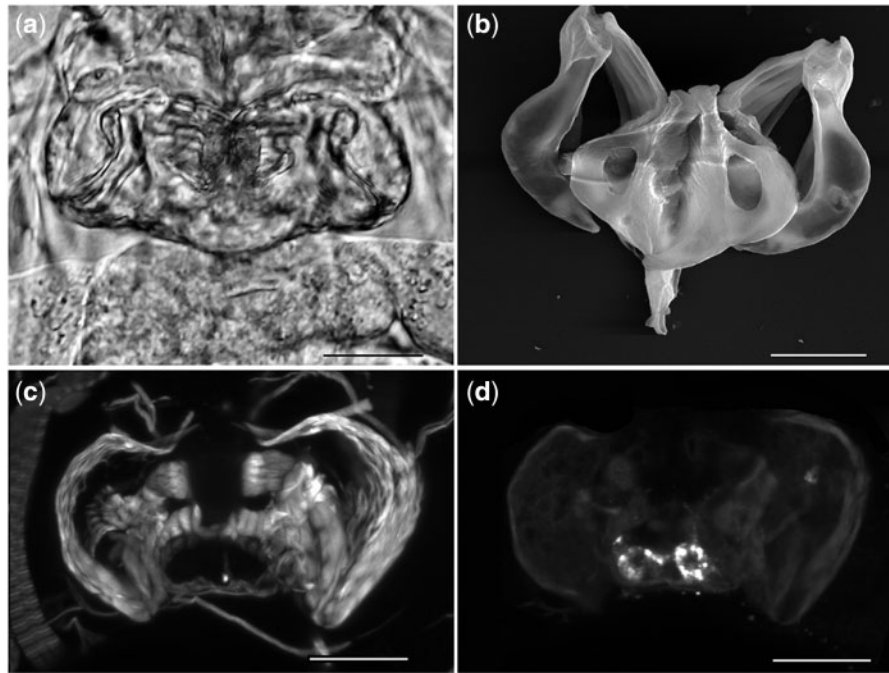
Fig. 4 The trophi of *Brachionus*. (**a**) Close-up of the mastax and trophi of *Brachionus plicatilis*. (**b**) SEM of the trophi of *B. calyciflorus*, dorsal view. (**c**) The musculature of the mastax of *B. plicatilis*. (**d**) FMRFamide-like staining in the mastax ganglia in *B. plicatilis*. Scale bars: a, c, d = 20 μm; β = 10 μm.

on the positions of insertions (e.g., the paired musculo fulcro-manubricus connects the fulcrum and manubria). However, since these early descriptions, relatively few studies have focused on how the mastax functions in a holistic sense: i.e., how prey is sensed, how that information is relayed to the mastax, and how individual muscles are stimulated to control movements of sclerites. To date, only Clément and Amsellem (1986) have attempted to tackle this topic through their neuro-ethological studies, which revealed that sensory information from the brain is related to the mastax ganglion, which is proposed to control the mastax's musculature and initiate piercing or pumping. If prey is captured, receptors of the mastax "taste" the prey and relay this information back to the ganglion, which if the prey is acceptable, signals the muscles of the sclerite to further process the prey. Because the sclerite's muscles are electrically coupled, contractions can happen quickly and do not appear to require multiple neural inputs. Since the study of Clément and Amsellem, several researchers have used CLSM and electron microscopy to reveal additional details about the complex process of capturing prey by a variety of microphagous and predatory rotifers (Riemann and Ahlrichs 2008; Wulfken et al. 2010; Wulfken and Ahlrichs 2012). Unfortunately, our knowledge about how the trophi function is based

on less than 1% of the described species and so confounds our abilities to generate a standard model of how rotifers' trophi function.

Novel methods of study and their challenges

To date, rotifers' jaws have received significant attention at the fine structural level (SEM) largely for the purposes of biological classification, with more recent studies using TEM and CLSM gaining further insights into their ultrastructure and mechanics. While these data have proven extremely valuable, much remains to be learned about the ecological and evolutionary reasons for so much variation in the structure of trophi, how it functions, and perhaps how further knowledge of rotifers' jaws can be of some practical use in the sciences. In an attempt to answer these questions and provide ideas for future consideration, we highlight two relatively novel methods of studying rotifers' trophi that we think will have interpretive value.

Elemental analyses

Rotifers are soft-bodied animals that nevertheless produce hard secretions (the trophi) with which they process their food. Given the kinds of foods consumed by rotifers (e.g., diatoms) their trophi must be very tough, wear-resistant, stiff, and hard. How

soft tissues secrete hardened materials (e.g., bone and cuticle) has been the subject of research for more than 100 years, but for soft-bodied invertebrates, this research is relatively new, especially concerning the production of sclerotic jaws. Perhaps the best-known examples are the jaws of polychaete worms and the radulae of gastropod mollusks, which have been subject to a wide variety of chemical and mechanical analyses, including back-scattered electron detection, X-ray diffraction, X-ray absorption spectroscopy, extended X-ray absorption fine structure (EXAFS), matrix-assisted laser desorption-ionization time-of-flight mass spectroscopy (MALDI-TOF), and nanoindentation (Broomell et al. 2007; Lichtenegger et al. 2008). In total, these analyses have revealed that the jaws of gastropods and polychaetes are composite secretions made of an organic matrix (chitin, protein) impregnated with either heavy-metal salts (e.g., Fe and Cu) that increases the toughness, as in gastropod radulae (Lichtenegger et al. 2008), or are impregnated with heavy metals (e.g., Zn) that function as cross-linkers in histidine-rich proteins, as in polychaetes' jaws (Broomell et al. 2007).

By way of comparison, studies of rotifers' jaws are sorely lacking. Histochemical procedures have shown that they consist of acid mucopolysaccharides (Klusemann et al. 1990) and chitin (Kleinow 1993), the latter being the primary structural compound and probably making up 50–75% of the jaw's matrix. Since then, no new methods have been applied to rotifers' jaws, perhaps because of their miniscule size. Recently however, we have used a well-known, but rarely employed, technique called energy dispersive X-ray spectroscopy (EDS, EDX) to determine the presence of trace elements in rotifers' jaws. This method relies on the production of atom-specific X-rays produced after stimulation by an electron beam (reviewed by Goldstein et al. 1992). Briefly, when an electron beam strikes a sample, it ionizes resident electrons and they are ejected from the inner-valence shells of all atoms, which are then replaced by electrons from outer-valence shells. The movement of electrons from outer to inner valence shells results in the production of X-rays that are specific to the atom and the valence shell. These X-rays are then detected by an EDX detector. This technique is particularly useful for the study of specimens that have been previously prepared for SEM because the EDX software can subtract the noise generated by the outer conductive coating (gold, gold-palladium) to determine the chemical composition of the specimen underneath.

While the benefits and constraints of EDX are well known (Goldstein et al. 1992), its utility for characterizing the chemical composition of miniscule jaws, like those of rotifers, has not been previously explored, and we are currently perfecting methods of analysis that will provide better resolution. Our recent analyses of the jaws of two species, *Dicranophorus forcipatus* (Monongononta: Ploima) and *Sinantherina socialis* (Monogononta: Gnesiotrocha) point to its potential. Our analyses have preliminarily identified that the trophi of both species are composed of carbon, oxygen, and nitrogen, the bulk of which are assumed to make up the chitinous and perhaps proteinaceous components of the jaws. Other elements have been detected, including calcium and magnesium, but the presence of these elements remains tentative because of the challenges of working with such small specimens (Fig. 5). Rotifers' jaws are miniscule—in most species the entire apparatus can be <50 μm wide with individual sclerites as small as 10 μm long and less than 5 μm in depth—making their characterization extremely difficult. The size is particularly problematic because an electron beam can penetrate up to 4 μm through a sample, therefore passing through the sclerites and penetrating (and stimulating) the background atoms of the sample substrate (Brundle et al. 1992). The background in most cases is either an aluminum stub or a glass coverslip. In neither case are the backgrounds homogeneous; this is particularly true of glass, which contains a wide variety of elements (e.g., Ca, Fe, Na, P, Si; R. Hochberg personal observations). The background noise therefore can interfere with the data from the sample and make interpretation extremely challenging. At present, we are searching for alternative background mountings (e.g., aluminum foil) to remove the elemental noise and provide a more homogeneous chemical signature.

Computer modeling

Biomechanical computer models are standard methods of exploring vertebrates' jaws so as to understand mechanical and trophic diversity (Lautenschlager et al. 2013), functional transformations during growth (O'Higgins et al. 2012), and evolutionary patterns in a phylogenetic context (Moazen et al. 2009). To date, such modeling has not been widely applied to the study of invertebrates' jaws, possibly because most are not multi-articulated structures like those present in vertebrates, and so mechanical complexity (numbers of levers, linkages, muscles) is assumed to be significantly less. However, as seen in rotifers and some of their gnathiferan relatives (e.g., Gnathostomulida and Micrognathozoa), complexity of the jaws can be elaborate and involve a wide variety of muscles supplying up to nine or more
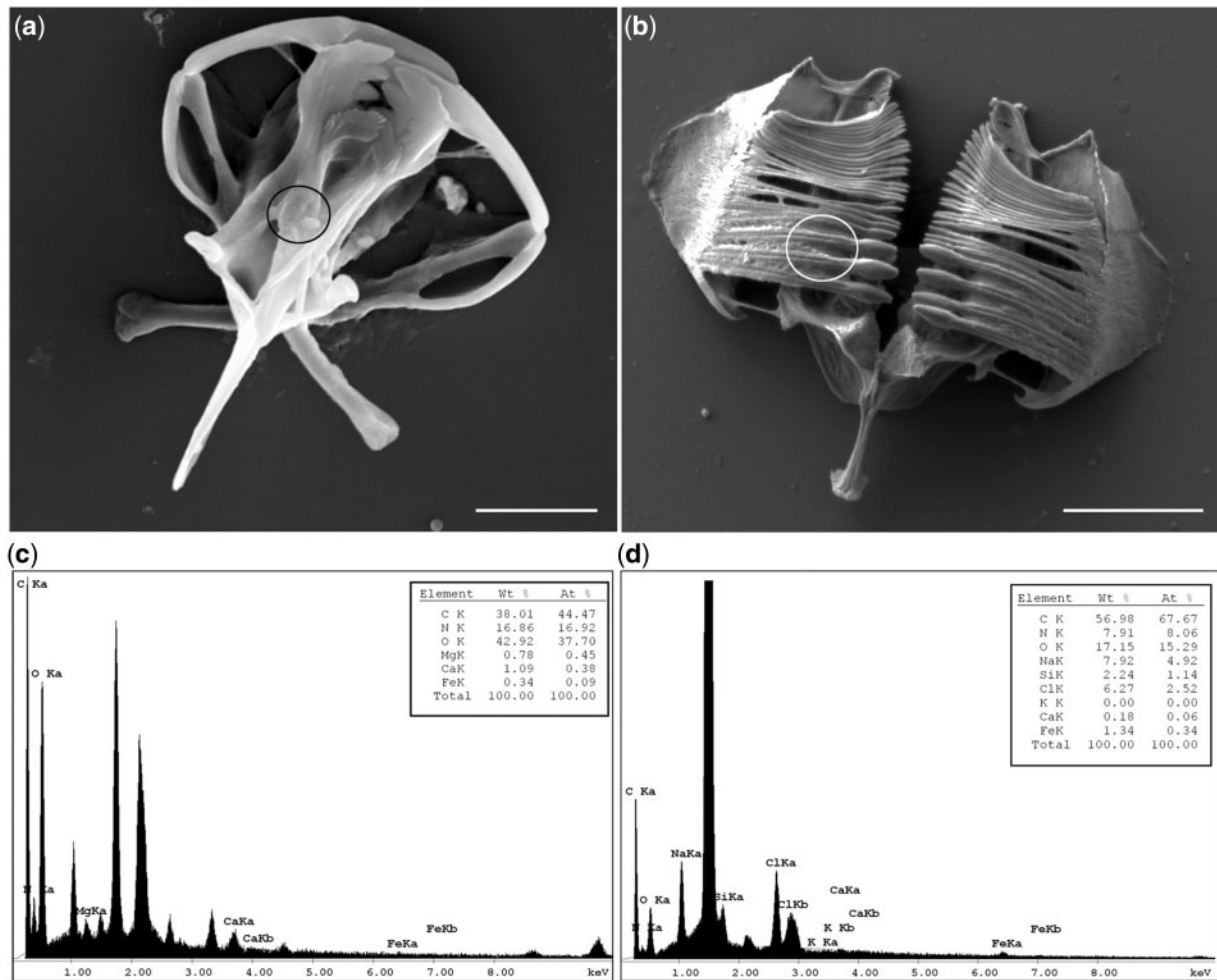
Fig. 5 Scanning electron micrographs of the trophi of *Dicranophorus forcipatus* (**a**) and *Sinantherina socialis* (**b**). Both specimens were prepared through the dissolution of soft tissues with sodium hypochlorite (bleach) and mounted directly on either an aluminum stub (a) or glass coverslip on an aluminum stub (b). EDX histograms showing the relative amounts of individual elements plotted against accelerating voltage for *D. forcipatus* (**c**) and *S. socialis* (**d**); both histograms are the results of scans (circles) on the thickest regions of the trophi and likely include background "noise" from the substratum. The labels Ka and Kb refer to K-alpha (lowest energy X-ray peak) and K-beta (highest energy X-ray peak), respectively, and represent the two energy X-ray peaks of the K shell (contains two electrons). When the electron beam from the SEM hits the K shell electrons, the K shell electrons are ionized (ejected) and replaced by electrons from outer valence shells, thereby producing X-rays. The inset shows the weight percent and atomic percent of each atom. Scale bars: a, b = 15 μm.

separate sclerites. We believe that rotifers' jaws are therefore complex enough for computer modeling and can, in theory, be modeled to explore their three-dimensional geometry, bite-force, material properties, and function.

Historically, most biomechanical modeling of animals' jaws was based on serial histological sections, and while the jaws of rotifers have yet to be modeled, serial sections via TEM remains the leading method of analysis. However, with the advent of non-destructive techniques such as CLSM and micro-computed tomography (micro-CT) that generate 3D digital reconstructions from stacks of 2D images, the possibility exists for the reconstruction

and modeling of rotifers' trophi. In these cases, the data are digital and ready for computer modeling, but to date, neither technique has been employed to study the mechanics of rotifers' jaws. Micro-CT should, in theory, be the method of choice (Metscher 2009; Faulwetter et al. 2013), but there appears to be limits on the level of resolution, with no studies publishing data on sections smaller than 5–10 μm in invertebrates (Betz et al. 2007); individual sclerites can have dimensions down to 1 μm or less (in depth), so the utility of micro-CT for their study remains unknown. Alternatively, synchrotron micro-tomography can achieve submicron-level spatial resolution, but numerous hurdles exist in the use of this
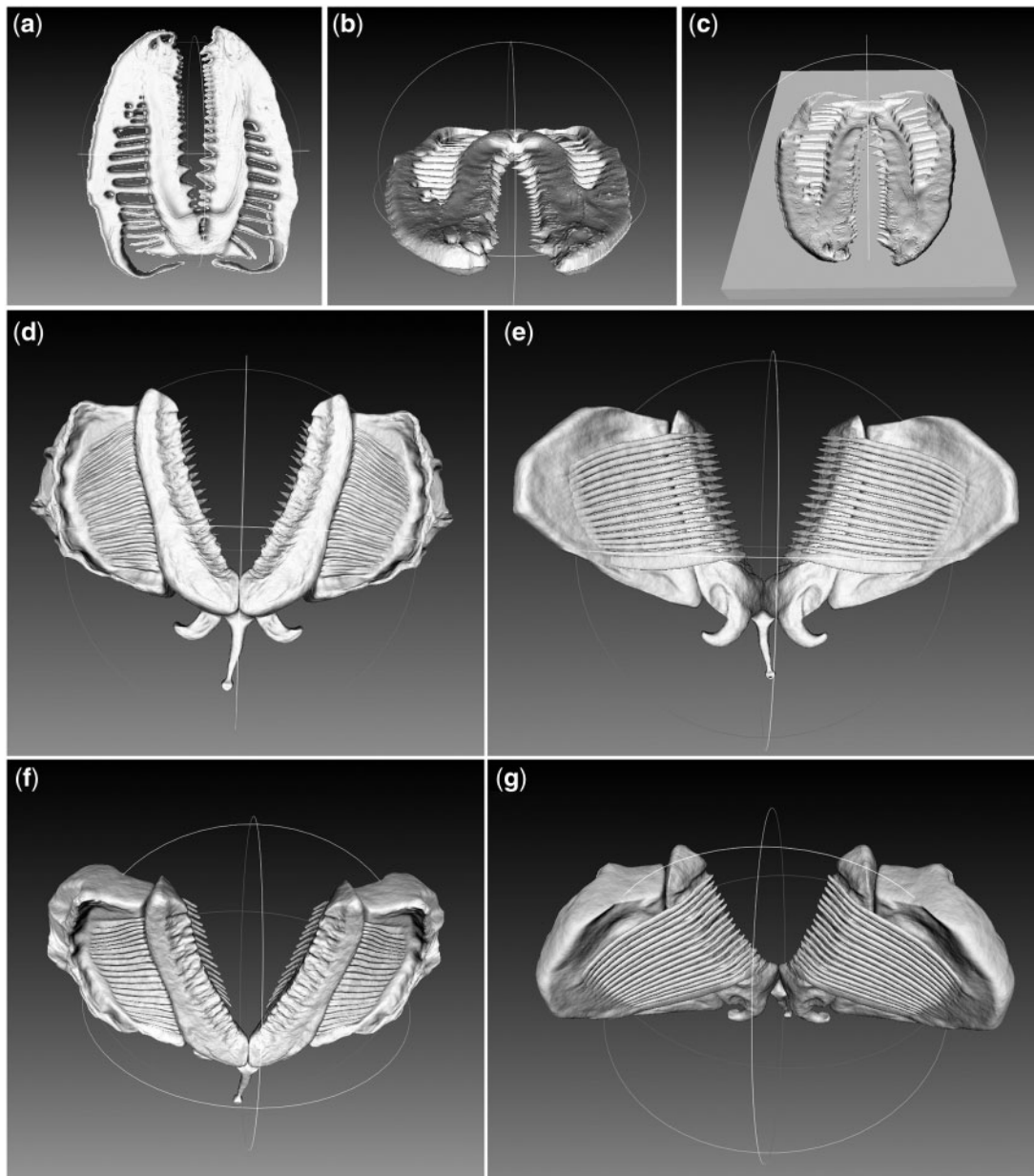
Fig. 6 3D models of rotifers' trophi based on brightfield and SEM images of (**a–c**) *Habrotrocha gracilis* (Bdelloidea) and (**d–g**) *Filinia longiseta* (Monogononta: Gnesiotrocha). The model of *H. gracilis* shows dorsal (a) and anterior (b) views, with (c) revealing a mold of the jaws that can be used for 3D printing. Models of *F. longiseta* in (d) dorsal, (e) ventral, (f) anterodorsal, and (g) anteroventral views.

technology, not the least of which is its availability (Betz et al. 2007); this makes it impractical for the study of rotifer's trophi.

Presently, we are working with computer modelers to generate 3D models so as to understand the form and function of rotifers' trophi. These models are generated in software packages such as MeshLab (Sourceforge.net) based on brightfield and SEM images, which achieve the necessary resolution to identify much of the structure and geometry of individual sclerites. However, there are several

limitations to this method, including the quality of the images, the ability to visualize all surfaces (requiring trophi to be mounted in multiple orientations), and the experience of the modeler. At present, we have designed basic models for the trophi of the bdelloid *Habrotrocha gracilis* (Fig. 6a–c) and the monogonont *Filinia longiseta* (Fig. 6d–g). These models can be rotated in 3D space to visualize all surfaces, and the models can also be made into molds for 3D printing. Nevertheless, even when models are designed and printed, videos of live

specimens are necessary to understand the precise movements of individual sclerites that can then be incorporated into the models to design the proper types of joints (e.g., sliding joints) that connect sclerites.

Practical applications

As mentioned by Wallace (2002), rotifers' trophi are similar in structure to complex tools such as micro-forceps, and based on this resemblance he posed a challenge for future researchers: "Could certain rotifer trophi serve as models for a new microtechnology?" We believe this challenge can be met once novel procedures for studying rotifers' jaws are combined with greater cross-disciplinary efforts (e.g., material science, physics, and robotics) to study and understand their potential uses. For example, microscale robotics is an emerging discipline and one that is predicted to have an impact on fields as disparate as environmental monitoring, homeland security, scientific research, and healthcare (Sitti 2007; Diller and Sitti 2013). As noted by Sitti (2007): "Handling μm- and nm-scale objects and accessing tiny spaces down to μm/nm sizes have become critical issues." However, one of the grand challenges for small-scale robotics is to understand the physics of extremely small size and then either adapting mesoscale robots to control microscale tools or miniaturizing the entire robot itself to engage in micromanipulation (Sitti 2007). An example of the former process already occurs in contemporary medicine, in which robotics-assisted micromanipulation of exceedingly small tools is used for delicate surgeries and to suppress the hand-tremors of surgeons (Camarillo et al. 2004). The latter process, whereby robots themselves are miniaturized, is developing at a fast pace, with one of the hopes being that miniature robots could be used to diagnose and treat diseases of humans (Sitti 2009). Regardless of their application, researchers acknowledge the difficulty of scaling down to meet the physical demands of extremely small size, which requires new materials that are compact, light-weight, can endure high mechanical stress, and require new grippers for precision manipulation (Sitti 2007). In this regard, we offer rotifers' jaws as a potential source of inspiration and study. At less than 50 μm, the jaw is composed of seven separate parts that grip, grind, crush, and pierce a wide variety of cellular materials, and all without showing significant wear or evidence of fracture. We think that with greater attention to their physical compositions and mechanics, rotifers' jaws might be revealed to be highly effective mechanical tools at the micron-scale and therefore to be of use in surgical and other precision-based micro-robotic applications.

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